

**High – Voltage Capacitively Coupled Contactless
Conductivity Detection
for Conventional and Microchip Capillary
Electrophoresis**

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For my father

Jabson Chikwanda Tanyanyiwa

Summary

This thesis focuses on the optimisation of capacitively coupled contactless conductivity detection for capillary and microchip electrophoresis and its applications in analytical chemistry.

First, the effect of high excitation voltages and operation frequencies on the capacitively coupled contactless conductivity detector cell for conventional capillary electrophoresis is evaluated. The detector electrodes comprised two steel tubes cut from hypodermic needles, through which the capillaries were inserted. It is demonstrated that increasing excitation voltages from 25 V_{pp} to 250 V_{pp} improves the detection limits by a factor of 10. The high actuator voltage approach was also investigated for contactless conductivity detection on glass-microchip devices with an 8 cm long channel. The detector electrodes formed part of the microchip and were placed on the microchip directly above the microchannel. In a separate project the simplification of on-microchip contactless conductivity detection was accomplished. This was achieved by integrating the detector electrodes on to a chip-holder specifically designed for this purpose. Thus the electrodes were a part of the holder, an improvement of the previous arrangement whereby the detector electrodes were situated on the microdevice.

Finally the applications and advantages of the optimised high-voltage capacitively coupled contactless conductivity detection for inorganic and organic analysis were demonstrated. The separation and detection of 14 metal ions was accomplished in less than six minutes. The compatibility of this detector with non-UV transparent, polymer capillaries has been demonstrated. The detection of native amino acids has been evaluated. Part of the work was dedicated to the on-chip analysis of various classes of organic ions. The two immunoproteins human immunoglobulin M (IgM) and immunoglobulin G (IgG), were analysed in their unlabelled state on both capillary and lab-on-chip platforms. All species involved in an immunological interaction between IgM and IgG could be detected. A method for the analysis of selected basic pharmaceutical drug substances was developed. Detection limits comparable to those supplied by direct UV detection were obtained. Main component assays of selected pharmaceutical preparations have been demonstrated.

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1 Introduction

1.1 Historical Background

Electrophoresis is the differential migration of charged ions by attraction or repulsion in an electric field. It was introduced by Tiselius in 1937. By applying an electric field to a mixture of proteins placed between buffer solutions in a tube, Tiselius discovered that sample components migrated in a direction and at a rate determined by their charge and mobility. However separation efficiency was limited by thermal diffusion and convection. Thus, electrophoresis has been traditionally performed in anti-convective support media, such as semi-solid slab gel or in non-gel media such as paper or cellulose acetate. The support media provide the physical support and mechanical stability for the carrier electrolyte. Alternatively, electrophoretic separations may be performed in narrow-bore tubes or capillaries. Since capillaries themselves are anti-convective, gel media are not essential for that function. This allows the performance of free-solution or open tube electrophoresis.

Initial work on open tube capillary electrophoresis was carried out by Hjerten in 1967 [1]. He rotated millimetre-bore quartz-glass capillaries coated with methylcellulose along their longitudinal axis to minimize the effects of convection. He employed UV detection. Later Mikkers' group performed zone electrophoresis in narrow-bore Teflon capillaries of 200 μm internal diameter [2]. They employed conductivity and UV detection. In the early 1980s Jorgenson and Lukacs used 75 μm id fused silica capillaries coupled with on-column fluorescence detection [3]. They reported that capillaries of small internal diameters reduced zone spreading due to convection and that these capillaries allow efficient dissipation of the heat generated by the application of high voltages. Jorgenson also clarified the theory, described the relationships between operational parameters and separation quality and demonstrated the potential of high performance capillary electrophoresis (HPCE) as an analytical technique.

In capillary zone electrophoresis, ionic analytes in solution migrate at different velocities from one another upon the application of an external field. Cations migrate towards the cathode, where they are visualised with a detector that may be situated on-column or end-column.

In modern capillary electrophoresis, separation is performed in even narrower capillaries of typically 25 - 75 μm inner diameters. These capillaries dissipate heat efficiently on one hand and at the same time compromise detection limits. Detectors that were employed in fundamental CE work namely UV and fluorescence were adapted from HPLC and are ill-suited to the narrow capillaries implemented in CE. This has provided the main drive for the quest for alternative electrochemical detection techniques.

A separate development in the capillary electrophoresis field is miniaturisation, whereby injection, separation and detection are carried out on microchannels embedded on planar devices [4, 5]. In the fundamental microchip work, mainly fluorescence detection was employed [6]. Although fluorescence detection offers very low detection limits, it is very expensive and not a universal detection method. Both factors contributing to further research for alternative electrochemical detection techniques.

Ever since Jorgenson's work, a variety of capillary separation techniques have been introduced. These are briefly discussed below.

1.2 Classification of Electrophoresis Modes

The most frequently used modes of capillary electrophoresis are:

- capillary zone electrophoresis (CZE),
- micellar electrokinetic capillary chromatography (MEKC),
- Capillary gel electrophoresis (CGE),
- capillary isoelectric focussing (CIEF),
- capillary isotachopheresis (CITP)

Capillary zone electrophoresis is the simplest and most frequently used mode. During CZE, separation occurs due to solute migration in discrete zones and at different velocities. Separation of both cationic and anionic solutes is possible due to a phenomenon known as electro-osmotic flow which will be discussed in the following section. Neutral solutes are co-eluted with electro-osmotic flow (EOF) rendering CZE an unsuitable method for their analysis

Micellar electrokinetic chromatography is a hybrid of CZE and chromatography. It is the only electrophoretic method that can be used for the separation of neutral and charged analytes. Separation of neutral solutes is accomplished by the inclusion of surfactants in quantities above their critical micellar concentration in the run buffer. The interaction of the micelles and the neutral solutes effect the separation.

Capillary gel electrophoresis is frequently used for bioscience applications. In this modus, size based separations are achieved by the inclusion of a suitable gel in the electrolyte which acts as a sieving medium.

Capillary isoelectric focussing is used to separate analytes with different isoelectric points. Mainly protein and peptides are analysed with this method. A pH gradient is created in the capillary by filling it with different ampholytes. Upon the introduction of an external electric field, the charged ampholytes and proteins migrate in the capillary until they reach the point where they are uncharged. CIEF may be used to determine the pI of unknown proteins.

Capillary isotachopheresis is performed by sandwiching a sample between a leading and terminating electrolyte and applying an electric field in the constant current mode. Anions and cations cannot be separated in the same run. In the case of cations, the leading electrolyte is chosen in such a way that its cations possess a higher mobility than the fastest migrating cation. The terminating electrolyte correspondingly contains the slowest cation. When an electric field is applied to the mixture, the analyte cations arrange themselves according to their mobilities between the fastest cation of the leading electrolyte and the slowest cation. After solute distribution, equilibrium is achieved and all the analytes migrate at the same speed towards the detector. Isotachopheresis is often employed for the concentration of dilute samples. Its main disadvantage is its unsuitability for analysing unknown samples.

For this thesis, all analysis were carried by capillary zone electrophoresis, therefore the following section and theory shall be limited to CZE. The term capillary zone electrophoresis is in itself misleading as it implies it s the only zonal technique, which is not the case as MEKC and CGE are zonal techniques too, whilst CIEF is described as a focussing techniques and CITP as a moving boundary technique. For simplicity, the term capillary electrophoresis (CE) shall be used instead of CZE in the subsequent discussions.

1.3 Basic Concepts of Capillary Zone Electrophoresis

In capillary zone electrophoresis, separation takes place in narrow-bore capillaries under the influence of an electric field.

Figure 1.1 illustrates a schematic diagram of capillary electrophoresis instrumentation. The main components required for electrophoresis are a capillary, a high-voltage power supply, detector, data acquisition system buffer and sample vials.

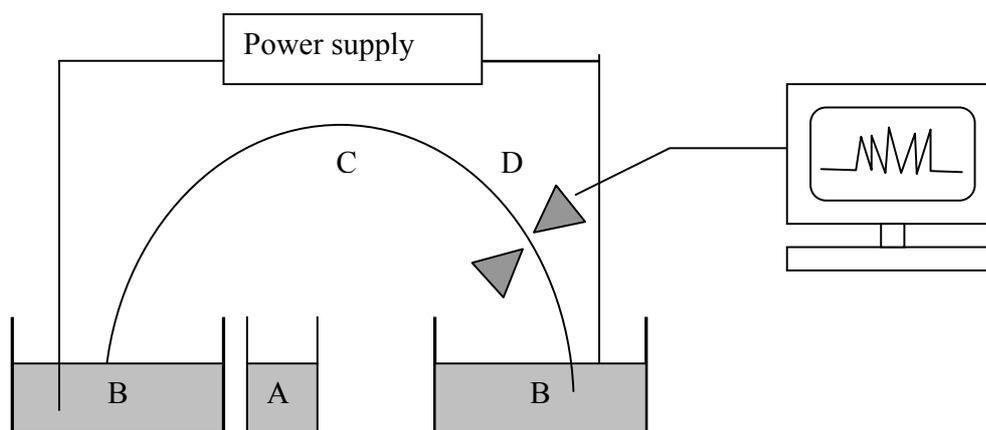


Fig. 1.1: Schematic representation of capillary electrophoresis; A: analyte vial; B: buffer vial; C: capillary; D: Detector

The contents of the two reservoirs and capillary are identical. Also situated in the reservoirs are electrodes, usually platinum, to make the electrical contact between the high voltage power supply and capillary. The sample vial contains the analyte which may be dissolved either in the background buffer or water depending on the type of injection to be implemented. To introduce a small quantity of the sample on to the column, one buffer vial is briefly replaced by the sample vial before applying either an electric field (electrokinetic injection) or pressure (hydrodynamic injection) for a specific period (usually seconds). The source vial is then replaced to the original position before switching the high voltage, which enables the ions to migrate towards the detector where they are visualised and the signal is recorded on the data acquisition device.

1.3.1 Electrophoretic mobility

Separation by electrophoresis is based on differences in analyte velocity in an electric field. The velocity of an ion is given by

$$v = \mu_e E \quad (1.1)$$

where $v =$ ion velocity in cm s^{-1}
 $\mu_e =$ electrophoretic mobility in $\text{cm}^2 \text{s}^{-1} \text{V}^{-1}$
 $E =$ applied electric field in V cm^{-1}

The electric field is a function of the applied voltage and capillary length. The electrophoretic mobility, for a given ion and medium, is a constant which is characteristic of that ion. It is determined by the electric force that the molecule experiences, balanced by its frictional drag through the medium i.e.

$$\mu_e \propto \frac{\text{Electric force } (F_E)}{\text{Frictional force } (F_F)} \quad (1.2)$$

where the electric force is given by

$$F_E = qE \quad (1.3)$$

and the frictional force for a spherical ion by

$$F_F = -6 \pi \eta r v \quad (1.4)$$

where $q =$ ion charge
 $\eta =$ solution viscosity
 $r =$ ion radius
 $v =$ ion velocity

During electrophoresis a steady state, defined by a balance of these forces is attained. At this point the forces are equal but opposite and

$$qE = 6 \pi \eta r v \quad (1.5)$$

Solving for velocity and substituting equation (1.5) in (1.1) yields

$$\mu_e = \frac{q}{6 \pi \eta r} \quad (1.6)$$

Equation (1.6) describes the mobility in terms of physical parameters. It is also evident from the equation that small, highly charged species have high mobilities whereas large, minimally charged species have low mobilities. Mobility is also strongly dependant on temperature and electroosmotic flow discussed below.

1.3.2 Electro-osmotic flow (EOF)

In capillary electrophoresis, in addition to the solutes, the buffer solution also moves through the capillary under the influence of an electric field. This bulk movement of liquid in the capillary is termed electro-osmotic or electroendosmotic flow and drags all analytes irrespective of charge in one direction. Consequently cations and anions may be simultaneously analysed in one run, which otherwise is not possible in the absence of EOF. A further advantage of EOF is it makes possible the analysis of analytes with different charge mass ratios within reasonable analysis times.

The EOF is a consequence of the charge on the surface of the interior capillary wall. When an aqueous buffer is placed inside a fused silica capillary, its inner surface acquires an excess of negative charges. This is due to the ionisation of the silanol groups (SiOH) that can exist in anionic form (SiO⁻) and or the absorption of ions from the buffer onto the capillary. Teflon capillaries also exhibit electro-osmotic flow, which is probably due to the absorption of the electrically charged ions in the buffer onto the capillary wall. In the case of fused silica, the EOF becomes significant above pH 4. The negatively charged silanoate groups attract counter ions from the buffer which form an inner layer of tightly held cations, also termed the fixed layer, at the capillary wall. However these cations are not of sufficient density to neutralise all negative charges, so a second outer layer of cations forms which makes up the diffuse layer. The fixed and diffuse layers make up the diffuse double layer of cations. When an electric field is applied, the outer layer of cations is pulled toward the negatively charged cathode. Since these cations are solvated, they drag the bulk buffer solution with them, thus causing electroosmotic flow. Between the two layers, an electrical imbalance is created which is a potential difference across the layers. This is known as the zeta-potential. Electroosmotic flow is proportional to the zeta potential and may be expressed as

$$v_{\text{EOF}} = (\epsilon \zeta / \eta) E \quad (1.7)$$

$$\text{or} \quad \mu_{\text{EOF}} = (\varepsilon \zeta / \eta) \quad (1.8)$$

where: v_{EOF} = velocity

μ_{EOF} = EOF “mobility”

ζ = zeta potential

ε = dielectric constant

The zeta potential is defined by the surface charge on the wall, which in turn is strongly pH dependant.

Electroosmotic flow in CE has a flat profile as illustrated in Fig. 1.2, compared to pumped or laminar flow, as in HPLC. The advantage of the flat flow profile is that all solutes experience the same velocity component caused by electroosmotic flow regardless of their cross-sectional position in the capillary, and they elute as narrow bands giving narrow peaks of high efficiency.

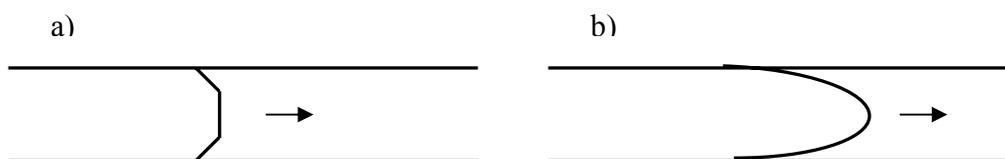


Fig. 1.2: Flow profile under: a) electroosmotic and b) laminar flow

The electroosmotic flow may be measured by injecting an uncharged compound, also termed neutral marker. It can be added to the sample or injected by itself. The main criteria in choosing a neutral marker are that it be uncharged at the pH of the buffer, detectable by detector in use, pure and have no interaction with the capillary wall. A variety of neutral markers have been used for CE with UV detection. These include mesityl oxide, formamide and methanol. The electroosmotic flow (μ_{EOF}) in cm^2/Vs may be calculated from the migration time of the neutral marker, effective length of the capillary (i.e. from inlet to detector) and applied electric field.

$$\mu_{\text{EOF}} = l L / Vt$$

where l = effective capillary

L = total capillary length

V = electric field

t = migration time of EOF marker

In the presence of EOF the measured ion mobility is the apparent mobility μ_a and thus:

$$\mu_a = \mu_e + \mu_{\text{EOF}} \quad (1.9)$$

Thus μ_e may be calculated from the μ_a by independently measuring EOF using a neutral marker. It is important that electroosmotic flow be constant. If it varies, the migration times of the solutes will change, which may result in incorrect analyte identification or errors in quantification.

Because the magnitude of EOF may affect the separation in CZE, it is imperative to employ it or even deliberately manipulate it. Its control requires alteration of the capillary surface charge or buffer viscosity. The most frequently used approaches being,

- variation of electric field; increasing the electric field effects an increase in EOF
- modifying buffer pH; lowering pH results in lower EOF
- varying the concentration of the buffer has an effect on the zeta-potential; increase in ionic strength results in double-layer compression, decreased zeta-potential and lower EOF
- temperature changes, high temperatures lead to low buffer viscosity and thus higher EOF.
- Inclusion of organic modifiers in run electrolyte, modifies the double layer
- Covalent coating of capillary wall

1.3.3 Reversing EOF

Not only can the magnitude of EOF be varied, it may be necessary to change its direction altogether. In normal operation i.e. injection at anode, the direction of the electro-osmotic flow is towards the negatively charged electrode, which means the buffer flows from the source vial, through the capillary, through the detector, to the destination vial. For anion analysis, it is desirable to reduce their migration times thereby reducing analysis times. Upon reversing EOF the bulk solution migrates from the detector to the source vial towards the anode. For the visualisation of the anions it is necessary to also reverse polarity so that the detector side becomes the anode.

A simple method of changing EOF is by adding a flow modifier to the buffer, usually a quaternary alkyl ammonium salt. A variety of salts have been used which include, cetyltrimethylammonium bromide (CTAB), tetradecyltrimethylammonium bromide (TTAB), diethylentriamine (DETA) and hexadimethrine bromide (HDB).

1.4 Microchip Capillary Electrophoresis

Capillary electrophoresis may be carried out on miniaturised analytical devices. In this approach, a capillary column is replaced with a microchip with much smaller channel length and dimensions, which offers dramatic decrease in analysis times and reagent consumption.

A schematic of a typical microchip used for electrophoretic separations is illustrated in Fig. 1.2. below.

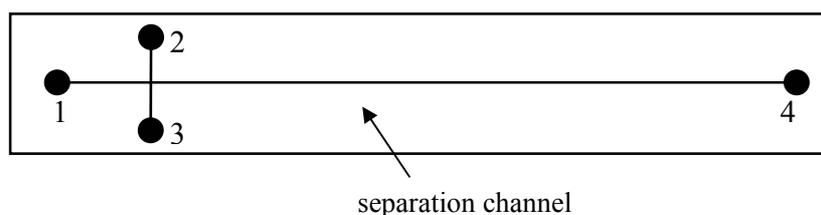


Fig. 1.3. Layout of a microchip; 1: electrolyte inlet, 2: sample inlet, 3: sample outlet, 4: outlet

These planar devices with micrometer dimensions, hence the name microchips, are mainly produced by photolithographic and wet etching techniques readily available from the semiconductor industry. Non-insulating substrates such as glass, silicon, and more recently polymers are used. A typical fabrication process involves metal film deposition, photolithography and etching of a CE channel with desired dimensions on a bottom plate. In the final step, a coverlid is then bonded on top. Fluid reservoirs usually plastic vials or pipet tips are glued on to the chips. They are connected to the microchannel through holes drilled into the cover plate. Platinum electrodes are placed into these reservoirs and connected to a high voltage power supply via relay.

1.5 Detection in Capillary and Microchip Electrophoresis

Polyimide coated columns of typical i.d. of 10-75 μm are used for CE. These have volumes ranging from tens of nanoliters to a few millimetres. Thus detection poses a significant challenge because of the small detection volumes available. The ideal detector must be versatile, provide high sensitivity and low noise level. It should supply a stable baseline and be responsive to all type of compounds, rugged and not too expensive. Most CE detectors have been adapted from HPLC. Detectors may be situated on-column, end-column or post column

1.5.1 Optical Detection Techniques

Optical detection schemes are relatively easy to implement with UV-transparent fused silica capillaries. This allows on-column detection, since the light source can be directly focussed on to the capillary whilst the electronic transduction of the signal remains galvanically separated from the DC influence of the high-voltage. As a result absorbance and fluorescence detectors were successfully implemented in commercial state-of-the-art CE instruments [7].

1.5.1.1 UV/Vis

The most commonly used detector is the UV/Vis absorbance detector. The reasons for its popularity include its relatively universal nature; any molecule possessing a chromophore can be detected by UV/Vis; and its availability from HPLC work. This detector responds only to those substances that absorb light at the wavelength of the source of the light. It is a non-destructive and on-column detector. Further advantages of the UV/Vis detector are:

- Its insensitivity to temperature and gradient changes
- Low cost
- Simplicity of use
- Its non-destructive nature means that other detectors may be connected to the same column downstream.

Its disadvantages are that it necessitates the presence of an optical window, which is created by removing a small section of the polyimide coating. This renders the capillary

fragile and vulnerable to breakage. Its detector sensitivity is pathlength dependant and is defined by the inner diameter of the capillary. Not all species of interest possess chromophores, examples of which include most amino acids, sugars and inorganic anions. This problem has partly been solved by detection in the indirect mode where a chromophore is added into the background electrolyte. This however yields lower sensitivity. Some restrictions in the running buffers exist due to the optical properties of the buffers themselves.

Up to date, there is no coupling of microchip electrophoresis with UV detection presumably from the pathlength limitations as detected by Lambert Beer's law. Extremely narrow channels would compromise detector sensitivity.

1.5.1.2 Fluorescence Detection

Fluorescent molecules absorb light at one wavelength and then re-emit it instantaneously at a longer wavelength. Hence when a fluorescent detector is used, two wavelengths are specified; the excitation and emission wavelengths respectively. Jorgenson et al employed the fluorescence detector in their pioneering work on CE [3]. With their system they obtained detection limits in the 10^{-7} – 10^{-6} M range for derivatized analytes. Many methods have been developed to improve sensitivity ever since.

One can distinguish between lamp-based and laser induced fluorescence detectors (LIF). In the former, light sources such as deuterium, tungsten or xenon lamps are used for excitation, whilst lasers are used as excitation sources in the latter.

Due to the high intensity of its incident light and the ability to accurately focus light to the smaller diameter capillaries, laser induced fluorescence gives rise to even higher sensitivities. Detection limits in the 10^{-12} M range have been reported. Sensitivity of fluorescence detection is proportional to the intensity of the emitted light. If more molecules are excited then more molecules emit light thereby increasing peak height. Lasers available include argon ion, helium-cadmium and helium-neon. The major disadvantages of LIF are that it is expensive and generally limited by the range of excitation wavelengths offered by the laser, there are also possibilities of photodegradation of the analytes caused by the high light intensity.

Laser induced fluorescence detection has been used for initial work on microchip electrophoresis. Jacobson et al. used an argon ion laser for excitation and a

photomultiplier tube to collect the fluorescence signal [8]. Labelled amino acids [6] [9, 10] and oligonucleotides [11] have been detected via laser induced fluorescence on micro-machined devices. In another paper, Jacobson et al. analysed fluorescent complexes of three metal ions on a quartz microchip platform [12].

Although the fluorescence detector is the most sensitive up to date; detection limits obtained are one to three orders of magnitude lower than those obtained with UV/vis; it is less versatile because many solutes of interest do not exhibit native fluorescence. For these analytes, pre or post column reactors for derivatisation may need to be implemented [3]. This supplies the main disadvantage of fluorescence detection as a significant part of the work is devoted to the tagging of the analytes.

Alternatively, by incorporation of the fluorophore in to the background buffer, indirect fluorescence detection has been demonstrated. As with indirect UV-Vis this approach provides less sensitive determinations.

1.6 Electrochemical detection methods

Three principle methods of electrochemical detection for CE may be distinguished; potentiometric, amperometric and conductimetric detection. From the start, the challenge in electrochemical detection for CE have been the isolation of the high-separation DC-potential across the separation capillary from the detector electronics and physical alignment of the detector electrodes with the capillary exit.

1.6.1 Potentiometric Detection

In this detection mode, a potential developing on an ion-selective electrode or membrane in contact with an analyte ion is measured. Nann and Simon [13] demonstrated sensitive determination of inorganic and organic cations with ion-selective microelectrodes (ISME).

Potentiometric sensors may be classified according to the nature and composition of the electrodes. Ion selective membrane electrodes were predominately used in the early investigations and consist of microelectrodes drawn out from capillaries or micropipets whose tips were filled with a lipophilic membrane containing an active ionophore and whose interiors contained an aqueous filling solution and internal reference electrode. Ion-selective electrodes can resemble bulk-property detectors if they are formulated such

that they respond to all species with a correct charge. More recently, potentiometric sensors are coated wire electrodes which consist of a wire coated with a thin polymer film containing the ionophore of interest. This detection mode is very sensitive although a lot of optimisation is required. For instance, a specific ionophore system required for a specific task has to be developed. The selectivity of the system depends upon the ionophore and thus response can be universal e.g. for cations and anions or it can be tuned for cations only. Schnierle et al. used coated-wire ion selective electrodes for the analysis of selected organic ions [14]. Reviews on potentiometric detection for capillary electrophoresis are available [15, 16].

Complications for potentiometric detection include sensor preparation, and handling, the preparation of ISME electrodes, fragile micromanipulations and limited lifetime. Up to this date, potentiometric detection on microchip platforms has not been reported.

1.6.2 Amperometric Detection

The earliest work on amperometric detection was carried out by Wallingford and Ewing [17]. In this detection mode, the redox current resulting from oxidation or reduction of electroactive species is measured. Oxidation occurs when an electron is transferred from a solute molecule to the working electrode in the amperometric cell, thereby increasing the charge of the solute. Conversely, reduction occurs when electron transfer is in the opposite direction. Amperometric detection requires three electrodes; a working electrode, reference electrode and an auxiliary electrode, which controls the potential difference between the working and reference electrode. A potential is applied across a supporting electrolyte between the working and reference electrode effecting solute oxidation or reduction. Amperometric detection may be carried out in oxidative or reductive mode. In the oxidative mode, a negative potential is applied by the auxiliary electrode. This results in a positive potential difference between the working and reference electrodes. As a result, electrons are transferred to the working electrode. In the reductive mode, the opposite occurs. Typical detection potentials in amperometric detection lie between 0.4 and 1.2 V. The current flowing through the working electrode is proportional to the number of electron transfers taking place and therefore to solute concentration. Amperometric detection however is applicable to oxidisable or reducible species. It has been used for the analysis of catecholamines [18] and carbohydrates [19]

which cannot be detected by fluorescence or UV. Many components are not easily oxidisable or reducible. Reviews on amperometric detection for capillary electrophoresis are available [20, 21].

Various groups have coupled amperometric detection to microchip electrophoresis. Hauser's group employed amperometric detection for the microchip analysis of neurotransmitters [22, 23], amino acids, sugars and chlorinated phenols [24]. Reviews on microchip capillary electrophoresis with amperometric detection are available [25, 26].

1.6.3 Conductivity Detection

In this universal detection mode, charged species in solution may be determined. Unlike potentiometric and amperometric detection, conductivity detection does not rely on electrochemical reactions on the surface of the electrode but measures an electrical signal (conductance) between electrodes contacting with solution.

Solutions of electrolytes obey Ohm's law i.e. the current (in amperes) passing through a given solution is denoted by :

$$I = V / R \quad 1.10$$

The resistance of an electrolyte is directly proportional to the length l of liquid through which the current passes, and inversely proportional to its cross-sectional area, A . It therefore follows that

$$R = \rho \ l / A \quad 1.11$$

ρ is a constant of proportionality and is called resistivity and is constant for an aqueous solution of a given electrolyte of fixed concentration at a particular temperature.

In practice it is more convenient to use the reciprocal of resistivity to characterise the behaviour of a particular electrolyte. This is called the electrolytic conductivity and is denoted by κ .

$$\kappa = 1/\rho = l / R \cdot A \quad 1.12$$

In conductivity detection it is useful to determine the electrolytic conductivity of each ion in the finite solution. Thus κ is given by

$$\frac{1}{1000} \sum_{i=1}^n c_i |z_i| \lambda_i \quad 1.13$$

where c_i = molar concentration of the ionic species i in solution
 z_i = charge number
 λ_i = the individual ionic equivalent conductance

The determination of the conductivity κ involves measuring the electrical conductance G of the solution. G is the reciprocal of resistance i.e.

$$G = 1 / R \quad 1.14$$

The equation 1.11 may be rearranged to

$$G = \kappa \cdot A / l \quad 1.15$$

with A being the surface area and l the distance between the electrodes.

A conductivity detector cell comprises two inert electrodes across which a high frequency AC signal is applied. It is necessary to use alternating current because a direct current (DC) would lead to electrolysis reactions on the surfaces of the electrodes. The signal arises from the difference in conductance between the analyte and the background electrolyte. The higher the conductivity differences between the analyte-molecules and background co-ion, the larger the detector response.

Conductivity detection may be carried out in the contact or contactless mode.

1.6.3.1 Contact Conductivity Detection

In contact conductivity detection, the electrodes are in galvanic contact with the electrolyte solution.

The detector maybe situated on-column or end-column. In their fundamental CE work, Mikkers et al. [2] demonstrated the use of conductivity detection in CE with instrumentation adapted from ion chromatography. They demonstrated the separation of organic and inorganic ions in 200 μm PTFE capillaries. An on-column conductivity detector for CE with narrow capillaries was constructed by Huang et.al. [27]. They used a computer-controlled CO_2 laser to drill 40 μm diametrically-opposite holes into 50 and 75 μm i.d. fused-silica capillaries. With the help of a microscope, they placed two 25 μm o.d. platinum wires exactly opposite to one-another in order to minimise the potential difference between the electrodes upon the application of a high electric field. They analysed an inorganic cation mixture of Rb^+ , K^+ , Na^+ and Li^+ and inorganic cations in blood serum samples. An electropherogram of organic cations, including two amino acids

was illustrated as well. The advantages of this arrangement were the excellent resolution due to the very small electrode cross-sectional area, absence of dead-volume in detector and very tiny detection volumes. A setback however is how to produce and align the electrodes inexpensively and reliably. In their subsequent paper Huang et. al. employed this on-column conductivity detector for the determination of low molecular weight carboxylic acids [28].

An end-column detector whereby the conductimetric sensor is situated at the capillary outlet is easier to construct as demonstrated by Huang's group [29]. They placed the sensing electrode at a distance of 1 to 2 μm behind the capillary in the effluent. The ground electrode for CZE was used as the second electrode. Although this arrangement demonstrated sensitivities comparable to the on-column scheme and eliminated difficulties due to electrode alignment, the dead-volume between the capillary outlet and sensing electrode increased band broadening by 25 %. The same group described a simplified end-column detector with improving its efficiency in their subsequent work [30]. They used a CO_2 laser to drill a hole of approximately 40 μm diameter in the walls of 50 and 75 μm capillary at a distance of 7 mm from the capillary outlet. They then placed a platinum wire along the length of the capillary close to but not protruding into the hole-structure. The conductance was measured between the sensing and electrophoretic ground electrode. The band broadening was reduced to less than 15 %. With this detector arrangement, they were able to couple two detectors on one column. Placing the detector at the capillary outlet enabled the coupling of the conductivity detector with a UV detector. Thus more analytical information could be obtained through detector coupling.

The only commercially available end-column conductivity detector was introduced in 1996 [31]. In this CE system the detection end of the capillary is encapsulated in a stainless-steel coupling connector, which additionally serves as a precision spacer and centres the detection end of the capillary. The conductivity electrode consists of a 150 μm platinum wire which is mounted through the centre opening of the connector and is permanently isolated from the surrounding stainless steel which forms the second electrode. The advantage of this system is its open-architecture design which provides interchangeability of capillary and sensor assembly and permits fast assembly and

disassembly for cleaning purposes and trouble shooting, factors which are important for executing accurate and reproducible analysis.

A number of papers illustrating applications of this Crystal CE system are available. This commercial detector has been used for determination of counterions and inorganic [32, 33] impurities and small amines [34] in pharmaceutical drug substances. Valsecchi and co-workers developed and validated a method for the determination of inorganic anions in rain water [35]. Gallagher and Danielson [36] used highly conductive background electrolytes for the indirect detection of cationic and anionic surfactants. With a sodium fluoride electrolyte, they were able to determine cationic and anionic surfactants simultaneously. Musial and co-workers [37] investigated the effect of a buffer additive on the separation of quaternary amines, potassium and sodium. Environmental samples were analysed for breakdown products of methylphosphonic acids by Rosso and Bossle [38]. Lucy and Wu characterised the the Crystal conductivity detector [39]. They examined the effect of the buffer composition on the detector sensitivities. Klampfl et al. detected inorganic and low-molecular-weight organic ions in beverage samples via indirect photometric ultraviolet and conductivity detection [40]. They latter optimised the sensitivity of the conductivity detector for the analysis of chloride, sulphate and low molecular weight carboxylate ions with respect to buffer composition and pH [41]. The same group developed a method for the rapid determination of anions in electrodeposition coatings [40, 42]. Govindaraju and coworkers utilised the commercially available conductivity detector for the analysis of lung airway surface fluid [43, 44].

Other groups opted for self-designed conductivity cells. Mo et al. [45] constructed an on-column miniature conductivity detector for CE applications by fixing two platinum electrodes between two capillaries of 75 μm id. They demonstrated the separation of amino acids in a hydrolysed hair sample. McWhorter and Soper [46] used a similar approach for their detector. They separated and purified polymerase chain reaction products via micro-reversed-phase high-performance liquid chromatography.

Several research groups have demonstrated contact conductivity detection on a microchip platform. Van den Berg manufactured glass-microchips by μTIC and powder blasting techniques and demonstrated the analysis of inorganic cations, organic anions detection and peptides with embedded electrodes [47, 48]. Soper and co-workers [49] used a

PMMA-device for the separation and detection of amino acids, proteins and DNA fragments with a built-in conventional, contacting conductivity detector. Stanislawski and co-workers have used a PMMA microchip platform for the analysis of oxalate in real samples[50, 51]. The same group also demonstrated microchip electrophoretic separations of proteins [52].

1.6.3.2 Contactless Conductivity Detection

In the early 1980s, Pungor et al. reported on a high-frequency conductance microcell for the determination of conductivity in streaming solutions or solvents, in which there was no galvanic contact of electrodes with solution [53]. They demonstrated the applicability of the cell for flow injection studies by measuring the conductance of potassium chloride and glucose solution at different concentrations. The same group later illustrated the feasibility of this detector cell with ion chromatography [54].

In 1998 a capacitively coupled contactless conductivity detector for capillary electrophoresis was introduced by two independent groups, Zemann and co-workers [55, 56] and by Fracassi da Silva and do Lago [57]. This configuration is illustrated in Fig. 1.4 below.

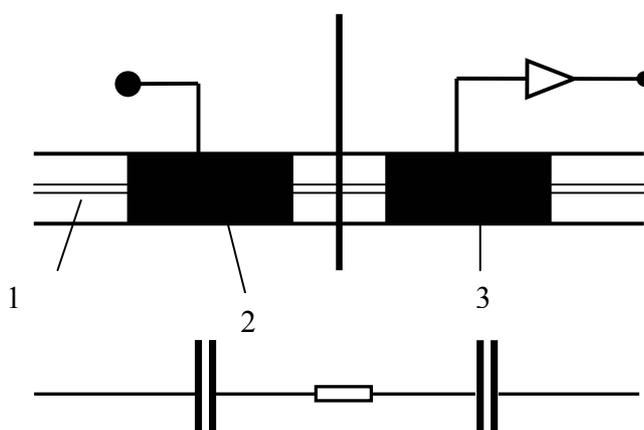


Fig. 1.4: Schematic drawing of a C⁴D. (1) capillary, (2) actuator electrode, (3) pick-up electrode

Two electrodes of a few millimetre lengths, consisting of either a conductive silver varnish or short metallic tubes, which are separated by a gap of several millimetres, are placed side by side around the capillary. As each electrode forms a capacitor with the internal electrolyte solution, it is possible to pass an ac-voltage of significantly higher

frequency through the cell. A different contactless conductivity detector arrangement has been reported by Kaniansky et al. [58, 59]. This detector cell is based on four small wire-electrodes of 200 μm diameter placed perpendicularly around the circumference of the capillary. However due to space restrictions this cell arrangement is only suitable for capillaries of 300 μm inner diameter and 500 μm outer diameter which are not commonly used in capillary electrophoresis. Detection limits of 1 μM have been reported for inorganic anions, which is comparable with those reported for the cell arrangement discussed above.

Both of these arrangements have the important advantages of easy alignment of the electrodes with the capillary and long-term robustness as prevention of electrode fouling is inherent. Several groups have therefore taken up the C^4D -configuration. Tuma and coworkers [60, 61] have presented a modified cell in which two strips of aluminium foil form semi-tubular electrodes and are clamped on to the capillary with a Perspex cell. Pumera et al. [62] have demonstrated the determination of cyclodextrins and Fracassi da Silva and do Lago [63] have performed indirect detection of non-ionic alcohols separated by micellar electrokinetic chromatography. Muzikar et al. have demonstrated that contactless conductivity detection is also possible in nonaqueous solvents [64]. Kappes et al. have adopted the detection method for a field-portable CE-instrument because of its simplicity [65]. Haddad and co-workers [66] have used contactless conductivity detection in electrochromatography. Zemann and co-workers [67, 68] and Kuban et al. [69, 70] have carried out simultaneous detection of cations and anions by injecting from both ends of the capillary while placing the detector in the middle. Chvojka et al. [71] have described a dual detector cell combining contactless conductivity detection and optical absorption. They used fibre optics to utilise the gap between the two electrodes for photometric detection. Detector optimisation and the effect of parameters such as electrode length and distance have been proposed. Fracassi da Silva et al. [72] have designed a miniaturised version of the C^4D in which the detector cell and associated electronic circuitry are all constructed on a printed circuit board. Because of its small size, this detector can be fitted into existing instruments, but it seems that the performance had to be compromised in order to achieve miniaturisation. Gas et al. constructed a conductivity cell with brass electrodes held in position on a printed board

by a glass epoxy laminate[73]. Macka and co-workers recently proposed a miniaturized movable cell for C^4D [74]. They assembled all the pieces required on a capillary impregnated with silicon grease in order to prevent adhesion to the finished cell. The parts were held in place and covered with epoxy resin, painted with a conducting silver vanish after hardening before being finally covered by a common nail polish for protection.

Several examples of applications of C^4D for inorganic and organic ions have since been reported. Zatkovskis et al determined mono and disaccharides in standards and beverage samples [75], Kuban et al. used the detector for the speciation of chromium (III) and chromium (VI) species [76]. Vuorinen et al. demonstrated the simultaneous UV and C^4D detection of catecholamines [77] whilst Lopez-Avila et al. used the two detectors for the determination of haloacetic acids [78]. Gas et al. determined underivatised amino acids [79]. Baltussen and co-workers separated nine peptides [80], whilst Surowiec [81] et al. analysed long-chain fatty acids and their degradation products in samples of drying oils. Contactless conductivity detector has been successfully used for flow injection- capillary electrophoresis systems [82-84].

Contactless conductivity detection has been performed on a microchip platform. Wang and co-workers have used [85, 86] PMMA microchips and demonstrated that contactless conductivity detection can be achieved by placing the electrodes on top of the cover plate rather than embedding them in the device as in contact conductivity detection. They demonstrated the detection of inorganic cations and anions as well as organic amines. In their subsequent work they analysed organosphosphates on PMMA microchips [87] and used non-aqueous solvents for the determination of aliphatic amines on glass microchips [88]. Wang's group also used a dual amperometric and contactless conductivity detection system for the simultaneous determination of nitroaromatic and ionic explosives [89]. Lichtenberg et al. [90] used a glass-based microchip with electrodes situated in-plane and at right angles to the channel. The microchips and electrodes were formed in the photolithographic steps. Alternatively a four-electrode arrangement was reported by Laugere's group [91]. They separated 6 organic acids on a microchip device.

In all the publications sited for contactless conductivity detection, excitation voltages in the range 20-24 V_{pp} were used. A review on capacitively coupled contactless conductivity detection for capillary electrophoresis is available [67].

1.7 Aims of this thesis

The main goal of this thesis was to evaluate the effect of implementing high excitation voltages with capillary and microchip electrophoresis. Ever since its introduction in 1998 [55, 57], capacitively coupled contactless conductivity detection has gained wide attention and at least fifty publications have appeared so far. Several questions of analytical relevance arise upon the introduction of a new detection technique, The most important of which may be formulated as follows: (A) Is there room for improving sensitivity without any ultimate compromise on robustness and reproducibility? (B) What are the ideal applications of this detector? (C) Does the detector possess other advantageous features and how can they be exploited? The goals of this thesis were three-fold.

(A) Optimisation of the capacitively coupled contactless conductivity detector

Several research groups have taken different approaches for characterisation of the detector, including the optimisation of frequency, distance between the electrodes. Some have examined the effect of cell geometry with regard to the number, shape and position of electrodes. All the research groups have adopted relatively low excitation voltages in the 20 –24 V_{pp} range for capillary electrophoresis and 5 -15 V_{pp} for microchip capillary electrophoresis. Thus one of the aims of this thesis was to develop a capacitively coupled contactless conductivity detection system for capillary and microchip platforms which employs higher excitation voltages. This new detection system is characterised with respect to sensitivity, reproducibility of measurements and robustness.

(B) Analytical Applications of Enhanced detector

In a second step the applications of this detector cell are explored. UV detection is the most commonly employed detection method in CE. It is however limited to molecules which possess chromophores. Its sensitivity is limited by the inherent short pathlengths. In principle, conductivity detection is suitable for the analysis of all charged substances. It has however been stressed in many texts that this detector is suitable for the analysis of

the inorganic ions possessing high mobility. Organic molecules are expected to give very small signals. The second goal is to examine its application for inorganic and organic analysis. We also examine its suitability for analysis of substance classes such as amino acids and sugars, which otherwise cannot be analysed by UV. We evaluate the detector for pharmaceutical drug analysis, which previously have been detected with UV.

(C) Exploration of other features of detector

The advantage of electrochemical detection schemes is that they are not limited by wavelengths as in optical detection. One group has exploited this aspect by using UV absorbing organic solvents in conjunction with contactless conductivity detection [64]. In this thesis the compatibility of this detector with non-transparent organic polymers is demonstrated

2 Results and Discussion

Parts of this thesis have been published in various journals of analytical chemistry. Thus the results and discussion chapter comprises reprints of ten published papers. Nine of the published papers are research articles, whilst one of them is a review article. The first two subsections (chapters 2.1 and 2.2) are concerned with the optimisation of the capacitively coupled contactless conductivity detector for capillary and microchip platforms with particular emphasis on actuator voltage. The effects of cell-arrangement, distance between the two electrodes and electronic arrangement are examined.

In their fundamental work on capacitively coupled contactless conductivity detection for capillary electrophoresis, both groups employed sinusoidal waves of relatively low amplitudes. Zemman et al. used voltages between 7 and 10 V_{pp} with low audio frequencies whilst Fracassi da Silva's group employed a signal of 20 V_{pp} at 600 kHz.

The optimisation of detection parameters in chapter 2.1 was achieved through the study of cell-behaviour at increasing frequencies for given voltages. The cell-current was plotted against frequency for 25 V_{pp} , 100 V_{pp} and 250 V_{pp} actuator voltages. The measurements were carried out on capillaries of different internal diameters filled with buffer. Increasing the frequency resulted in an initial sharp increase in cell-current. At 25 V_{pp} the current reached a maximum at 100 kHz and remained constant up to 450 kHz. The plots were similar for all voltages, although the cell currents were higher for higher voltages. The frequency patterns were similar for capillaries of different internal diameters. At identical frequency and voltage, larger cell currents were observed for capillaries of larger inner diameters. It is clear from these results that the applied frequency is not critical for detector performance and that larger capillaries are most ideal for optimal sensitivity as they give larger cell currents and thus cell volume. That increasing the actuator-voltage results in increased detector sensitivity is demonstrated with electropherograms of metal ions at 20 μM each recorded at 25 V_{pp} , 100 V_{pp} and 250 V_{pp} . An increase in peak height is observed at constant background noise. The three times the signal-to-noise detection limits for potassium and magnesium were $1.2 \cdot 10^{-7}$ and $1.4 \cdot 10^{-7}$ M for 250 V_{pp} respectively. For the same ions Fracassi da Silva and do Lago obtained detection limits that were eight to nine times higher at 20 V_{pp} actuator voltage.

From these results, it is evident that higher excitation potentials result in increased detector sensitivity. Therefore, it should be possible to use these conditions for other applications.

Chapter 2.2 is concerned with the evaluation of actuator voltages of up to 500 V_{pp} for microchip capillary electrophoresis. For these measurements, a Faradaic shield, which prevented direct coupling of the signal from the actuator to the pick-up electrode, was placed between the two electrodes situated above the microchannel. In its absence an unstable baseline was observed. Also examined were the implications of electrode distance from the microchannel. Electropherograms of ammonium and magnesium were recorded with the detectors situated at distances of 1 mm and 200 μm from the channel respectively. Although detection was still possible with a 1mm gap, the best sensitivity is obtained by placing the electrodes in the proximity of the channel. As expected, higher actuator voltages were accompanied with an increase in sensitivity. Wang and coworkers[85] also noted an increase in sensitivity with increasing actuator voltage between the 0-15 V_{pp} range. They however reported a corresponding increase in background noise. Based from the observations we made in our measurements, we strongly suspect that their omission of a shield between the two electrodes resulted in the observed increase in baseline noise. Fast separations and detection of organic species were demonstrated in this paper. 4-acetamidophenol (paracetamol), ibuprofen and salicylic acid were all separated and detected within 30 seconds.

The detector arrangement for on-microchip determinations was further simplified in chapter 2.3. For this purpose a chip-holder which accommodates the detector electrodes was built, enabling the easy assembling and disassembling of bare microchips. This is an improvement of the former arrangement where the electrodes were mounted on the microchip. This feature also allows detection at any desired point of the microchip. For this project plastic poly (methylmethacrylate) (PMMA) chips with 100 μm thick chip-covers were employed. These microchips possess an EOF much lower than that of glass microdevices. All other detection parameters i.e. actuator voltage and frequency were similar to those used in chapter 2.2. Demonstrations of possible applications included the successful baseline separation of four alkali metal ions and the analysis of five organic

acids at low concentrations. The fast inorganic ions chloride, nitrate and perchlorate were detected within 80 seconds in the absence of an EOF-modifier in the buffer.

After optimisation of the detection parameters, applications of high-voltage capacitively coupled contactless conductivity detection for metal ion analysis (chapter 2.4), native amino acids (chapter 2.5), inorganic ion analysis with non transparent organic capillaries (chapter 2.6) are demonstrated. The lab-on-chip analysis of selected organic ions is the subject of chapter 2.7. The feasibility of a life science application with capillary electrophoresis in combination with this electrochemical detector is demonstrated with the immunological reaction between unlabelled IgG and IgM (chapter 2.8). The universality of contactless conductivity detection is the subject of chapter 2.8 in which pharmaceutical drug substances with chromophores are analysed.

In addition to the electronic configuration, the composition of the separation electrolyte for capillary electrophoresis is of key importance as its composition determines the migration behaviour of all analytes. Thus in all the work presented in this thesis, care was taken to choose an optimal buffer for each task. In chapter 2.4, a method for the capillary electrophoretic separation of alkali, alkaline earth and heavy metal ions was developed. First, ten alkali and alkaline earth metal ions were separated in less than four minutes in the commonly used, low mobility MES/ His buffer at pH 6. A crown-ether, 18-crown-6, which forms inclusion complexes with small metal ions was added in the buffer to effect the separation of rubidium, ammonium and potassium which comigrate in one peak in its absence. The same buffer was then used for the simultaneous analysis of alkali, alkaline earth and transition metals in the presence and absence of 18-crown-6 additive respectively. In the absence of 18-crown-6, the two pairs, potassium and ammonium; strontium and sodium co-migrated. Barium migrated faster than lithium. However the four transition metals zinc, cobalt, copper and nickel showed a negative response at this pH. Alternatively transition metal ions were analysed in a MES/His buffer containing different concentrations of α -hydroxyisobutyric acid (HIBA), which complexes with heavy metal ions. In the presence of 3 mM HIBA, the direction of the zinc and cobalt peaks could be reversed in a MES/His buffer containing 3 mM HIBA, eight transition metal ions were baseline resolved. Iron, which initially could not be quantitatively analysed at pH 6, does not precipitate in this electrolyte. Another observation was that

zinc and cobalt ions gave positive responses in this electrolyte, whereas copper and nickel gave negative responses with respect to peak orientation.

Four of the twenty most common naturally occurring amino acids possess chromophores. The rest cannot be detected by means of direct UV. They have to be tagged with an absorbing group before detection. Amino acids are however charged at low and high pH. At low pH they are positively charged, whilst they are anionic at high pH. This makes them amenable to conductivity detection. In chapter 2.5 the analysis of native amino acids in acidic and basic media is evaluated. Twelve amino acids were analysed in glycolic acid, HIBA and lactic acid at pH values lower than three. They all gave indirect peaks; arginine, histidine and lysine were the fastest migrating ions at low pH. However, baseline separations of valine, serine, threonine and proline were not achieved in all three buffers. Aspartic acid was detected after the EOF peak in all three electrolytes. Lactic acid buffer provided the most stable baseline and further optimisation was carried out on this buffer. Undecanesulfonic acid, an ion-pairing agent was included in the lactic acid background electrolyte at low concentrations. It lowered the EOF and effected better separation of the amino acids, but an unstable baseline was noted in its presence even at 1 mM. The second approach was to lower the pH of the buffer instead of using additives. At a pH of 2.4, all amino acids were baseline resolved except for threonine and phenylalanine. Alternatively, low mobility buffers were used for amino acid separation at high pH. Indirect detection of amino acids was accomplished in 2-amino-2-methyl-1-propanol (AMP) and 2-amino-2-methyl-1,3-propanediol (AMPD) buffers. The AMP buffer was deemed the most suitable of the two buffers for indirect detection as it resulted in symmetrical peaks, however some amino acids could not be resolved. These were successfully resolved in a buffer containing 50 μ M of magnesium ions. An AMP buffer containing the zwitterionic 3-(cyclohexylamino)-1-propane sulfonic acid (CAPS) resulted in peaks showing direct responses, with the exception of proline. A 50 mM AMP/ 10 mM CAPS buffer provided reproducible determinations of amino acids. Compared to the acidic buffer, lower detection limits were generally obtained with high pH buffers with three exceptions, namely arginine, histidine and lysine. Arginine may only be analysed at low pH. At high pH it is neutral and co-migrates with the EOF. The detection limits of histidine and lysine at low pH were at least ten times less than those calculated for

alkaline conditions. Urine and beer samples were successfully analysed for amino acids with an alkaline buffer.

Quartz capillaries have gained acceptance for capillary electrophoresis applications because of their commercial availability and their optical transparency for use with on-column absorption detectors. The high EOF of glass is unfavourable for some analytical applications. Where required, the EOF is manipulated either by including an organic modifier to the run-buffer or chemically modifying the silanol surface of the capillary. The latter procedure is time consuming and is sometimes difficult to reproduce. The advantage of the contactless conductivity detector is that it may be coupled to non-UV transparent capillaries which may offer other advantageous characteristics for analyte separations than quartz. The applications of organic PEEK capillaries, which have recently become commercially available, in combination with contactless conductivity detection are explored in chapter 2.6. At pH 6 the calculated EOF of PEEK was $2.6 \cdot 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ which is lower than that of glass, ($4 \cdot 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$) at the same conditions. We successfully separated and detected seven high-mobility inorganic anions in a PEEK capillary with a buffer devoid of EOF-modifier, just by reversing the polarity. It is not possible to analyse these anions in quartz capillaries just by reversing the instrument configuration without surface modification since the EOF is in the wrong direction. For anion analysis in quartz capillaries, it is therefore necessary to include an organic surfactant, which reverses the EOF and thereby decreases analysis times. However these buffer additives have some disadvantages. The commonly employed quaternary amines such as TTAB and CTAB increase the background conductivity of the buffer. Some groups have used polymers such as hexadimethrine bromide and hexadimethrine hydroxide [55, 56] in minute amounts. These do not increase background conductivity of the buffer, but they bind irreversibly on the capillary wall. Hence the capillaries cannot be used for cation analysis. The effect of EOF modifiers on the separation of six aliphatic organic acids was demonstrated by Huang and co-workers[28]. They demonstrated that just reversing polarity leads to the detection of anions that possess higher mobilities than EOF. Thus in this configuration they were able to detect formate. The other components including acetate have mobilities less or equal to EOF and escaped detection. With PEEK

capillaries we demonstrated the detection of organic acids including the acetate ion with reversed polarity in the absence of a flow-modifier.

Dual detection of cations and anions including the relatively slow organic acids was carried out on a PEEK capillary with a 10 mM MES/ His buffer. The detector was placed in the middle of the capillary with injection of cations and anions from the anode and cathode respectively. Several groups have demonstrated dual detection of anions and cations with the contactless conductivity detector situated in the middle of the capillary[67-70]. They however added EOF modifier in the electrolyte to facilitate the fast migration of organic anions. An advantage of contactless conductivity detection over UV detection is that the detector may be slid along one capillary until the optimum position for dual detection is found without having to make numerous optical windows as would be the case with absorption detection.

The three times signal-to-noise detection limits obtained for chloride, bromide, sulphate, nitrate and oxalate ions were 3.3×10^{-7} M, 1.8×10^{-7} M, 1.8×10^{-7} M, 2.1×10^{-7} M and 5.4×10^{-8} M are comparable to those obtained for quartz capillaries in a buffer containing hexadimethrine bromide (see chapter 2.1). Thus PEEK capillaries are a viable option for the analysis of these ions. Finally qualitative and quantitative analysis of cations and anions in water and wine samples were demonstrated.

In chapter 2.7, the on-microchip analysis of different organic molecules with high-voltage contactless conductivity detection is illustrated. The determinations were carried out in PMMA and glass microchips mounted on the chip-holder described in chapter 2.3. Two surfactants, ethane sulfonate and heptane sulfonate were separated and detected in 30 seconds at pH 7. Mono and dicarboxylic acids, which cannot be detected by direct UV were separated and detected in 30 seconds. Further illustrated examples include amino acids, artificial sweeteners, sugars and basic drugs. Although on-chip analysis facilitates fast analysis, the peak capacities are generally lower, owing to the short separation channels. The detection limits obtained on-microchips are generally higher than those obtained in capillaries.

Immunoassays are highly specific interactions between an antibody and an antigen and are used for the selective recognition and determination of analytes that may be antibodies or antigens. Recently the advantages of CE, have been exploited for

immunoassays in a technique known as capillary electrophoretic immunoassays (CEIA). Fluorescence detection methods, which necessitate a prelabelling step with a fluorophore, have been predominately used for these reactions. Up to date, a limited number of immunological reactions have been carried out with non-labelled species. In chapter 2.8 high-voltage contactless conductivity is evaluated for the detection of species formed in the interaction between unlabelled human immunoglobulin M (IgM) and monoclonal anti-human IgM (IgG). A low mobility buffer containing equimolar proportions of N-tris-(hydroxymethyl)-methyl-3-amino propanesulfonic acid (TAPS) and AMPD with pH 8.7 was ideal for the detection of these species since its pH is higher than the pIs of both immunoproteins. At this pH both reagents possess a net negative charge and do not interact strongly with the negatively charged capillary wall. A surfactant, Tween 20 was added to this buffer to further suppress the interactions. The suitability of the 20 mM TAPS / AMPD buffer, 0.01 % Tween for protein separation was assessed by comparing the migration times and peak areas after successive injections of 0.05 ng / ml IgM. The relative standard deviation of peak area was 1 % (n=3). A calibration curve was acquired for IgM in order to determine the dynamic range of the assay. The curve was linear with respect to peak area in the 0.05 – 1 ng/ml range. The detection limit of human IgM was 0.15 ng / ml. This was calculated as the concentration corresponding to three times the standard deviation of the intercept. For the capillary electrophoresis assisted immunological reaction 10 µg / ml IgM and 10 µg / ml were mixed in a microtiter plate for 15 minutes before diluting with TAPS/ AMPD buffer and injecting into the capillary. All three species namely, the complex, and unreacted IgM and IgG were detected via contactless conductivity detection. Alternatively the detection of these species on PMMA and quartz microchips was investigated. IgM was detected in a PMMA chip in a 20 mM TAPS / AMPD buffer containing 0.05 % of Tween 20. The plastic device was not ideal for the detection of all species involved in the immunological reaction; the two unreacted immunoglobulins co-migrated and were not resolved. An unstable EOF was observed in the plastic microdevice. Better results were obtained with glass microchips. All three species were successfully baseline separated in less than 90 seconds. The on-microchip detection limit was 34 ng / ml for IgM.

In chapter 2.9 methods for separation of basic pharmaceutical drug substances were developed. Their analysis on capillary and microchip platforms was evaluated. Analytes amenable to UV detection were deliberately chosen in order to compare the detection limits offered by high-voltage contactless conductivity with those published for direct UV methods. A pH optimisation was carried out for the analysis of the six pharmaceutical amines octopamine, ephedrine, noradrenaline, adrenaline, isoproterenol and doxylamine. Complete baseline separation of all six amines was achieved at pH 3 with a citric acid buffer. Lactic acid buffer provided a stable baseline but noradrenaline and ephedrine were not baseline resolved. The separation of the six beta-blockers pindolol, propranolol, atenolol, metoprolol, labetalol and acebutolol was examined at low pH. In both lactic acid and acetic acid the first two analyte-pairs were not baseline resolved. The inclusion of low concentrations of histidine in both buffers facilitated baseline separation of all components. The acetic acid/ histidine buffer however gave the best detection limits. They were calculated as the three times signal-to-noise ratio and were $1.5 \cdot 10^{-8}$ g/ml, $1 \cdot 10^{-7}$ g/ml and $3.5 \cdot 10^{-7}$ g/ml for pindolol, atenolol and labetalol respectively. These are one to two magnitudes lower than those reported by Lin et al. for CZE with UV detection [92]. Main component analysis was conducted on three pharmaceutical preparations. The optimised conditions for basic drug separation were transferred to on-microchip analysis. Only three beta-blockers could be baseline separated in an 8cm long channel. From this work, it is evident that contactless conductivity detection is a viable alternative for UV detection.

The final chapter 2.10 is a review on potentiometric and conductivity detection for capillary electrophoresis. The recent developments in conductivity detection for electrophoresis are reported.

The individual contributions of the various co-authors are stated on a separate page at the end of this thesis.

Improved capacitively coupled conductivity detector for capillary electrophoresis

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The new features of the capacitively coupled contactless conductivity detector for capillary electrophoresis described are higher peak-to-peak excitation voltages for the detector cell of up to 250 V, a pick-up amplifier in close proximity to the electrode and synchronous detection. The electrical performance of the cell was characterized and found to follow readily predictable patterns. The alterations led to a higher signal strength, a better signal-to-noise ratio (S/N) and improved stability. The $3 \times$ S/N detection limits obtained for inorganic cations and anions are in the range 0.1–0.2 μM . For the indirect detection of model compounds of organic cations and anions (aliphatic amines and sulfonates), detection limits of typically 1 μM were achieved.

Introduction

Detection in capillary electrophoresis is a persistent challenge because of the low liquid volumes available. In routine analysis, most commonly UV absorbance detection is employed. However, many analyte species, in particular inorganic ions, are transparent to UV radiation and therefore indirect absorbance methods are widely used for such analytes. Furthermore, the indirect method provides only limited sensitivity. Fluorescence, in contrast, is a highly sensitive method, also available commercially for detection in capillary electrophoresis, but generally requires chemical derivatization of the analyte species. Its use is therefore limited to certain classes of analytes. In order to overcome these limitations of the two optical detection techniques, in recent years many researchers have been working on the development of alternative electrochemical methods. Although the high voltage used for separation in capillary electrophoresis appears to conflict with sensitive electrode measurements, in practice this is not a problem when appropriately configured detector cells are employed. Amperometry is an electrochemical technique with good detection limits and methods for many different analytes have been described, but is possible only for species which can be oxidized or reduced. Conductometry, in contrast, is almost universal as in principle any charged species which can be separated by electrophoresis may be detected. However, the highest sensitivity is obtained for inorganic species because of their high equivalent conductivity. Several recent reviews on electrochemical detection techniques in capillary electrophoresis are available.^{1–6}

Different arrangements for conductometric detector cells have been described. An early configuration for capillary zone electrophoresis consisted of a pair of Pt wires of 25 μm diameter inserted through laser-drilled holes into a capillary.⁷ Later, the simpler wall-jet configuration was developed⁸ and employed in a commercial instrument.^{9,10} The wall-jet arrangement was also adopted for suppressed conductivity detection, a method in which the background conductivity is lowered by ion-exchange before detection in order to obtain higher sensitivity.^{11–16} Other detector configurations reported include a cell formed by depositing platinum directly on the capillary outlet¹⁷ and the use of a strip of a hydrophilic polymer material attached to the end of the capillary to form a thin liquid film whose resistance is measured.¹⁸

Recently, Zemmann and co-workers^{19,20} and Fracassi da Silva and do Lago²¹ introduced a capacitively coupled contactless

conductivity detector (C⁴D) which is based on two tubular electrodes placed over the capillary. Each of the electrodes forms a capacitor with the buffer solution inside the capillary through which an ac signal can be coupled into and out of the capillary. This configuration has the advantage that the solution does not come in contact with the electrode and therefore any fouling is prevented. Perhaps more significantly, the alignment of electrodes and capillary is also greatly simplified compared with conventional arrangements which rely on direct contact with the solution and require precisely machined cells or micromanipulators.

Zemmann and co-workers fashioned their detector electrodes from syringe needles of the appropriate diameter, cut to typically 2 cm length and separated by about 2 mm.²⁰ Fracassi da Silva and do Lago employed electrodes of 2 mm length formed on the capillary with silver paint.²¹ Recently, a further modification of the cell design was described by Tuma and co-workers,²² who brought two narrow strips of aluminium foil into close contact with the capillary by using a purpose-made, grooved Perspex clamp. Excitation voltages between about 10 and 20 V peak-to-peak (V_{p-p}) at frequencies ranging from 40 to 600 kHz were employed by all workers. Detection limits reported for inorganic cations and anions for these arrangements were of the order of 1 μM .

The application of a contactless conductivity detector of different configuration for capillary zone electrophoresis was reported by Kaniansky and co-workers.²³ This detector had been designed for isotachopheresis^{24,25} and requires capillaries of id 300 μm , which is unusually large for capillary zone electrophoresis. The cell consists of four lengths of copper wire of 200 μm diameter, which are arranged perpendicularly around the circumference of the capillary, and is operated at a frequency of 4 MHz. The detection limits reported for inorganic anions are also approximately 1 μM .²³

Recently, the usefulness of contactless conductivity detection has been further proved by demonstrating new fields of application. Fracassi da Silva and do Lago have shown that contactless conductivity detection may be successfully employed for the detection of non-ionic species in micellar electrokinetic chromatography (*via* depression of the background conductivity).²⁶ Muzikár *et al.* extended the contactless technique to non-aqueous buffer systems for the detection of organic ions.²⁷ Hilder and co-workers employed the detection technique in capillary electrochromatography²⁸ and have shown that the concurrent detection of anions and cations is possible by simultaneous injection from both ends with the contactless

detector positioned in the centre of the capillary.²⁹ Chvojka *et al.* constructed a detector cell which incorporates two modes, contactless conductivity and optical absorption, and demonstrated simultaneous measurements.³⁰ A review of the recent progress in conductometric detection in capillary electrophoresis is also available.²⁹

Experimental

Apparatus

A block diagram of the detector circuitry is given in Fig. 1(A). A standard function generator (GFG-8019G, Good Will Instruments, Taipei, Taiwan) was used to create a sine-wave excitation signal of the desired frequency. Its amplitude is boosted by a purpose-made unit based on a high-voltage operational amplifier (Model 3584, Burr-Brown, Tucson, AZ, USA) capable of a maximum peak-to-peak voltage of 250 V. The pick-up amplifier consists of a wideband, FET-input operational amplifier (OPA655, Burr-Brown) in the current follower (transimpedance) configuration. The amplifier was chosen for its high bandwidth and for its gain stability at high frequency. Many operational amplifiers show a very pronounced gain peak (the gain is much higher than defined by the external resistor) at frequencies just before the bandwidth limit. This feature, which will compromise the evaluation of the frequency behaviour of an experimental set-up, is absent for the particular amplifier employed in this case. Feedback resistors between 100 k and 1 M were used. The output was fed *via* coaxial cable to a separate unit where the signal was rectified, low-pass filtered, offset and further amplified. The rectification was carried out with a monolithic synchronous detector (AD630, Analog Devices, Norwood, MA, USA), which allows phase-sensitive detection (lock-in detection) and therefore discrimination against noise.

The detector cell itself is shown in Fig. 1(B). The electrodes and the pick-up amplifier are contained in a small electrically grounded die-cast metal box. The detector electrodes are based

on steel tubes of 4 mm length cut from hypodermic needles as used by Zemann and co-workers.^{19,20} The diameter of the electrodes was chosen to match closely capillaries with the common outer diameter of 360 μm . The use of capillaries with thinner walls and therefore smaller outer diameter, in order to achieve a tighter coupling of electrodes to the fluid channels, was considered, but the thinner capillaries were deemed too fragile for routine use. A grooved Perspex clamp is used to hold the electrodes in place, but the tube itself is not clamped. Capillaries are simply pushed through the assembly and can easily be exchanged without dismantling the cell, opening the case or loosening any clamps. The polyimide coating of the capillaries is not removed. The detector case is divided into two compartments by a Faraday-shield between the two electrodes which completely separates the two sections except for a small hole just large enough for the capillary to pass through. The electrodes were separated by a gap of 1 mm unless stated otherwise. The shield eliminates direct coupling between the excitation and pick-up electrodes and, as experimentally confirmed with no capillary present, completely prevents any interference of the electrical field created on the actuator side directly on the pick-up amplifier. The input amplifier was mounted in close proximity to the pick-up electrode and connected directly to it in order to eliminate stray capacitance which would be introduced if a coaxial connection to a remote amplifier was used. This would be expected to lead to a deterioration of the bandwidth considering the high impedance of the signal.

The frequency behaviour of the capacitor-resistor-capacitor arrangement was modelled with the software package Electronic Workbench (Interactive Image Technologies, Toronto, Ontario, Canada). The electrophoretic experiments were carried out using a purpose-made set-up, consisting of a benchtop dual polarity HV unit (CZE1000R, Start Spellman, Fulborough, UK), an injection cage made from Perspex and fitted with a microswitch to interrupt the high-voltage supply for safety, a compartment for detection which is lined with aluminium sheet to form a Faraday cage and a MacLab 4e data acquisition system (ADInstruments, Hastings, UK).

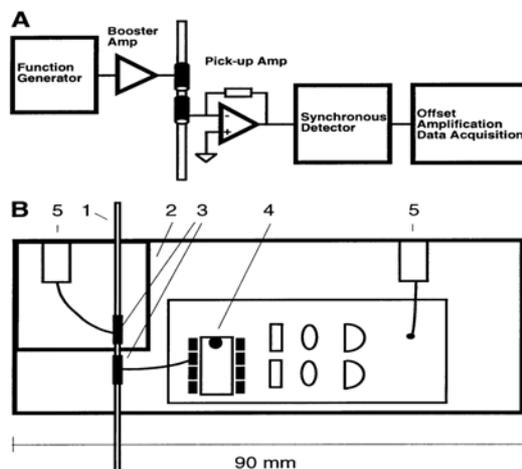


Fig. 1 (A) Block diagram of the detector electronics. (B) Sketch of the detector cell drawn approximately to scale and close to real size: 1, capillary; 2, Faraday shield; 3, electrodes; 4, amplifier; 5, coaxial connectors to the actuator source and signal processing circuitry.

Reagents and methods

All chemicals were of analytical-reagent grade and obtained from Fluka (Buchs, Switzerland), with the exceptions of tetramethylammonium bromide, caesium chloride and potassium bromide, which were obtained from Merck (Darmstadt, Germany), and glacial acetic acid, which was purchased from Riedel-de-Haën (Buchs, Switzerland). A 10 mM MES-His (1 + 1) buffer (MES = 2-morpholinoethanesulfonic acid monohydrate; His = histidine) was used in the determination of both inorganic cations and anions and was employed without adjustment at pH 6. Hexadimethrine bromide was added to the running buffer to modify the electroosmotic flow for anion analysis. An acetic acid-sodium acetate buffer and an acetic acid-lithium acetate buffer at pH 5.2 was used for the indirect conductivity determination of organic cations and anions, respectively.

Stock standard solutions of the inorganic cations and anions were prepared from their chloride and potassium salts, respectively. Solutions of tetramethylammonium, tetraethylammonium and benzyltriethylammonium were obtained from the bromide, perchlorate and chloride salts, respectively, and of propanesulfonate and heptanesulfonate from their sodium salts. Calibrated flasks made from poly(methylpentene) (PMP) and supplied by Semadeni (Ostermundigen, Switzerland) were used for the preparation and dilution of inorganic cations. Polypropylene vials from Agilent Technologies (Anwil, Switzerland) were used for buffers and standards. All solutions were ultrasonicated and filtered through 0.2 μm nylon filters. Fused-

silica capillaries of 360 μm od and different inner diameters were obtained from Polymicro Technologies (Phoenix, AZ, USA). These were preconditioned with a 0.1 M sodium hydroxide solution and thoroughly rinsed with de-ionised water prior to flushing with buffer. All standard solutions were contained in the background buffer in order to carry out electrokinetic injections under non-stacking conditions. Detection was carried out at 190 kHz and 250 V_{p-p} for actuation unless stated otherwise.

Results and discussion

The electrical characteristics of the cell and associated electronic circuitry were tested by determining the peak-to-peak cell currents obtained for a range of frequencies of the applied ac signal at different voltages. These values were determined as peak-to-peak voltage at the output of the pick-up amplifier with an oscilloscope and converted to input current according to its circuit function: $V_0 = iR$ (where V_0 is the output voltage, i the input current and R the value of the feedback resistor). The results obtained for a capillary of 50 μm id are given in Fig. 2. Note that the highest frequency which could be employed is limited by the bandwidth of the high-voltage amplifier on the actuator side and dependent on the voltage. No such limitation applies to the pick-up side. The performance of the pick-up amplifier was tested on its own and it was confirmed that in the configuration used, its frequency response was almost flat up to 2 MHz, the limit of the function generator. The data in Fig. 2 therefore represent the behaviour of the cell and are not compromised by artefacts introduced by the circuitry employed. For the lowest voltage applied to the cell (25 V_{p-p}), the current is found to increase up to a frequency of about 100 kHz and then to reach a plateau which extends up to the highest frequency of 450 kHz tested. This is characteristic of a high-pass filter and corresponds to the expected response (as confirmed by modelling) if the cell is assumed to consist of the serial arrangement of capacitor-resistor-capacitor. The behaviour shows that in the application of the cell the applied frequency is not critical as long as it falls between the boundaries. On increasing the voltage, a proportional increase in cell current is obtained. Even though the available frequency range is restricted for the higher voltages, the plateau is reached in all cases. These findings suggest that even higher cell currents could be expected with excitation at still higher voltages, but that an extension of the available frequency range would not lead to a further improvement.

The same measurements were also carried out for capillaries of 10, 25 and 75 μm id and similar frequency patterns were

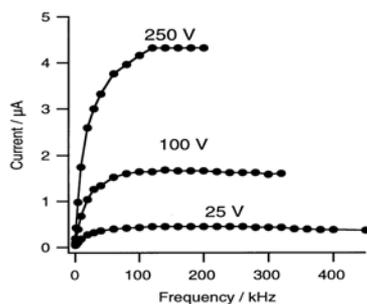


Fig. 2 Frequency characteristics of the cell with a capillary of 50 μm id filled with MES-His buffer at 10 mM (pH 6.0) for actuator voltages of 25, 100 and 250 V_{p-p} .

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obtained. The maximum currents were also found to be approximately proportional to the square of the capillary diameter (0.22, 1.4, 4.4 and 9.7 μA peak currents for capillaries of 10, 25, 50 and 75 μm id, respectively, at 250 V_{p-p}) and hence the cell volume. Capillaries of 75 μm id were therefore expected to give the best results. This size corresponds to the largest capillary diameter considered compatible with the requirements for running the electrophoretic separation. It is interesting that by coincidence the electrokinetic currents passing through the capillaries are almost identical with the detector currents. For capillaries of 25, 50 and 75 μm id, the separation currents were measured as 0.48, 1.71 and 3.93 μA , respectively, using 10 mM MES-His buffer and a separation voltage of 25 kV. In order to achieve a high signal-to-noise ratio, at least in principle, it is desirable that the detector current should be high compared with the separation current.

In Fig. 3, electropherograms for cations acquired at excitation voltages of 25, 100 and 250 V_{p-p} are shown. Clearly, the larger cell currents obtained for higher voltages translate into an increased sensitivity in peak height. On all three traces a small level of random high-frequency noise is present. The level of this noise was of the order of a few millivolts and similar for the three electropherograms. Therefore, the highest sensitivity corresponds to the best signal-to-noise ratio. It was found that the level of this noise is not dependent on the separation voltage as no difference was detected when this was turned off. In a different experiment, the cell itself was replaced with a resistor with a value appropriate to yield the same detected current. Again, the noise level was still the same. This clearly shows that, at least for the present configuration, the noise is mainly generated in the electronic circuitry itself and not in the detector cell. In conclusion, the observations indicate that the signal-to-noise ratio may be further increased by using an even higher excitation voltage and/or additional refinement of the electronic circuitry. Note that the fronting and tailing observed on the peaks is normal for capillary electrophoretic separation and not a feature of the detector.

When the distance between the two electrodes was extended from 1 mm to approximately 1.5 and 2.5 mm, it was found that the cell current was reduced to 66 and 44%, respectively. This corresponds to the expected behaviour considering that the cell resistance must increase with increase in its length. The peak heights for the 1.5 and 2.5 mm gaps were also found to decrease to approximately 78 and 60% of the sensitivity at 1 mm, respectively.

Evidence of the performance of the cell is also given in Fig. 4, where several runs for inorganic cations at 20 μM are overlaid. The high stability of the baseline and reproducibility of the peak heights observed at this relatively low concentration are ascribed not only to the performance of the electronics but

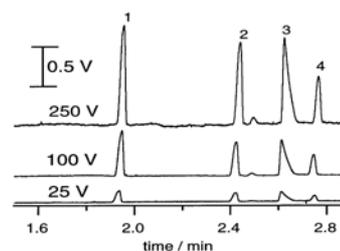


Fig. 3 Electropherograms for cations at 20 μM in 10 mM MES-His buffer (pH 6.0) for cell excitation voltages of 25, 100 and 250 V_{p-p} : 1, potassium; 2, sodium; 3, magnesium; 4, lithium. Sample injection: electrokinetic, 7 s at +5.0 kV. Separation: +25 kV. Capillary: fused silica, 74 cm length, 50 μm id.

also to the fact that short-term temperature fluctuations of the system are minimized by complete enclosure of the assembly to prevent any air draughts. This is an important consideration as conductivity measurements have a high intrinsic temperature coefficient, a factor which is not related to the electronic arrangement of the detector itself.

The detection of inorganic cations and anions at the level of $1 \mu\text{M}$ is illustrated in Fig. 5(A) and (B), respectively. Well defined peaks are obtained even at this low concentration. Note that the peak shapes observed, in particular for Fig. 5(A), are typical for the electrokinetic mode of injection employed (non-stacking conditions). The detection limits based on the peak heights corresponding to three times the signal-to-noise ratio (S/N) were determined as 1.2, 2.0, 1.4 and 2.7×10^{-7} M for K^+ , Na^+ , Mg^{2+} and Li^+ , respectively. For the anions, the values obtained were 2.1, 1.0, 0.89, 1.5 and 1.5×10^{-7} M for bromide, chloride, nitrate, sulfate and oxalate, respectively. Note that the use of polymer containers and clean-room conditions is required for reliable work at a level of $1 \mu\text{M}$. In particular, contamination

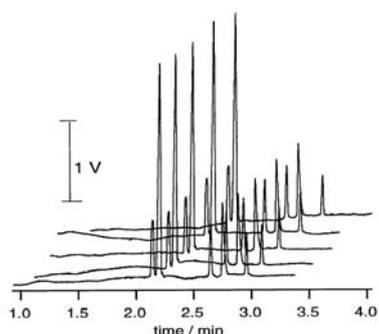


Fig. 4 Sequential separations of $20 \mu\text{M}$ cesium, potassium, sodium, magnesium and lithium (in order of appearance in the electropherograms). The traces are offset against each other for clarity. Buffer: 10 mM MES-HIS (pH 6.0). Sample injection: electrokinetic, 7 s at $+5 \text{ kV}$. Separation: $+25 \text{ kV}$. Capillary: fused silica, 74 cm length, $75 \mu\text{m}$ id.

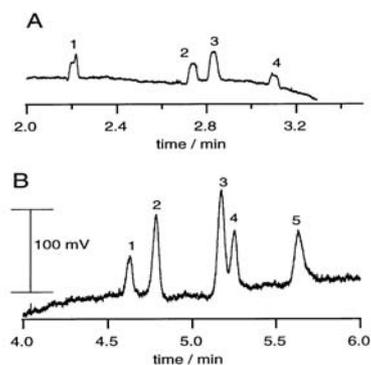


Fig. 5 Electropherograms of (A) cations and (B) anions at $1 \mu\text{M}$. (A) 1, Potassium; 2, sodium; 3, magnesium; 4, lithium. (B) 1, Bromide; 2, chloride; 3, nitrate; 4, sulfate; 5, oxalate. Buffer: 10 mM MES-HIS (pH 6.0), 0.00015% hexadimethrine bromide (for anions only). Sample injection: electrokinetic, 7 s at 5 kV . Separation: $+25 \text{ kV}$ (cations), -25 kV (anions). Capillaries: fused silica, 74 cm length, $75 \mu\text{m}$ id for cations and $50 \mu\text{m}$ id for anions.

by Na^+ and K^+ was observed at this concentration when using standard glassware. The two electropherograms were acquired with two different capillaries, of 75 and $50 \mu\text{m}$ id for the cations and anions, respectively. The lower apparent sensitivity for the cations arises from the use of a smaller feedback resistor on the pick-up amplifier, which was necessary in order to keep the baseline signal within its dynamic range ($560 \text{ k}\Omega$ for cation determination and $1 \text{ M}\Omega$ for anion determination). However, in contrast to the effect on the sensitivity, at least for the two relatively large diameter capillaries and the present conditions, there does not appear to be a systematic effect of the capillary diameter on the detection limits. Calibration curves for the inorganic anions and cations were found to be linear between 1 and about $200 \mu\text{M}$, but to show a slight curvature between $200 \mu\text{M}$ and 1 mM , the latter being the highest concentration tested. The regression data were determined for the lower concentration range for three of the ions ($y = ax + b$, where $y = \text{peak area}$ in arbitrary units and $x = \text{concentration in } \mu\text{M}$): Li^+ , $y = 0.0348x - 0.0384$; K^+ , $y = 0.0861x - 0.0297$; Mg^{2+} , $y = 0.0980x + 0.0271$ (correlation coefficients: 0.9996 , 0.9987 and 0.9999 , respectively). At $1 \mu\text{M}$ the peak areas could be reproduced to within 8.1 , 8.8 and 6.1% for these three ions, respectively, and at $200 \mu\text{M}$ the corresponding values were 1.4 , 2.3 and 1.9% (standard deviation, $n = 5$).

A similar performance was obtained for indirect detection using a buffer of ions with relatively high conductivity, as evidenced by the data presented in Fig. 6(A) and (B). As can be seen, the detection of organic ions presents no problems at a level of $10 \mu\text{M}$. The $3 \times S/N$ detection limits for tetramethylammonium, tetraethylammonium and butyltriethylammonium were determined as 1.2 , 0.7 and $0.6 \mu\text{M}$, respectively, and the corresponding data for the anions propanesulfonate and heptanesulfonate were 1.7 and $0.5 \mu\text{M}$, respectively.

Conclusion

The capacitively coupled contactless conductivity detector for capillary zone electrophoresis has been improved by optimization of the electronic circuitry (in particular the cell voltage) and its layout. The electrical behaviour of the cell was found to be straightforward and predictable. As indicated by the results obtained, it should be possible to employ the same set of optimized operating conditions for the detector (excitation voltage, frequency, capillary diameter) for all possible applications. The detection limits achieved are lower than the 50 – 500

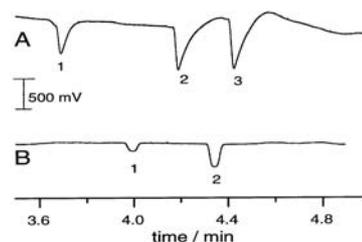


Fig. 6 Electropherograms for the indirect detection of (A) organic cations and (B) organic anions at $10 \mu\text{M}$. (A) 1, Tetramethylammonium; 2, tetraethylammonium; 3, benzyltriethylammonium. (B) 1, Propanesulfonate; 2, heptanesulfonate. Conditions for organic cation separation. Buffer: 2.5 mM sodium acetate (pH 5.2). Sample injection: electrokinetic, 10 s at $+5 \text{ kV}$. Separation: $+20 \text{ kV}$. Capillary: fused silica, 74 cm length, $75 \mu\text{m}$ id. Anion separation. Buffer: 2.5 mM lithium acetate (pH 5.2), 0.0007% hexadimethrine bromide. Sample injection: electrokinetic, 10 s at -5.0 kV . Separation: -20 kV . Capillary: fused silica, 74 cm length, $75 \mu\text{m}$ id.

ppb quoted for the indirect optical detection methods for inorganic ions,³¹ which are currently used with commercial instrumentation. These values are also at least as good as for conventional conductivity detection in capillary electrophoresis, even when employing chemical suppression of the background conductivity. This is achieved with a cell arrangement which is much simpler than required for optical or conventional electrochemical detection. Electrical interference of the electrophoretic voltage is not a problem and the electronic circuitry is straightforward.

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References

- 1 S. Polesello and S. M. Valsecchi, *J. Chromatogr., A*, 1999, **834**, 103.
- 2 L. A. Holland and S. M. Lunte, *Anal. Commun.*, 1998, **35**, 1H.
- 3 T. You, X. Yang and E. Wang, *Electroanalysis*, 1999, **11**, 459.
- 4 R. P. Baldwin, *Electrophoresis*, 2000, **21**, 4017.
- 5 T. Kappes and P. C. Hauser, *J. Chromatogr., A*, 1999, **834**, 89.
- 6 T. Kappes and P. C. Hauser, *Electroanalysis*, 2000, **12**, 165.
- 7 X. Huang, T. J. Pang, M. J. Gordon and R. N. Zare, *Anal. Chem.*, 1987, **59**, 2747.
- 8 X. Huang and R. N. Zare, *Anal. Chem.*, 1991, **63**, 2193.
- 9 C. Haber, W. R. Jones, J. Soglia, M. A. Surve, M. McGlynn, A. Caplan, J. R. Reineck and C. Krstanovic, *J. Capillary Electrophoresis*, 1996, **3**, 1.
- 10 C. Haber, R. J. van Saun and W. R. Jones, *Anal. Chem.*, 1998, **70**, 2261.
- 11 P. K. Dasgupta and L. Bao, *Anal. Chem.*, 1993, **65**, 1003.
- 12 S. Kar, P. K. Dasgupta, H. Liu and H. Hwang, *Anal. Chem.*, 1994, **66**, 2537.
- 13 P. K. Dasgupta and S. Kar, *Anal. Chem.*, 1995, **67**, 3853.
- 14 P. K. Dasgupta and S. Kar, *Anal. Chim. Acta*, 1999, **394**, 1.
- 15 N. Avdalovic, A. Pohl, R. D. Rocklin and J. R. Stillian, *Anal. Chem.*, 1993, **65**, 1470.
- 16 M. Harrold, J. Stillian, L. Bao, R. Rocklin and N. Avdalovic, *J. Chromatogr., A*, 1995, **717**, 371.
- 17 D. Müller, I. Jelinek, F. Opekar and K. Stulik, *Electroanalysis*, 1996, **8**, 722.
- 18 P. Tuma, F. Opekar, I. Jelinek and K. Stulik, *Electroanalysis*, 1999, **11**, 1022.
- 19 A. J. Zemmann, E. Schnell, D. Volgger and G. Bonn, *Anal. Chem.*, 1998, **70**, 563.
- 20 K. Mayrhofer, A. J. Zemmann, E. Schnell and G. K. Bonn, *Anal. Chem.*, 1999, **71**, 3828.
- 21 J. A. Fracassi da Silva and C. L. do Lago, *Anal. Chem.*, 1998, **70**, 4339.
- 22 P. Tuma, F. Opekar and I. Jelinek, *Electroanalysis*, 2001, **13**, 989.
- 23 D. Kaniansky, V. Zelenská, M. Masár, F. Iványi and S. Gazdíkova, *J. Chromatogr., A*, 1999, **844**, 349.
- 24 B. Gas, M. Demjanenko and J. Vacik, *J. Chromatogr.*, 1980, **192**, 253.
- 25 J. Vacik, J. Zuska and I. Muselasova, *J. Chromatogr.*, 1985, **320**, 233.
- 26 J. A. Fracassi da Silva and C. L. do Lago, *Electrophoresis*, 2000, **21**, 1405.
- 27 J. Muzikár, T. van de Goor, B. Gas and E. Kenndler, *J. Chromatogr., A*, 2001, **924**, 147.
- 28 F. E. Hilder, A. J. Zemmann, M. Macka and P. R. Haddad, *Electrophoresis*, 2001, **22**, 1273.
- 29 A. J. Zemmann, *Trends Anal. Chem.*, 2001, **20**, 346.
- 30 T. Chvojka, I. Jelinek, F. Opekar and K. Stulik, *Anal. Chim. Acta*, 2001, **433**, 13.
- 31 W. R. Jones, in *Handbook of Capillary Electrophoresis*, ed. J. P. Landers, CRC Press, Boca Raton, FL, 1997, p. 156.

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High-Voltage Capacitively Coupled Contactless Conductivity Detection for Microchip Capillary Electrophoresis

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Contactless conductivity detection was carried out on a planar electrophoresis device by capacitive coupling using an ac excitation voltage of 500 V_{p-p} and a frequency of 100 kHz. It was possible to carry out detection in this way through a cover plate of 1 mm thickness. Better sensitivity is obtained, however, by placing the electrodes into troughs that allow tighter coupling to the separation channel. The 3 × S/N detection limits are 0.49, 0.41, and 0.35 μM for the small inorganic ions K⁺, Na⁺, and Mg²⁺. The detection of heavy metals is demonstrated with the example of Mn²⁺, Zn²⁺, and Cr³⁺ with detection limits of 2.1, 2.8, and 6.8 μM, respectively. The universal nature of the method is further illustrated by the detection of citric and lactic acids, which are of interest in food and beverage analysis, and detection of three antiinflammatory nonsteroid drugs, 4-acetamidophenol, ibuprofen, and salicylic acid, as examples of species of pharmaceutical interest.

Detection on micromachined electrophoretic devices has often been carried out by laser-induced fluorescence. This method is highly sensitive but limited in scope, because it is only amenable to species that can be derivatized to render them fluorescent. For this reason, alternative detection techniques are increasingly desired.¹ Detection by UV absorption, the standard technique in conventional capillary electrophoresis, is not easily possible on chips because of the shortness of the optical path lengths available. The electrochemical methods of amperometry, potentiometry, and conductometry are particularly attractive alternatives, because their inherent simplicity makes them well-suited for miniaturized systems. For this reason, considerable effort has been spent on developing amperometric detection (see, for example, the reviews in refs 1–3). Amperometric detection, however, is only possible for electroactive species. Conductometric detection, on the other hand, may be considered almost universal in electrophoresis because it is based on the same property of the analyte, namely electrophoretic mobility, as the separation itself.

It was established early on in the development of conventional capillary zone electrophoresis that conductometric detection is

feasible, despite the possible conflict between separation voltage and electrochemical detection, by using a wall jet/electrode arrangement.⁴ The introduction of a commercial instrument in the late 1990s^{5,6} brought on a flurry of publications, clearly demonstrating the versatility of the method. Detection limits on the order of 1 μM were obtained for inorganic ions,^{7–15} and it was shown that the technique can be extended to organic species, such as detergents, that cannot be detected by optical means.^{16,17} Great impetus was given to conductometric detection by the independent introduction of the capacitively coupled contactless conductivity detector (C⁴D) by Zemann and co-workers and Fracassi da Silva and do Lago in 1998, because this approach overcomes problems with the alignment and electrode fouling present in the wall jet arrangement.^{18,19} The C⁴D has since been adopted by several research groups, and a range of applications of this detector have been demonstrated.^{20–30} It has very recently also been shown by

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(1) Schwarz, M. A.; Hauser, P. C. *Lab Chip* **2001**, *1*, 1–6.
(2) Lacher, N. A.; Garrison, K. E.; Martin, R. S.; Lunte, S. M. *Electrophoresis* **2001**, *22*, 2526–2536.
(3) Wang, J. *Talanta* **2002**, *56*, 223–231.

(4) Huang, X.; Zare, R. N. *Anal. Chem.* **1991**, *63*, 2193–2196.
(5) Haber, C.; Jones, W. R.; Soglia, J.; Surve, M. A.; McGlynn, M.; Caplan, A.; Reineck, J. R.; Krstanovic, C. *J. Capillary Electrophoresis* **1996**, *3*, 1–11.
(6) Haber, C.; van Saun, R. J.; Jones, W. R. *Anal. Chem.* **1998**, *70*, 2261–2267.
(7) Katzmayer, M. U.; Klampfl, C. W.; Buchberger, W. *J. Chromatogr. A* **1999**, *850*, 355–362.
(8) Gebauer, P.; Caslavská, J.; Thormann, W.; Bocek, P. *J. Chromatogr. A* **1997**, *772*, 63–71.
(9) Williams, R. C.; Boucher, R.; Brown, J.; Scull, J. R.; Walker, J.; Paolini, D. *J. Pharm. Biomed. Anal.* **1997**, *16*, 469–479.
(10) Bowman, J.; Tang, L.; Silverman, C. E. *J. Pharm. Biomed. Anal.* **2000**, *23*, 663–669.
(11) Govindaraju, K.; Cowley, E. A.; Eidelman, D. H.; Lloyd, D. K. *Anal. Chem.* **1997**, *69*, 2793–2797.
(12) Klampfl, C. W.; Katzmayer, M. U.; Buchberger, W.; Basener, N. *J. Chromatogr. A* **1998**, *804*, 357–362.
(13) Hissner, F.; Mattusch, J.; Heinig, K. *J. Chromatogr. A* **1999**, *848*, 503–513.
(14) Valsecchi, S.; Tartari, G.; Polesello, S. *J. Chromatogr. A* **1997**, *760*, 326–332.
(15) Schlegel, D.; Mattusch, J.; Wennrich, R. *Fresenius' J. Anal. Chem.* **1996**, *354*, 535–539.
(16) Lucy, C. A.; Wu, Q. *J. Chromatogr. Sci.* **1998**, *36*, 33–36.
(17) Gallagher, P. A.; Danielson, N. D. *J. Chromatogr. A* **1997**, *781*, 533–540.
(18) Zemann, A. J.; Schnell, E.; Volgger, D.; Bonn, G. *Anal. Chem.* **1998**, *70*, 563–567.
(19) Fracassi da Silva, J. A.; do Lago, C. L. *Anal. Chem.* **1998**, *70*, 4339–4343.
(20) Tuma, P.; Opekar, F.; Jelinek, I. *Electroanalysis* **2001**, *13*, 989–992.
(21) Pumera, M.; Jelinek, I.; Jindrich, J. *Fresenius' J. Anal. Chem.* **2001**, *369*, 666–669.
(22) Fracassi da Silva, J. A.; do Lago, C. L. *Electrophoresis* **2000**, *21*, 1405–1408.
(23) Muzikár, J.; van de Goor, T.; Gas, B.; Kenndler, E. *J. Chromatogr. A* **2001**, *924*, 147–154.
(24) Kappes, T.; Galliker, B.; Schwarz, M. A.; Hauser, P. C. *Trends Anal. Chem.* **2001**, *20*, 133–139.

our group that the sensitivity of this detector can be improved by using a voltage of several hundred volts for cell excitation (HV-C⁴D), and detection limits between 5×10^{-8} and 10^{-7} M could be achieved for small inorganic ions, which is better than what had been reported for the conventional contacted wall jet configuration.^{31,32}

Conductometric detection has also been demonstrated for micromachined electrophoresis devices. A number of researchers have used the contacted approach, based on electrodes embedded in the chip that are in direct contact with the solution, for detection of different inorganic and organic ions in on-chip isotachopheresis.^{33–42} Other workers have implemented standard zone electrophoresis on microfabricated devices. Van den Berg and co-workers demonstrated the detection of inorganic ions and peptides with embedded electrodes.^{43–45} Soper and co-workers have also used a device with a built-in conventional contacting conductivity detector and shown the separation and sensitive detection of amino acids, proteins, and DNA fragments.⁴⁶

Wang and co-workers recently demonstrated that capacitively coupled contactless conductivity detection is also possible in the chip format.^{47,48} A device manufactured in PMMA was employed,

- (25) Hilder, F. E.; Zemmann, A. J.; Macka, M.; Haddad, P. R. *Electrophoresis* **2001**, *22*, 1273–1281.
- (26) Zemmann, A. J. *Trends Anal. Chem.* **2001**, *20*, 346–354.
- (27) Unterholzner, V.; Macka, M.; Haddad, P. R.; Zemmann, A. *Analyst* **2002**, *127*, 715–718.
- (28) Chvojka, T.; Jelinek, I.; Opekar, F.; Stulik, K. *Anal. Chim. Acta* **2001**, *433*, 13–21.
- (29) Fracassi da Silva, J. A.; Guzman, N.; do Lago, C. L. *J. Chromatogr., A* **2001**, *942*, 249–258.
- (30) Kuban, P.; Karlberg, B.; Kuban, P.; Kuban, V. *J. Chromatogr., A* **2002**, *964*, 227–241.
- (31) Tanyanyiwa, J.; Galliker, B.; Schwarz, M. A.; Hauser, P. C. *Analyst* **2002**, *127*, 214–218.
- (32) Tanyanyiwa, J.; Hauser, P. C. *Electrophoresis*, in press.
- (33) Prest, J. E.; Baldock, S. J.; Bektas, N.; Fielden, P. R.; Brown, B. J. *T. J. Chromatogr., A* **1999**, *836*, 59–65.
- (34) Prest, J. E.; Baldock, S. J.; Fielden, P. R.; Brown, B. J. *Analyst* **2001**, *126*, 433–437.
- (35) Kaniansky, D.; Masar, M.; Bielikova, J.; Ivanyi, F.; Eisenbeiss, F.; Stanislawski, B.; Grass, B.; Neyer, A.; Jöhnck, M. *Anal. Chem.* **2000**, *72*, 3596–3604.
- (36) Masar, M.; Zuborova, M.; Bielikova, J.; Kaniansky, D.; Jöhnck, M.; Stanislawski, B. *J. Chromatogr., A* **2001**, *916*, 101–111.
- (37) Bodor, R.; Madajova, V.; Kaniansky, D.; Masar, M.; Jöhnck, M.; Stanislawski, B. *J. Chromatogr., A* **2001**, *916*, 155–165.
- (38) Masar, M.; Kaniansky, D.; Bodor, R.; Jöhnck, M.; Stanislawski, B. *J. Chromatogr., A* **2001**, *916*, 167–174.
- (39) Bodor, R.; Zuborova, M.; Ölvecka, E.; Madajova, V.; Masar, M.; Kaniansky, D.; Stanislawski, B. *J. Sep. Sci.* **2001**, *24*, 802–809.
- (40) Grass, B.; Neyer, A.; Jöhnck, M.; Siepe, D.; Eisenbeiss, F.; Weber, G.; Hergenröder, R. *Sens. Actuators, B* **2001**, *72*, 249–258.
- (41) Grass, B.; Siepe, D.; Neyer, A.; Hergenröder, R. *Fresenius' J. Anal. Chem.* **2001**, *371*, 228–233.
- (42) Zuborova, M.; Masar, M.; Kaniansky, D.; Jöhnck, M.; Stanislawski, B. *Electrophoresis* **2002**, *23*, 774–781.
- (43) Guijt, R. M.; Baltussen, E.; van der Steen, G.; Schasfoort, R. B. M.; Schlautmann, S.; Billiet, H. A. H.; Frank, J.; van Dedem, G. W. K.; van den Berg, A. *Electrophoresis* **2001**, *22*, 235–241.
- (44) Schlautmann, S.; Wenskink, H.; Schasfoort, R.; Elwenspoek, M.; van den Berg, A. *J. Micromech. Microeng.* **2001**, *11*, 386–389.
- (45) Guijt, R. M.; Baltussen, E.; van der Steen, G.; Frank, H.; Billiet, H.; Schalkhammer, T.; Laugere, F.; Vellekoop, M.; Berthold, A.; Sarro, L.; van Dedem, G. W. K. *Electrophoresis* **2001**, *22*, 2537–2541.
- (46) Galloway, M.; Stryjewski, W.; Henry, A.; Ford, S. M.; Llopis, S.; McCarley, R. L.; Soper, S. A. *Anal. Chem.* **2002**, *74*, 2407–2415.
- (47) Pumera, M.; Wang, J.; Opekar, F.; Jelinek, I.; Feldman, J.; Löwe, H.; Hardt, S. *Anal. Chem.* **2002**, *74*, 1968–1971.
- (48) Wang, J.; Pumera, M.; Collins, G.; Opekar, F.; Jelinek, I. *Analyst* **2002**, *127*, 719–723.

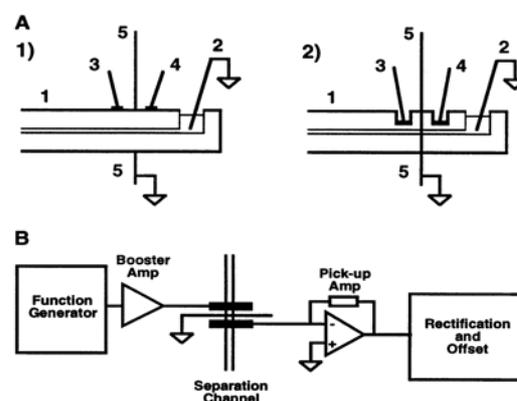


Figure 1. (A) Cross-sectional view of the two cell arrangements without (1) and with (2) troughs for the detector electrodes. (B) Block diagram of the electronic circuitry: (1) detector end of the separation device, (2) electrophoretic ground electrode, (3) actuator electrode for detection, (4) pick-up electrode for detection, and (5) Faraday shield.

and for detection, a pair of electrodes was placed on top of a 150- μm -thick cover sheet, sealing the separation channel. The fact that the electrodes do not need to be embedded in the device represents, of course, a significant simplification of the manufacturing process. The performance of this arrangement was illustrated with the separation and detection of small inorganic cations and anions with concentrations on the order of 0.5 mM. The aim of the current project was the evaluation of the high-voltage approach to capacitively coupled contactless conductivity detection (HV-C⁴D) for detection on microfabricated planar electrophoresis devices.

EXPERIMENTAL SECTION

Glass microchips containing a manifold in the standard elongated cross configuration were purchased from Micralyne (model MC-BF4-TT100, Edmonton, Canada). The separation channel is 85 mm long with a semicircular cross section of 50- μm width and 20- μm depth buried ~ 1 mm below the surface. The arrangement of the detector electrodes is illustrated in Figure 1A. These consisted of two parallel strips of ~ 0.5 -mm width and 5-mm length across the channel and separated by 1 mm and were formed with silver paint. The wells for placement of the electrodes were created either by ultrasonic abrasion, carried out at the Technical Institute of the Friedrich Schiller University of Jena, Germany, with a purpose made imprinting tool, or manually by using a cutting wheel attached to a high-frequency spindle (15 000 rpm). The bottoms of the troughs were situated ~ 0.2 mm from the channel. The chip was placed on a specially made holder that features a Faradaic shield located between the electrodes and completely encompassing the device. If the shield is omitted, significant direct coupling from the actuator to the pick-up electrode is observed, even when the separation channel is dry. This causes an undesirably high background signal, which leads to a significant deterioration of the signal-to-noise ratio. A block

diagram of the detection circuitry is given in Figure 1B. It chiefly consists of a function generator (GFG-8019C, Goodwill Instruments, Taipei, Taiwan) to create a sinusoidal excitation signal and a high-voltage amplification stage (PA94, Apex Microtechnology Corporation, Tucson, AZ). The circuitry on the pick-up side comprises a current-to-voltage converter (OPA627, Texas Instruments, Dallas, TX), followed by rectification, low-pass filtering, and an offset stage for baseline suppression. More details have been given elsewhere.^{31,32} Injection and separation voltages were produced by two high-voltage power supplies (model CZE1000R, Start-Spellman Ltd., Pulborough, England), which are controlled by a specially built interface connected to a multifunctional I/O-card (model PCI-MIO-16XE-50, National Instruments, Austin, Texas) located in a standard personal computer. All parameters were set, and data acquisition was performed using a program written in LabVIEW (National Instruments). Detection limits were determined as the concentrations giving peak heights corresponding to 3 times the baseline noise. Please note that both electrophoretic and detector voltages are hazardous, and appropriate precautions to avoid accidental contact must be taken.

Stock solutions of ammonium, potassium, sodium, magnesium, strontium, and lithium were prepared from their chloride salts. Manganese, chromium and zinc solutions were prepared from the chloride, nitrate, and acetate salts, respectively. Citrate and lactate stock solutions were prepared from the sodium salts. These salts were purchased from Merck (Darmstadt, Germany). Ibuprofen sodium salt was supplied by Sigma; 4-acetamidophenol (paracetamol), by Acros; and salicylic acid, by Aldrich. Fresh running buffers were prepared daily. A 10 mM 2-(*N*-morpholino)ethanesulfonic acid (MES)/10 mM histidine(His) buffer in equimolar amounts was used for the determination of inorganic cations. 18-Crown-6 (optional) and α -hydroxyisobutyric acid (HIBA) were used as complexing agents for potassium and heavy metals, respectively. A 10 mM 2-(cyclohexylamino)ethanesulfonic acid (CHES)/6 mM arginine (Arg) electrolyte (pH 9) was used for the separation of the organic anions lactate and citrate. A 10 mM 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS)/10 mM arginine buffer at pH 10 was used for the determination of 4-acetamidophenol, ibuprofen, and salicylic acid. These buffers were adapted from Klampfl et al.¹² All solutions were degassed in an ultrasonic bath and filtered through 0.2 μ m nylon filters before use. Hypodermic needles were used to introduce the buffer and solutions into the microchip reservoirs. The channel was preconditioned with 1 M sodium hydroxide solution before thoroughly rinsing with Millipore water and subsequently flushing with buffer. The chip was rinsed with buffer between runs to ensure reproducibility and maintain a stable baseline. After use, it was rinsed with Millipore water to prevent clogging of the narrow channel. The required standards were prepared by diluting the stock solutions in the running buffer to ensure nonstacking conditions on injection.

RESULTS AND DISCUSSION

The detection of ammonium and magnesium at 200 μ M with electrodes placed on an unmodified device is shown in Figure 2A. Clearly, the use of the high actuator voltage enables detection through the 1-mm-thick cover plate at acceptable sensitivity. However, when employing electrodes located in troughs, which leave a smaller gap of only 0.2 mm between the electrodes and

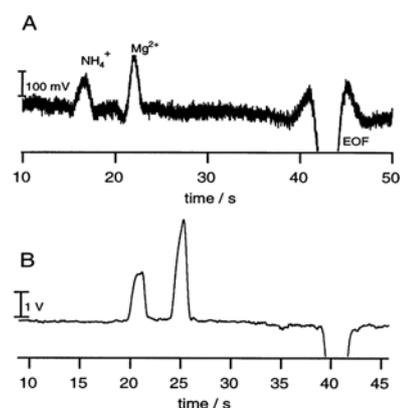


Figure 2. Electropherograms of ammonium and magnesium at 200 μ M for the cell configuration with and without electrode wells: (A) electrodes placed on an unaltered chip at 1 mm distance from separation channel and (B) electrodes placed in electrode wells at 0.2 mm from the separation channel. Buffer, 10 mM MES/His, 2 mM 18-crown-6 (pH 6); injection, 1 kV for 2 s; separation, 3 kV; actuator voltage, 500 V_{p-p} ; frequency, 100 kHz.

channel, the detector sensitivity to the same mixture of standards is further improved. The resulting electropherogram is given in Figure 2B. Note the different scales for the two sections of Figure 2. The ammonium peak shows signs of peak broadening. This is caused by the relatively high injection voltage used. The higher level of baseline noise apparent on the electropherogram for the unmodified device is largely due to the expanded voltage scale on the plot, and evidently the detection limits are worse under these conditions. In comparison to the results reported by Wang and co-workers,^{47,48} who used peak-to-peak excitation voltages on the order of 10 V for a similar arrangement, the sensitivity is much improved.

The effect of applied voltage and frequency on the peak height is illustrated in Figure 3. It can be seen that the expected proportionality between peak height and applied voltage is obtained. However, the reason the maximum sensitivity is encountered around 50 kHz in the frequency profiles is not so clearly understood. It does not match a similar behavior, though, obtained for actuator voltages higher than 350 V_{p-p} on conventional capillaries.³² For lower applied voltages, it had been established that the electrical behavior of a C^1D cell corresponds to that of a serial arrangement of capacitor-resistor-capacitor, the most simple possible model.^{31,32} This results in a plateau in the plot of peak height vs frequency extending up to \sim 800 kHz. It is, therefore, thought that the exception encountered for the higher applied voltages is most likely due to an electronic feature of the system that is not understood rather than an intrinsic behavior of the cell itself. Please note that the maximums encountered in this case at 50 kHz are not related to a peak in the frequency plot reported by other workers^{19,28,47} around 400–600 kHz, which could be traced to an artifact due to the electronic circuitry used.³² Highest sensitivity was obtained with 500 V_{p-p} and 50 kHz. All subsequent electropherograms were, however, recorded at 500 V_{p-p} and 100 kHz as the higher frequency led to a somewhat more

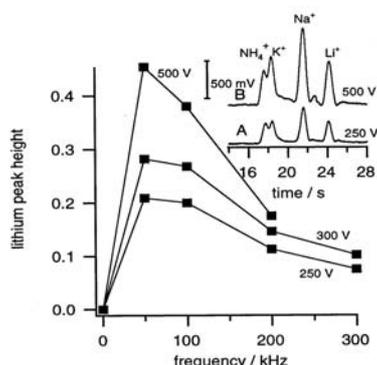


Figure 3. Effect of applied frequency on the detector response to 200 μM lithium at 250 V_{p-p} , 300 V_{p-p} , and 500 V_{p-p} . The insert shows two electropherograms of ammonium, potassium, magnesium, and lithium at 200 μM corresponding to actuator voltages of 250 V_{p-p} (A) and 500 V_{p-p} (B) recorded at 100 kHz frequency. Buffer, 10 mM MES/His, 2 mM 18-crown-6 (pH 6); injection, 0.5 kV for 3 s; separation, 3 kV.

stable baseline. The reason for this behavior is not understood. The insert in Figure 3 illustrates electropherograms of ammonium, potassium, sodium and lithium recorded at two different actuator voltages, namely 250 V_{p-p} and 500 V_{p-p} . All other parameters were held constant. The arrangement with the detector electrodes situated in the wells was used for these and all subsequent determinations. It can be deduced from the peak heights that increasing the actuator voltage leads to a proportional increase in sensitivity. A 10 mM MES/His, 2 mM 18-crown-6 electrolyte solution was used for these separations. With 2 mM crown ether, it is not possible to resolve ammonium and potassium peaks on a glass chip. In the capillary however, complete resolution is achieved with crown ether concentrations as low as 1 mM. The incomplete separation on chip can be attributed to the short separation distance (8.5 cm). Wang et al. investigated the effect of 18-crown-ether on the separation of cations.⁴⁷ They established that concentrations as high as 7.5 mM 18-crown-6 were required for the separation of ammonium, potassium, methylammonium, and sodium on a poly(methyl methacrylate) microchip, which, however, shows a lower electroosmotic flow.

For Figure 4, 20 μM potassium, sodium, and magnesium were separated with 2, 3, and 4 kV, respectively. At 2 kV, the potassium peak has a migration time of 28 s, and all peaks are severely broadened. Increasing the separation voltage leads to shorter migration times, sharper peaks, and better detection limits. These electropherograms were recorded at 500 V_{p-p} and 100 kHz. At these conditions, concentrations as low as 20 μM can be detected with good sensitivity. The peak heights we obtained for 20 μM potassium, sodium, and lithium for a lower actuation voltage. Therefore, increasing the actuator voltage may be considered the first step toward improving detection limits. Detection limits of 0.49, 0.41, and 0.35 μM were obtained for potassium, sodium, and magnesium, respectively, using a separation voltage of 4 kV.

In Figure 5, it is shown that 50 μM strontium, manganese,

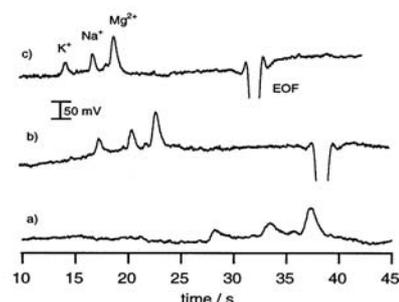


Figure 4. Electropherograms for the cell configuration with wells for potassium, sodium, and magnesium at 20 μM for separation voltages of 2 kV (A), 3 kV (B), and 4 kV (C). Buffer, 10 mM MES/His; injection, 0.5 kV for 3 s; actuator voltage, 500 V_{p-p} ; frequency, 100 kHz.

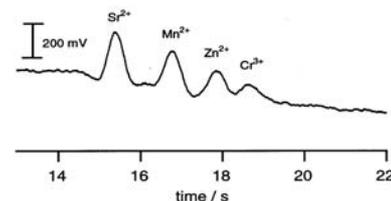


Figure 5. Electropherogram of heavy metal ions. Concentrations, 50 μM Sr^{2+} , Mn^{2+} , Zn^{2+} and Cr^{3+} ; buffer, 5 mM His, 3 mM HIBA, pH 4.5; injection, 500 V, 3 s; separation, 5 kV; actuator voltage, 500 V_{p-p} ; frequency, 100 kHz.

zinc, and chromium could be separated in <20 s. Two buffers were examined for the determination of heavy metal ions. A 5 mM MES/His, 3 mM HIBA buffer at pH 5.2 was tested first.³² This background electrolyte solution was found to be not suitable for the on-chip analysis of heavy metals, because the peaks comigrated, and a high electroosmotic flow and high background conductivity were obtained. The electropherogram in Figure 5 was recorded with a 5 mM His, 3 mM HIBA buffer at pH 4.5. This buffer was better suited for the separation of heavy metals, although a baseline drift is evident in the electropherogram. The detection limits are estimated to be 1.4, 2.1, 2.8, and 6.8 μM for Sr^{2+} , Mn^{2+} , Zn^{2+} , and Cr^{3+} , which are better than those obtained with a MES/HIS-HIBA buffer on conventional capillaries.³² Excluding MES from the electrolyte solution compensates for the additional background conductivity introduced by HIBA. Hence, the improved detection limits can be attributed to an optimization of the buffer.

The low mobility anions lactate and citrate were separated using a 10 mM CHES/ 6 mM Arg electrolyte solution at natural pH 9. Under these conditions, a high electroosmotic flow is obtained, leading to the sweeping of the slow anions to the cathodic end. An electropherogram of the two ions at 100 μM with +5 kV is shown in Figure 6. The anions could also be separated with 3 kV; the peaks were, however, broadened. Lactate and citrate are relevant analytes in beverage analysis. The $3 \times$ S/N detection limits for lactate and citrate were 5.5 and 2.3 μM , respectively.

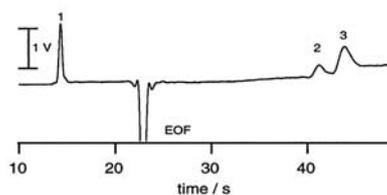


Figure 6. Electropherogram of 100 μM lactate and citrate sodium salts. Buffer, 10 mM CHES/6 mM Arg (pH 9). Peak identification: (1) sodium, (2) lactate, (3) citrate; Injection, 500 V, 3 s; separation, 5 kV; actuator voltage, 500 V_{p-p} ; frequency, 100 kHz.

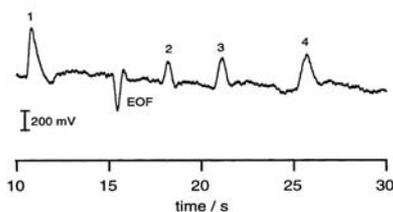


Figure 7. Electropherogram of pharmaceutically relevant compounds at 100 μM . Peak identification: (1) sodium, (2) 4-acetamidophenol, (3) ibuprofen, and (4) salicylic acid. Buffer, 10 mM CAPS/Arg at pH 10; injection, 500 V, 3 s; separation, 5 kV; actuator voltage, 500 V_{p-p} ; frequency, 100 kHz.

In Figure 7, the separation of 100 μM 4-acetamidophenol, ibuprofen, and salicylic acid is illustrated. 4-Acetamidophenol and ibuprofen are antiinflammatory agents and also analgesics and antipyretics. Acetylation of salicylic acid yields acetylsalicylic acid (aspirin), a widely used drug for the reduction of pain and fever. These drugs have unpleasant side effects, such as gastric irritation

and bleeding. 4-Acetamidophenol and ibuprofen are orally administered in relatively high doses. Pharmaceutical preparations of 500 mg of ibuprofen and 4-acetamidophenol are available. A 10 mM CAPS/Arg buffer (pH 10) was used for the analysis of these compounds, which have pK_a 's between 4.5 and 10.2. At a pH value of 10, all three analytes are anions. The analyte peaks illustrate a direct response; thus, the electrolyte possesses a background conductivity lower than that of the analytes. Estimated detection limits for the three species are all $\sim 10 \mu\text{M}$.

CONCLUSIONS

Increasing the excitation voltage for capacitively coupled conductivity detection on a microfabricated electrophoresis device did, indeed, lead to a much improved signal strength, which allowed detection through a thick cover and led to improved sensitivity when placing the electrode into troughs to bring them closer to the separation channel. Detection limits of $\sim 0.5 \mu\text{M}$ could be obtained for small alkali and alkaline earth metal cations. It could furthermore be shown that conductivity detection may not only be useful for the to-date well-known determination of small inorganic ions, but also holds great promise for the versatile analysis of organic species as well, although the detection limits for the organics (5–10 μM) do not quite match the values obtained for the inorganic species.

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High-voltage contactless conductivity-detection for lab-on-chip devices using external electrodes on the holder

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The detection of ionic species in a polymeric planar electrophoresis device by contactless conductivity measurement is described. To our knowledge this is the first report of such measurements carried out with external electrodes which are part of the holder rather than the separation chip itself. The approach allows the use of bare devices as used for optical measurements, which greatly simplifies the method. The use of a sine wave of 100 kHz of a high amplitude of 500 V for cell excitation assures high sensitivity which is demonstrated with electropherograms for alkali and heavy metal ions as well as inorganic anions and carboxylates at concentrations between 10 and 50 μM . The determination of underivatized amino acids was also possible by using a buffer in the alkaline region where these species are present in anionic form. Detection limits were found to be in the order of 1–5 μM for the inorganic ions and between about 5 and 50 μM for the organic species.

Introduction

Detection on microscale separation devices, so-called electrophoresis chips, has mainly been carried out with fluorescence detection using a laser focused onto the separation channel of a cross-sectional dimension of typically 25 μm . Laser induced fluorescence is highly sensitive and therefore works well for detection in small volumes. Most analyte species are not fluorescent however. Derivatization, in order to render the analyte amenable for detection, is successfully used for amino acids and more complex species of biological interest. On the other hand, most other important classes of analytes of importance in industry and in environmental monitoring are not suitable for fluorescence detection. Therefore, in order to broaden the scope of the lab-on-a-chip devices, alternative detection methods are sought, and for this reason, amperometry¹ and conductometry² have recently gained considerable interest. Conventionally these techniques are well suited to miniaturization because the signal transduction from the chemical to the electronic domain is carried out in a single step only.

Conductometry is, at least in principle, a universal detection method for electrophoresis as it is based on the same property of the analyte as the separation itself, namely the mobility in the electric field. Electrophoresis chips with electrodes embedded in the device for conductometric detection have been described by several groups. Van den Berg and coworkers^{3–5} as well as Lichtenberg *et al.*⁶ have demonstrated the detection of inorganic ions in zone electrophoresis and Soper and coworkers^{7,8} as well as Zuborová *et al.*⁹ have shown the detection of biochemically relevant species with a pulsed conductivity detector. Conductometry has also been used for detection in isotachopheresis implemented in the chip format (see for example refs. 10–12).

Conductometric detection has also experienced a veritable renaissance for conventional capillary electrophoresis since the concurrent introduction of a capacitively coupled contactless arrangement by Zemmann and coworkers¹³ and do Lago and coworkers¹⁴ in 1998. The contactless approach is based on two tubular electrodes through which the capillary is pushed. This greatly simplifies the cell construction and precludes electrode fouling. Because of these advantages, several groups have adopted the technique since its introduction (for reviews see refs. 2 and 15). Wang and coworkers have also successfully transferred the technology to the chip format by gluing a pair of planar electrodes on a polymeric chip which has the separation channel covered with a thin cover sheet of only 125 μm thickness.^{16,17} Tanyanyiwa and Hauser have subsequently shown that the use of an elevated excitation voltage (several hundred volts) in contactless conductivity detection not only leads to enhanced sensitivity on conventional capillaries,^{18–20} but also when employed with a microchip which had electrodes attached to it.²¹ A further improvement is presented here, the placement of the electrodes on the chip holder, rather than on the device itself. This then allows the use of bare chips, which can be as easily mass produced as the ones employed for fluorescence detection, a considerable simplification in the application of electrochemical detection for lab-on-a-chip devices.

Experimental

The separation microchips were fabricated from poly(methylmethacrylate) (PMMA) at the Forschungszentrum in Karlsruhe using a proprietary procedure. They consist of a plate of about 1 mm thickness which contains a longitudinal separation

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channel of 50 μm depth, 50 μm width and a length of 80 mm at the bottom and access holes to the top. The injection cross is formed by intersection of the separation channel with a shorter channel of identical cross-sectional dimensions. The channel is sealed underneath with a thin foil of 100 μm thickness, also made of PMMA, which allows close coupling to a pair of electrodes when the device is clamped onto its holder. The chips were fabricated *via* hot embossing with a steel master followed by sealing of the microchannel with the foil by applying heat and pressure. Microchannels of good surface quality and reliable sealing without leakage are obtained by this technology. The chip is placed on a holder also constructed from PMMA, on which two electrodes for detection are mounted so that they are positioned approximately 1 cm from the channel end. These two electrodes consist of strips of adhesive copper tape of a thickness of about 50 μm and a width of 2 mm separated by a gap of 2.5 mm. The holder also features a vertical earth plane which is employed to minimise direct capacitive coupling between the electrodes. The arrangement is illustrated in Fig. 1. Cell excitation is effected by applying a sine wave with a frequency of 100 kHz and a peak-to-peak amplitude of 500 V to one of the electrodes, *i.e.* the actuator electrode. The resulting cell current is picked up *via* capacitive coupling by the second, transducer electrode. The current is converted to voltage at the input stage of the amplification circuitry. This is followed by rectification, an offset stage and further amplification before digitization of the signal. More details of the detector circuitry have been given previously.^{18,21}

All chemicals were of analytical reagent grade and were used as received. Rubidium, potassium, sodium and lithium stock solutions were prepared from their chloride salts. Those of chloride, nitrate and perchlorate were prepared from the sodium salts. Stock solutions of iron and cobalt were prepared from nitrate salts and cadmium from the acetate respectively. A 1:1 morpholinoethanesulfonic (MES)/histidine (His) buffer at its natural pH value was used for the analysis of alkaline earth, inorganic anions and organic acids. 18-Crown-6, required for the resolution of rubidium and potassium, was included in the buffer for cation analysis. 200 μM hexadecyltrimethylammonium bromide (CTAB) was used to reverse the EOF for organic acid analysis. No EOF modifier was used for the analysis of inorganic anions. For the heavy metal analysis a histidine, α -hydroxyisobutyric acid (HIBA) at pH 4.5 was employed. A 10 mM 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS)/50 mM 2-amino-2-methyl-1-propanol (AMP) buffer at pH 10.8 was used for the analysis of amino acids.

Pipette tips were used to introduce the buffer and solutions into the microchip reservoirs. All solutions were ultrasonicated and filtered through 0.2 μm nylon filters before analysis. The channel was rinsed with water and then flushed with buffer before runs. At the end of the day it was rinsed with water in order to prevent clogging of the microchannel. All standards were diluted in the background buffer to ensure injection under non-stacking conditions.

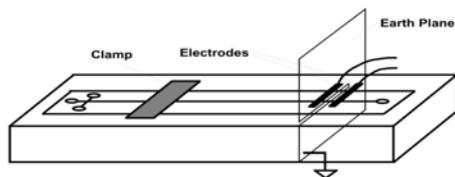


Fig. 1 Sketch of the chip holder. The chip is inserted into the slit in the earth plane from the front and held in place with the clamp on the left hand side. The electrodes for injection and separation are not shown.

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Results and discussion

The performance of the arrangement is illustrated in Fig. 2 with an electropherogram for 4 alkali cations at 10 μM . Separation of the 4 ions could be achieved within 15 s. The measurements were carried out at a peak-to-peak excitation voltage of 500 V, which is the maximum available with the current system. It was found that the signal strength increases roughly proportionally with the amplitude of the applied sine wave. Optimization of the frequency showed a maximum of the signal strength at about 100 kHz. The performance of the current arrangement with regard to voltage and frequency response are almost identical to what was obtained previously with a glass chip which had electrodes permanently attached.²¹ The lower limits of detection for the small inorganic ions are around 1.5 μM based on the peak height and a signal-to-noise ratio of 3, which is only slightly higher than the results obtained previously for this type of ion with high voltage contactless conductivity detection using attached electrodes (approximately 0.5 μM).²¹ The reproducibility of the peak area for sodium was determined as 1.1% (standard deviation, $n = 5$). The number of theoretical plates for this peak was calculated as 3100. This is somewhat better than the value of 1500 obtained for the same ion with the arrangement previously reported,²¹ and therefore the use of external electrodes does not lead to a loss of resolution in comparison to measurements with attached electrodes.

It can be seen from the electropherogram of Fig. 3 that the system is not only suitable for the small alkali cations but also for the fast separation of heavy metal ions. Note that higher concentrations were injected, but from the reduced scale on the signal axis it is evident that the sensitivity is nearly as good also for the larger transition metals. The detection limits ($3 \times \text{S/N}$)

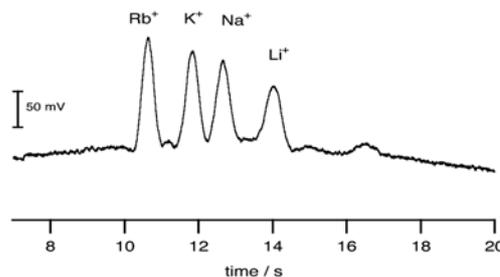


Fig. 2 Electropherogram for alkali metal ions at 10 μM in 10 mM MES/His buffer, 4 mM 18-crown-6, pH 6.0. Injection: +1 kV for 3 s. Separation: +3 kV.

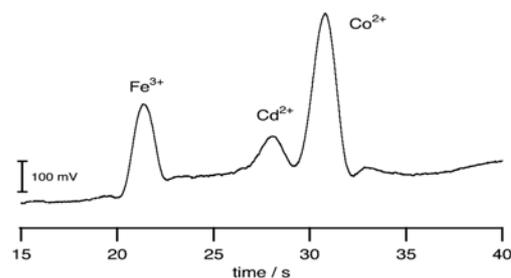


Fig. 3 Heavy metal ions at 50 μM in 5 mM His, 3 mM HIBA, pH 4.5. Injection: +1 kV for 3 s. Separation: +3 kV.

were determined as 3.5, 8 and 2 μM for Fe^{3+} , Cd^{2+} and Co^{2+} respectively.

A further application is demonstrated in Fig. 4 with anionic species. The separation of 3 inorganic anions at 50 μM is shown. Note that in the PMMA device an electroosmotic flow towards the cathode was found to be present. This is somewhat less than in fused silica or glass devices but its presence means that the anions migrate against the electroosmotic flow. This explains the longer analysis time (approximately 1 min) encountered in comparison to the cations reported in Figs. 2 and 3. It is also noteworthy that on the absolute time scale, the peaks in Fig. 4 are much wider than in Fig. 2, which must have been caused by further diffusional band broadening because of the extended time delay. The detection limits ($3 \times \text{S/N}$) were determined as 2.5, 3 and 2.5 μM for Cl^- , NO_3^- and ClO_4^- respectively.

In Fig. 5 it is shown that the approach is also readily suitable for organic species. An electropherogram for five organic anions in the form of carboxylates is given. This separation, in contrast to the separation of the inorganic ions, was obtained by adding an EOF modifier to the run buffer. The surface active substance causes a reversal of the direction of the EOF, which was now towards the anode located at the detection end of the device. It was therefore possible to separate the relatively more slowly migrating larger organic anions in the short time of 30 s. The reduction in peak height with analysis time for the 5 species, which were all present at the identical concentration of 50 μM is a systematic effect. The larger the ion is, the slower is its electrophoretic migration and the smaller the signal in conductometric detection. The detection limits ($3 \times \text{S/N}$) were determined as 4.4, 7, 10, 25 and 30 μM for oxalate, tartrate, succinate, acetate and lactate respectively.

The separation of a further class of organic ions, namely amino acids, is demonstrated in Fig. 6. Owing to their zwitterionic nature, in electrophoresis amino acids must be

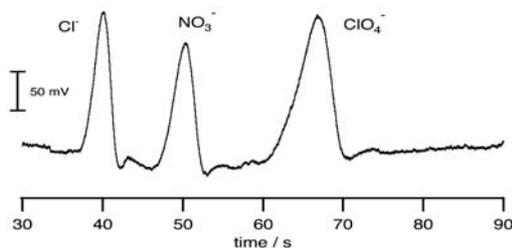


Fig. 4 Inorganic anions at 50 μM in 10 mM MES/His buffer. Injection: -1 kV for 3 s. Separation: -4 kV.

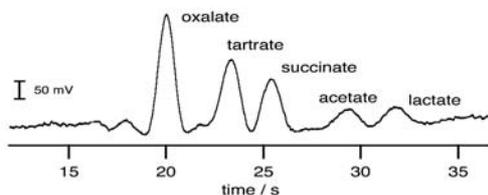


Fig. 5 Organic acids at 50 μM in 10 mM MES/His buffer containing 200 μM CTAB. Injection: -1 kV for 3 s. Separation: -3 kV.

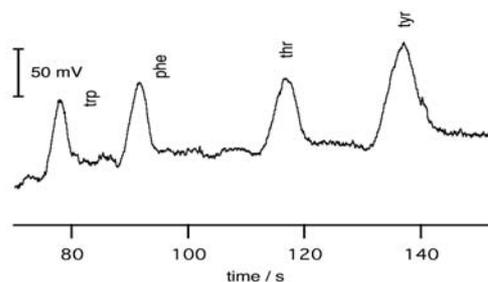


Fig. 6 Amino acids at 100 μM in 10 mM CAPS/50 mM AMP at pH 10.8. Injection: $+1$ kV for 3 s. Separation: -4 kV.

either determined at acidic pH values in order to render them cationic, or at basic pH values to obtain the anionic form. Both approaches have been found suitable for detection by contactless conductivity measurements in conventional capillaries.²² Here basic conditions were adopted for the separation of 4 amino acids. The detection limits ($3 \times \text{S/N}$) were found to be 50, 43, 45 and 32 μM for tryptophan, phenylalanine, threonine and tyrosine, respectively. Please note however, that the PMMA chip employed was not suitable for long term measurements under the alkaline conditions employed for this separation.

Conclusion

It could be shown that by using a high ac voltage for excitation it is possible to carry out conductometric detection in microfluidic channels with high sensitivity using external electrodes which are not part of the separation device itself. Our own experience in the construction of devices with embedded or attached electrodes clearly tells us that this is a significant simplification. The approach combines the advantage of spectrometric measurements, *i.e.* the use of simple chip devices bare of any electrodes, with the inherent simplicity of electrochemical detection methods. However, the sensitivity and separation efficiency of fluorescence detection cannot be matched. The detection limits achieved with the electrodes in the holder are comparable to what was reported previously for the high voltage detection approach with attached electrodes, and certainly as good or better than any results with any microfabricated device employing conductometric detection. Conductometric detection is a versatile technique which does not require pretreatment of samples. In particular, it could be demonstrated that the facile detection of underivatized amino acids on a chip is possible by contactless conductivity measurement. The polymeric devices may be mass produced inexpensively by embossing, an approach not possible when using glass as substrate, or when electrodes need to be incorporated.

Acknowledgement

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References

- 1 W. R. Vandaveer, S. A. Pasas, R. S. Martin and S. M. Lunte, *Electrophoresis*, 2002, **23**, 3667.
- 2 J. Tanyanyiwa, S. Leuthardt and P. C. Hauser, *Electrophoresis*, 2002, **23**, 3659.
- 3 R. M. Guijt, E. Baltussen, G. van der Steen, R. B. M. Schasfoort, S. Schlautmann, H. A. H. Billiet, J. Frank, G. W. K. van Dedem and A. van den Berg, *Electrophoresis*, 2001, **22**, 235.
- 4 R. M. Guijt, E. Baltussen, G. van der Steen, H. Frank, H. Billiet, T. Schalkhammer, F. Laugere, M. Vellekoop, A. Berthold, L. Sarro and G. W. K. van Dedem, *Electrophoresis*, 2001, **22**, 2537.
- 5 S. Schlautmann, H. Wenskink, R. Schasfoort, M. Elwenspoek and A. van den Berg, *J. Micromech. Microeng.*, 2001, **11**, 386.
- 6 J. Lichtenberg, N. F. de Rooij and E. Verpoorte, *Electrophoresis*, 2002, **23**, 3769.
- 7 M. Galloway, W. Stryjewski, A. Henry, S. M. Ford, S. Llopis, R. L. McCarley and S. A. Soper, *Anal. Chem.*, 2002, **74**, 2407.
- 8 M. Galloway and S. A. Soper, *Electrophoresis*, 2002, **23**, 3760.
- 9 M. Zuborová, Z. Demianová, D. Kaniansky, M. Masár and B. Stanislawski, *J. Chromatogr., A.*, 2003, **990**, 179.
- 10 J. E. Prest, S. J. Baldock, P. R. Fielden and B. J. T. Brown, *Analyst*, 2001, **126**, 433.
- 11 B. Grass, D. Siepe, A. Neyer and R. Hergenröder, *Fresenius' J. Anal. Chem.*, 2001, **371**, 228.
- 12 M. Masár, D. Kaninansky, R. Bodor, M. Jöhnck and B. Stanislawski, *J. Chromatogr., A.*, 2001, **916**, 167.
- 13 A. J. Zemmann, E. Schnell, D. Volgger and G. Bonn, *Anal. Chem.*, 1998, **70**, 563.
- 14 J. A. Fracassi da Silva and C. L. do Lago, *Anal. Chem.*, 1998, **70**, 4339.
- 15 A. J. Zemmann, *TrAC, Trends Anal. Chem.*, 2001, **20**, 346.
- 16 M. Pumera, J. Wang, F. Opekar, I. Jelinek, J. Feldman, H. Löwe and S. Hardt, *Anal. Chem.*, 2002, **74**, 1968.
- 17 J. Wang, M. Pumera, G. Collins, F. Opekar and I. Jelinek, *Analyst*, 2002, **127**, 719.
- 18 J. Tanyanyiwa, B. Galliker, M. A. Schwarz and P. C. Hauser, *Analyst*, 2002, **127**, 214.
- 19 J. Tanyanyiwa and P. C. Hauser, *Electrophoresis*, 2002, **23**, 3781.
- 20 J. Tanyanyiwa, S. Leuthardt and P. C. Hauser, *J. Chromatogr., A.*, 2002, **978**, 205.
- 21 J. Tanyanyiwa and P. C. Hauser, *Anal. Chem.*, 2002, **74**, 6378.
- 22 J. Tanyanyiwa, K. Schweizer and P. C. Hauser, *Electrophoresis*, in press.

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High-voltage contactless conductivity detection of metal ions in capillary electrophoresis

The detection of alkali, alkaline earth and heavy metal ions with a high-voltage capacitively coupled contactless conductivity detector (HV-C⁴D) was investigated. Eight alkali, alkaline earth metal ions and ammonium could be separated in less than 4 min with detection limits in the order of 5×10^{-8} M. The heavy metals Mn²⁺, Pb²⁺, Cd²⁺, Fe²⁺, Zn²⁺, Co²⁺, Cu²⁺ and Ni²⁺ could also be successfully resolved with a 10 mM 2-(*N*-morpholino)ethanesulfonic acid/*D,L*-histidine (MES/His)-buffer. Zn²⁺, Co²⁺, Cu²⁺ and Ni²⁺ showed an indirect response. The detection limits for the heavy metals were determined to range from about 1 to 5 μ M.

Keywords: Capillary electrophoresis / High-voltage contactless conductivity detection / Metal ions
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1 Introduction

Conductivity detection methods are increasingly being utilized both for conventional and on-chip capillary electrophoretic methods. A change in electrolyte conductivity caused by the presence of analyte ions is detected. Conductometric methods, in which the detector electrodes are directly in contact with the running electrolyte have been widely investigated and such an instrument has been commercially available. A major disadvantage of this arrangement is detector fouling. In 1998 a novel capacitively coupled contactless conductivity detector (C⁴D) for capillary electrophoresis was introduced independently by two groups. Zemann and co-workers [1] aligned two cylindrical detector electrodes, made from steel tubes, side by side on a fused-silica capillary and demonstrated the analysis of inorganic cations and anions. Fracassi da Silva and do Lago [2] used silver varnish to create their electrodes directly on the capillary. They demonstrated the use of the detector with the direct detection of inorganic cations and indirect determination of organic cations. The electrodes each form a capacitor with the solution inside the capillary and the liquid phase between the two electrodes corresponds to a resistor which is dependent on the total electrolyte concentration. The detectors were operated with sine wave excitation

voltages of typically 200 kHz at 15–20 V peak-to-peak (V_{p-p}). Fracassi da Silva and do Lago obtained detection limits in the range of 1.1–1.6 μ M for inorganic cations. Several other research groups have since adopted the C⁴D [3–7]. Wang and co-workers [8, 9] have very recently also described a capacitively coupled conductivity detector implemented on a microfabricated planar device for electrophoretic separations. A capacitively coupled contactless conductivity detector, which can be operated with higher excitation voltages of up to 250 V_{p-p} (HV-C⁴D), was reported by our group [10]. The high excitation voltage yielded a higher detector sensitivity and improved detection limits of 0.1–0.2 μ M for small inorganic cations and anions on conventional capillaries.

The application of the contactless conductivity mode of detection have been demonstrated for alkali and alkaline earth metal ions, small inorganic anions and surfactants [4, 11, 12]. The detection of heavy metal ions has to our knowledge not been investigated. For the detection of heavy metal ions in capillaries mainly UV detection methods have been described [13–15]. This detection method owes its widespread use to its commercial availability. Transition metals and alkaline earth metal ions possess similar mobilities and thus necessitate the use of a complexing agent to achieve good separation. Various complexation methods, which include partial and complete complexation, on-column and pre-column complexation have been utilized. Carboxylic acids and hydroxycarboxylic acids such as α -hydroxyisobutyric acid, tartaric acid, lactic acid and malonic acid have been used as chelating agents. Extensive research has been carried out and reviews are available [16, 17]. In this publication, we describe the use of a second-generation HV-C⁴D to the determination of alkali, alkaline earth and heavy metal ions.

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Abbreviations: HIBA, α -hydroxyisobutyric acid; His, *D,L*-histidine; HV-C⁴D, high-voltage capacitively coupled contactless conductivity detection

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2 Materials and methods

2.1 Instrumentation

A purpose-made electrophoretic instrument was used for all measurements. It consists of a perspex box divided in two compartments, the injection cage and a detector compartment that houses the detector cell. Other components are a dual polarity high voltage power supply with ± 30 kV maximum output, function generator, excitation and detection electronics, oscilloscope, and a data acquisition system. The perspex box is fitted with a microswitch, which interrupts the power supply on opening the box. In Fig. 1 a schematic representation of the detector is given. A function generator (GFG-8019G; Goodwill Instruments, Taipei, Taiwan) was used to create a sinusoidal excitation signal, which is boosted by a high voltage amplification stage. The detector electrodes and pick-up amplifier for current to voltage conversion are encased in a small electrically grounded die-cast metal box. The 4 mm long electrodes were cut from hypodermic needles and are separated by a gap of approximately 1 mm. An internal metallic shield completely encloses the excitation part of the detector cell, except for a small hole through which the capillary is passed, in order to eliminate any direct coupling between the electrodes. The detection circuitry consists of rectifier, low pass filter and offset/gain circuitry for baseline suppression. A more detailed description of the detector cell and circuitry is given in a previous paper [10]. Alterations made to the circuitry comprise the use of a high voltage booster amplifier capable of a higher maximum output voltage of 900 V_{p-p} (PA94, supplied by Apex Microtechnology Corporation, Tucson, AZ, USA), the use of a OPA627 (Texas Instruments, Dallas, TX, USA) as the pick-up amplifier because it allows a higher output voltage (and hence better signal-to-noise ratio), and the inclusion of a two-pole low pass filter with a 3dB-frequency of 100 Hz at the output stage. For the frequency plots the OPA655 (Texas Instruments) was used as pick-up amplifier. An oscilloscope (TDS 360, supplied by Tektronix, Beaverton, OR, USA) was

employed for the visualization and measurement of wave parameters during the characterization experiments. Data acquisition was carried out with a MacLab 4e system (ADInstruments, Hastings, UK).

2.2 Reagents and methods

All chemicals were of analytical grade. Stock solutions of sodium, potassium, caesium, rubidium, magnesium, calcium, manganese and ammonium ions were prepared from their chloride salts. These, with the exception of rubidium chloride and caesium chloride, were purchased from Fluka (Buchs, Switzerland). The stock solutions of all other metal ions were prepared from acetate (Cd²⁺, Zn²⁺) and nitrate (Ni²⁺, Co²⁺, Pb²⁺, Sr²⁺, Fe³⁺, Ba²⁺) salts, respectively. Rubidium chloride, caesium chloride, lead nitrate, soluble salts of transition metals and buffer substances, 2-(*N*-morpholino)ethanesulfonic acid (MES), DL-histidine (His) and acetic acid were supplied by Merck (Darmstadt, Germany). 18-Crown-6, α -hydroxyisobutyric acid (HIBA) and oxalic acid were used as complexing agents. Dilutions were carried out in deionized water. Fresh electrolyte solutions were prepared daily before use. Analyte solutions and running buffers were degassed in an ultrasonic bath and filtered with 0.2 μ m syringe filters supplied by BGB-Analytik (Anwil, Switzerland). Fused-silica capillaries of 360 μ m outer diameter were obtained from Polymicro Technologies (Phoenix, AZ, USA). New capillaries were washed with 1 M solution sodium hydroxide, water and then flushed with running electrolyte. Otherwise preconditioning was carried out with 0.1 M sodium hydroxide on a day-to-day basis. The capillary was rinsed with buffer in between runs to improve reproducibility. All analytes were contained in the background electrolyte solution for electrokinetic injection under non-stacking conditions.

3 Results and discussion

3.1 Detector characterization

The effect of excitation voltage and frequency on detector sensitivity was investigated. For this purpose electropherograms of a cation mixture containing 20 μ M caesium, ammonium, potassium, sodium, magnesium and lithium ions were recorded and the peak heights were evaluated. At each examined voltage a frequency scan was carried out. These measurements were performed with a 820 k Ω feedback resistor on the pick-up operational amplifier in the current follower mode. In Fig. 2, the effect of excitation voltage and frequency is shown for the peak height of lithium as a representative example. The frequency plot

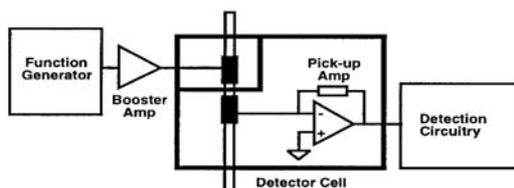


Figure 1. Sketch of the contactless conductivity detector.

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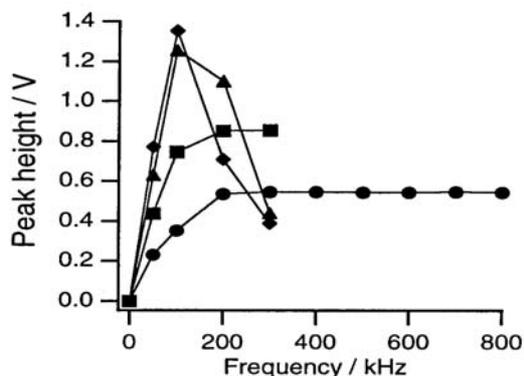


Figure 2. Peak heights for 20 μM Li^+ in dependence of the applied frequency for different peak-peak actuator voltages. (●) 100 V_{p-p} ; (■) 200 V_{p-p} ; (▲) 300 V_{p-p} ; (◆) 400 V_{p-p} . Electrolyte solution, 10 mM MES/His, 2.5 mM 18-crown-6, pH 6.0; sample injection, electrokinetically 7 s at 5.0 kV; separation potential, 25 kV; capillary, fused-silica, 75 μm ID, 74 cm length.

for a peak-to-peak voltage of 100 V shows a steady increase in peak height up to about 200 kHz and then a plateau which is almost perfectly flat up to 800 kHz, the highest frequency investigated. This feature corresponds exactly to what is expected from a simple serial arrangement of capacitor-resistor-capacitor (corresponding to a high pass filter) and from the plot the cell capacitances can be calculated as approximately 0.1 pF and the resistance as 50 $M\Omega$. Thus the electrical behavior of the cell is very straight-forward and predictable. Note, that this finding is in stark contrast to all previous reports on the frequency behavior for the C⁴D, where invariably a pronounced and broad peak is shown with a maximum somewhere between 300 and 600 kHz [2, 6–8, 18]. The explanation for this discrepancy is thought to largely stem from the fact that apparently in all cases the same type of operational amplifier was used for pick-up of the cell current (OPA606). This amplifier shows a not uncommon phenomenon known as gain peaking (*i.e.*, the gain is higher than defined by the feedback resistor at frequencies approaching the bandpass limit). The behavior is dependent on the amplifier configuration and by testing with a dummy cell was experimentally found to be particularly pronounced for the OPA606 in the arrangement used in the C⁴D-application. The frequency plots presented in Fig. 2 were therefore acquired with an amplifier (OPA655) which is classified as unity gain stable, denoting absence of the instrumental artefact.

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It can further be seen from Fig. 2, that by increasing the excitation voltage up to 300 V_{p-p} , improved peak heights were obtained. Note, that beyond 100 V_{p-p} , the generation of frequencies above 300 kHz is not possible with the amplifier employed. However, for voltages higher than 300 V_{p-p} no further gain could be achieved and the maximum achievable peak height for lithium remained approximately constant between 300 and 400 V_{p-p} . A further observation was that increasing the voltage led to achievement of maximum detector response at lower frequencies. For instance, the maximum detector response at 200 V_{p-p} was obtained with 200 kHz excitation frequency, whereas at 400 V_{p-p} it was observed at 50 kHz. The reason for the loss of effectiveness when increasing the excitation voltage beyond 300 V_{p-p} is thought to be most likely a slew rate (rate of voltage change) limitation in the system. It is not clear which part of the signal chain is causing this restriction, however, and it appears likely that the approach of using a high excitation voltage in order to gain sensitivity cannot be further extended.

3.2 Determination of alkali, alkaline earth and ammonium ions

In Fig. 3A an electropherogram of alkali, alkaline earth metal ions and ammonium at 20 μM is depicted. Cs^+ , Rb^+ , NH_4^+ , K^+ , Ca^{2+} , Na^+ , Mg^{2+} , Li^+ , Sr^{2+} and Ba^{2+} were resolved in this order and detected in less than 4 min with 10 mM MES/His buffer containing 2.5 mM 18-crown-6. The crown ether, which complexes with potassium, thereby increasing its retention time slightly, is added to achieve the resolution of ammonium and potassium ions, but affects other ions as well. Skewed peak shapes are common in capillary electrophoresis, but it is not clear why in Fig. 3A Sr^{2+} and Ba^{2+} show pronounced tailing.

In Fig. 3B an electropherogram for some of these cations at the low concentration of 1 μM is given, indicating the high sensitivity of the system. The noise visible on the baseline of the electropherogram in Fig. 3B is electronic noise, originating in the detector circuitry itself, which is normally below the resolution of data acquisition systems (typically < 100 μV). Because of the high-voltage amplification necessary in the final stage of the detector circuitries used in C⁴D, it is rendered measurable and is generally visible on all the C⁴D electropherograms published by the different workers. This means that the detection limit is directly determined by the electronic noise level in a text book fashion. The domination by this electronic baseline noise is the reason why the high voltage excitation scheme adopted leads to a better signal-to-noise ratio and therefore the improved detection limits. Generally, the earlier in the signal chain the amplification is taking place, the lower is the resulting relative noise level.

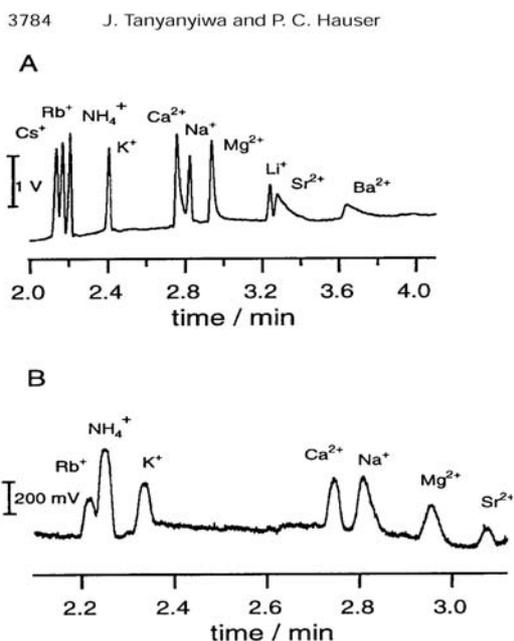


Figure 3. Alkali and alkaline earth metals at (A) 20 μM and (B) 1 μM . Conditions as in Fig. 2. Detection parameters: 300 V_{p-p} , 100 kHz.

The detection limits (the concentrations giving peak heights which are 3 times as tall as the baseline noise) for ammonium, potassium, sodium, magnesium, and lithium were determined as 4.4, 3.8, 3.1, 3.7 and 12.5×10^{-8} M at 300 V_{p-p} and 100 kHz excitation frequency. These values are approximately half an order of magnitude lower than the ones reported for these ions with the previous version of the HV-C⁴D [10] and this is the result of the overall improvements made to the detector circuitry. Note, that electrokinetic injection was used with the standards contained in the background buffer. This means that preconcentration through stacking was not taking place. In a separate experiment it was found that the peak sensitivities obtained in our procedure were similar to those obtained with pressure injection when standards in buffer were injected. When pressure injection was employed for pure standard solutions, increased peak heights were found (which must have been caused by a transient on-column stacking effect). Therefore, our detection limits must be considered somewhat conservative compared to those of other reports where other injection procedures were used.

3.3 Heavy metal analysis

MES/His buffer is most commonly used for direct conductivity detection due to its low background conductivity. Therefore, this buffer system was also investigated for its suitability in heavy metal analysis. In Fig. 4 an electropherogram of 50 μM Mn^{2+} , Pb^{2+} , Cd^{2+} , Fe^{3+} , Zn^{2+} , Co^{2+} , Cu^{2+} and Ni^{2+} is shown. In spite of similar ionic mobilities, it was possible to resolve the heavy metal ions in the absence of a deliberately added complexing agent. However, iron was found to partially precipitate at this pH value, thus the peak in the electropherogram is not representative of 50 μM . The Mn^{2+} , Pb^{2+} , Cd^{2+} and Fe^{3+} peaks are positive going whilst those of Zn^{2+} , Co^{2+} , Cu^{2+} and Ni^{2+} are negative. The background conductance of the electrolyte must be higher than that of these four ions. The ionic mobilities in water of Mn^{2+} , Pb^{2+} , Cd^{2+} , Fe^{3+} , Zn^{2+} , Co^{2+} , Cu^{2+} and Ni^{2+} ions are 5.54, 7.36, 5.60, 7.05, 5.47, 5.70, 5.56, 5.14 (10^{-8} $\text{m}^2 \text{s}^{-1} \text{V}^{-1}$), respectively (calculated from the equivalent ionic conductivities given in [19]). Clearly, the separation order does not follow the sequence of the mobilities, which must be caused by individual degrees of complexation with the buffer. This must also be the explanation for the negative going peaks. The species with the lowest mobility are of course also the ones with the lowest ionic conductivity so that the last eluting species show the negative peaks.

In Figs. 5A and B it is shown that the MES/His buffer is also suitable for the simultaneous separation of alkali, alkaline earth and heavy metals without and with 2 mM 18-crown-6 respectively. The high detector response to the smaller cations with their high conductivity is appa-

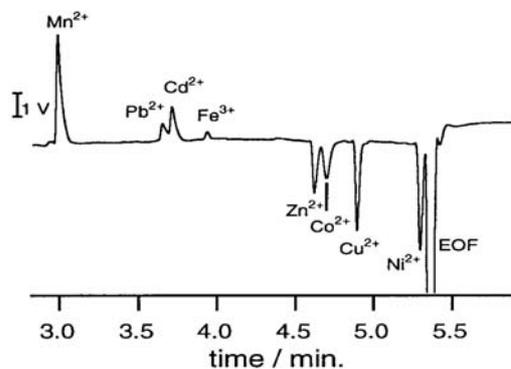


Figure 4. Electropherogram of transition metal ions at 50 μM . Conditions as in Fig. 3.

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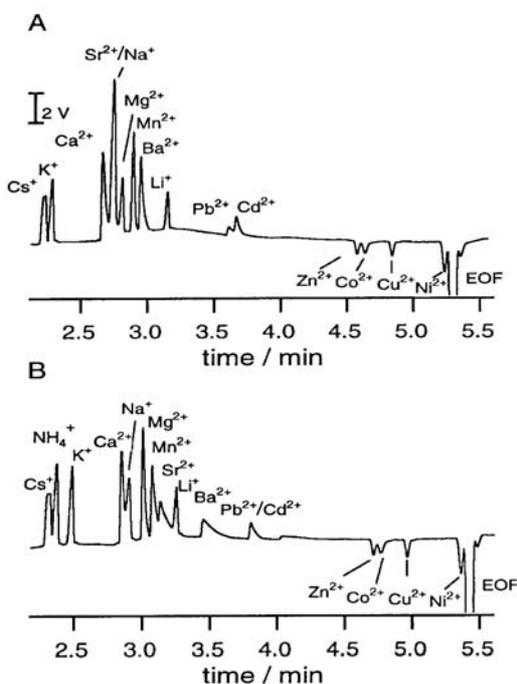


Figure 5. Simultaneous separation of alkali, alkaline earth, and transition metals at $50\ \mu\text{m}$ (A) without and (B) with 18-crown-6. Conditions as in Fig. 4.

rent. In the presence of crown ether, lead and cadmium comigrate. Note that the slightly lower crown ether concentration used here (compare with Fig. 3) was found to lead to a change in the sequence of elution of some of the alkali and earth alkali metals. Sixteen alkali, alkaline earth and heavy metal ions could be separated in less than 6 min with 10 mM MES/His buffer and 2 mM 18-crown-6.

In Fig. 6 an electropherogram of Mn^{2+} , Pb^{2+} , Cd^{2+} , Fe^{3+} , Zn^{2+} , Co^{2+} , Cu^{2+} and Ni^{2+} at $50\ \mu\text{m}$ is given. These ions were resolved in a 5 mM MES/His buffer containing 3 mM of HIBA. The small peak immediately prior to the EOF peak is thought to be due to an impurity. This buffer has a pH value of 5.3. HIBA forms weak complexes with heavy metals; it also lowers the pH of the running electrolyte. Iron does not precipitate in this electrolyte solution. Zinc and cobalt gave positive going peaks in the presence of complexing agent. The baseline was less stable,

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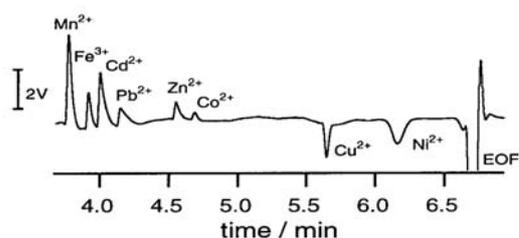


Figure 6. Electropherograms of heavy metal ions at $50\ \mu\text{m}$. Electrolyte solution, 5 mM MES/His, 3 mM HIBA pH 5.2; separation potential, 20 kV. Other conditions as in Fig. 3.

thus the separation potential was lowered to 20 kV. The effect of HIBA concentration on metal ion resolution was investigated. The peaks tailed strongly in the absence of HIBA (*i.e.*, with only 5 mM MES/His electrolyte), zinc and cobalt gave indirect peaks. With 1 and 2 mM HIBA, sharp peaks and comigration of Fe^{3+} and Cd^{2+} , Zn^{2+} and Co^{2+} were observed. 4 mM of HIBA produced an even more unstable baseline, the nickel peak did not appear in the electropherogram. The concentration of HIBA is therefore highly critical in the separation of cations. It is not clear why the inclusion of this complexing agent leads to positive going peaks for zinc and cobalt as complexation should lead to the opposite effect. Possibly this is related to the change of the pH value of the buffer system.

Calibration curves for manganese and copper as representative examples for species showing positive and negative going peaks were determined with a buffer solution consisting of 5 mM MES/His and 3 mM HIBA for the concentration range from 10 to 200 μM . The corresponding regression data was $y = 223.15x + 0.6067$ (correlation coefficient: 0.9988) for manganese and $y = -82.93x + 0.2409$ (correlation coefficient: 0.998) for copper. The detection limits were determined as 0.8 μM and 4.8 μM (at 300 V_{p-p} and 100 kHz) for Mn^{2+} and Cu^{2+} , respectively, and were obtained for nonstacking electrokinetic injection at 5 kV for 7 s. It is evident that the reduced sensitivity for the heavy metals in comparison to the lighter metal ions leads to higher detection limits. This is particularly true for copper which shows a negative going peak. Therefore, other buffer systems were investigated in the hope of finding conditions leading to lower detection limits for the heavy metals. A His/Lac-electrolyte solution was examined. 40 μM of Mn^{2+} , Cd^{2+} , Co^{2+} , Pb^{2+} , and Cu^{2+} eluted in that order with a 5 mM His/4 mM Lac (pH 5.2) electrolyte solution. The detector showed a direct response to all species except to copper. Overall the results obtained were similar to the ones with the MES/

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His-buffer. A buffer consisting of glycolic acid and oxalic acid was found to yield indirect peaks for all heavy metals but the retention times for some of the species were found to be exceedingly long (15 min).

Analysis of metal ions *via* indirect conductivity detection has also been reported by Haber and co-workers [20]. It was thought that by adopting indirect detection it might be possible to obtain negative going peaks with similar high sensitivity for all species. A buffer adapted from Haber and co-workers (20 mM acetic acid and 1 mM oxalic acid) [20] was examined for the determination of 20 μM alkali, alkaline earth, manganese, cadmium, iron, cobalt, lead, zinc, copper and nickel. All peaks were indeed found to be negative going. The alkali and alkaline earth metal ions demonstrated the highest response. However, the retention times for the heavy metals were very long and the peaks were severely broadened on arrival to the detector. Also, the high background conductivity of this electrolyte solution was found to lead to a highly unstable baseline. Indirect detection, which always requires a buffer of relatively high background conductivity, was therefore deemed not desirable in conductivity detection, at least not for this particular application.

In conclusion further improvement of the HV-C⁴D led to yet lower detection limits for alkali and alkaline earth metals. For the heavy metals the MES/His buffer appears to remain the best choice. The sensitivity obtained is not as good as that of the lighter species, precluding trace analysis in environmental applications. However, the sensitivity achieved is more than adequate for such applications as the analysis of electroplating baths.

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Electrophoresis 2002, 23, 3781–3786

4 References

- [1] Zemmann, A. J., Schnell, E., Volgger, D., Bonn, G. K., *Anal. Chem.* 1998, 70, 563–567.
- [2] Fracassi da Silva, J. A., do Lago, C., *Anal. Chem.* 1998, 70, 4339–4343.
- [3] Pumera, M., Jelinek, I., Jindrich, J., *Fresenius' J. Anal. Chem.* 2001, 369, 666–669.
- [4] Tuma, P., Opekar, F., Jelinek, I., *Electroanalysis* 2000, 13, 989–992.
- [5] Hilder, F. E., Zemmann, A. J., Macka, M., Haddad, P. R., *Electrophoresis* 2001, 22, 1273–1281.
- [6] Chvojka, T., Jelinek, I., Opekar, F., Stulik, K., *Anal. Chim. Acta* 2001, 433, 13–21.
- [7] Kuban, P., Karlberg, B., Kuban, P., Kuban, V., *J. Chromatogr. A* 2002, 964, 227–241.
- [8] Pumera, M., Wang, J., Opekar, F., Jelinek, I., Feldman, J., Löwe, H., Hardt, S., *Anal. Chem.* 2002, 74, 1968–1971.
- [9] Wang, J., Pumera, M., Collins, G., Opekar, F., Jelinek, I., *Analyst* 2002, 127, 719–723.
- [10] Tanyanyiwa, J., Galliker, B., Schwarz, M. A., Hauser, P. C., *Analyst* 2002, 127, 214–218.
- [11] Mayrhofer, K., Zemmann, A. J., Schnell, E., Bonn, G. K., *Anal. Chem.* 1999, 71, 3828–3833.
- [12] Muzikar, J., Van de Goor, T., Gas, B., Kenndler, E., *J. Chromatogr. A* 2001, 924, 147–154.
- [13] Francois, C., Morin, P., Dreux, M., *J. Chromatogr. A* 1995, 717, 393–408.
- [14] Fung, Y. S., Lau, K. M., Tung, H. S., *Talanta* 1998, 45, 619–629.
- [15] Shakulashvili, N., Faller, T., Engelhardt, H., *J. Chromatogr. A* 2000, 895, 205–212.
- [16] Janos, P., *J. Chromatogr. A* 1999, 834, 3–20.
- [17] Liu, B. F., Liu, L. B., Cheng, J. K., *J. Chromatogr. A* 1999, 834, 277–308.
- [18] Fracassi da Silva, J. A., Guzman, N., do Lago, C. L., *J. Chromatogr. A* 2001, 942, 249–258.
- [19] Lide, D. R. (Ed.), *CRC Handbook of Chemistry and Physics*, CRC Press, Boca Raton, FL 1994.
- [20] Haber, C., Jones, W. R., Soglia, J., Surve, M. A., McGlynn, M., Caplan, A., Reineck, J. R., Krstanovic, C., *J. Capil. Electrophor.* 1996, 3, 1–11.

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High-voltage contactless conductivity detection of underivatized amino acids in capillary electrophoresis

High-voltage contactless conductivity detection of underivatized amino acids in both acidic and basic media is demonstrated. The suitability of different acidic buffer solutions at pH values of about 2.5 was investigated with 12 amino acids. Lactic acid as background electrolyte gave the best results in terms of detection limits for arginine, lysine and histidine, which were approximately 2×10^{-7} , 3×10^{-7} and 4×10^{-7} M, respectively. However, the sensitivity for other species was not quite as good and the detection limits in the order of $0.5\text{--}1 \times 10^{-6}$ M. The use of basic conditions at pH 10–11 generally led to more stable baselines and more consistent sensitivities. A range of 20 amino acids was investigated with alkaline buffers and detection limits were typically about 10^{-6} M. Urine and beer samples were analyzed. Nine and eleven amino acids could be identified, respectively.

Keywords: Amino acid / Capillary electrophoresis / High-voltage contactless conductivity detection
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1 Introduction

Amino acids are the basic building blocks of many biochemically relevant species, and therefore their determination is important in clinical diagnostics and in the life sciences in general. Detection in capillary electrophoresis is hampered however, as most of the species do not contain UV-absorbing moieties nor fluorophores and are therefore not directly accessible with the commercially available UV or fluorescence detectors. Commonly, amino acids are therefore derivatized in order to render them amenable to optical detection. Reviews on the commonly used fluorophores and chromophores for the derivatization of amino acids in capillary electrophoresis analysis are available [1, 2]. These methods are usually complicated, labor-intensive, expensive and time-consuming. Detection of native amino acids may also be achieved, albeit only at moderate sensitivities, by indirect UV absorption or fluorescence methods, in which a chromophore is displaced by the analyte leading to negative going peaks [3, 4].

Electrochemical detection techniques are an alternative, but amino acids are not electroactive and thus also not suitable for direct amperometric detection, the most commonly used variant of this group of methods. An indirect amperometric method which is based on the complexa-

tion of free copper ions on the surface of a copper electrode has however been used with success and detection limits of about $1 \mu\text{M}$ were achieved [5–7]. Kappes and Hauser [8] have also demonstrated the analysis of a nine-component mixture of amino acids with potentiometric detection using a copper electrode.

Preliminary results on conductometric detection of amino acids were first reported by Huang *et al.* [9] who used a detector based on microelectrodes inserted into the capillary through laser-drilled holes. The conductivity detector has been much simplified in 1998 by Zemann [10] and Fracassi da Silva and co-workers [11] by the introduction of a contactless capacitively coupled version (commonly termed CCD, for contactless conductivity detector). In the contactless approach the electrodes are in the shape of tubes through which the capillary is passed, and besides simplification a further advantage is its inertness to electrode fouling. A number of workers have since adopted this method and demonstrated a variety of applications, see, for example, [12–20].

In work carried out in our laboratory, we have demonstrated the advantages of using elevated excitation voltages of several hundred volt for contactless conductivity detection in conventional capillaries and on electrophoresis chips (HV-CCD) [21–24]. High-excitation voltages lead to better sensitivities, improved signal-to-noise ratios and therefore lower limits of detection. It is becoming increasingly clear that the method, which is in principle universal, not only works well for the initially investigated inorganic ions but is also well suited for many organic species. To our knowledge, the application of the CCD to amino acids has not previously been assessed.

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Abbreviations: AMP, 2-amino-2-methyl-1-propanol; AMPD, 2-amino-2-methyl-1,3-propanediol; CCD, contactless conductivity detector

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2 Materials and methods

2.1 Instrumentation

Separations were carried out with a purpose-built instrument. It comprises a perspex box, function generator, CCD, and detector electronics. The perspex box is subdivided into injection and detection compartments. The detector is housed in a Faraday cage for minimization of electrical interferences. A high voltage power supply (CZE 100R; Start Spellman, Pulborough, UK) with interchangeable polarity was used for the electrophoretic separations. The electrophoresis instrumentation has been described in detail elsewhere [22]. For all measurements, a sine-wave of peak-to-peak voltage 450 V at 100 kHz was used for excitation. The electropherograms were recorded on a MacLab/4e data acquisition system (AD Instruments, Castle Hill, Australia).

2.2 Reagents and methods

All chemicals were of analytical reagent grade. Amino acids were purchased from Fluka (Buchs, Switzerland) and were used as received. Stock solutions of amino acids (0.01 M) were prepared with deionized water. The background electrolyte substances, lactic acid hydroxyisobutyric acid, glycolic acid, 2-amino-2-methyl-1-propanol (AMP), 2-amino-2-methyl-1,3-propanediol (AMPD), 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS) were also obtained from Fluka. Fresh buffer solutions and standards were prepared daily. Fused-silica capillaries of 25 μm ID and 360 μm OD were used for recording all electropherograms. These were purchased from Polymicro Technologies (Phoenix, AZ, USA) and preconditioned with a 0.1 M sodium hydroxide solution before flushing with water followed by running buffer. All capillaries had a total length of 60 cm. Sample injection was carried out electrokinetically at +5.0 kV for 7 s, the separation voltage was +25 kV. The standards were diluted in the background electrolyte solution to ensure injection under nonstacking conditions. A urine sample supplied by a healthy volunteer was diluted tenfold with buffer. The beer sample was diluted 1:5 with run buffer. Prior to injection, all solutions were ultrasonicated and filtered through 0.2 μm nylon filters.

3 Results and discussion

3.1 Determination at acidic pH values

Owing to their amphoteric nature, amino acids may be analyzed in their cationic or anionic form. This requires either relatively strong acidic or basic conditions to avoid the zwitterionic forms which are neutral overall and pre-

clude electrophoretic separation. Three acidic background electrolytes, glycolic, α -hydroxyisobutyric and lactic acid, were examined. Their suitability for separation of a selection of 12 amino acids was evaluated. The resulting electropherograms are illustrated in Fig. 1. The migration sequence is identical in all three electrolyte solutions and all amino acids exhibited an indirect response, thus the mobility of acidic electrolytes was higher than that of the analytes. The electropherograms in Fig. 1 were recorded with a capillary of 25 μm ID, which yielded the most stable of baselines. Initial measurements were carried out with capillaries of larger ID, 75 μm and 50 μm , respectively. With these, unstable, noisy backgrounds were obtained, an effect attributed to Joule heating caused by the high-mobility electrolytes. The detector response to amino acids however differed. The detector was highly sensitive to arginine, histidine and lysine; the peaks illustrated in Fig. 1 represent 10 μM as opposed to 100 μM for the other eight amino acids. The reason for the faster migration as well as the heightened sensitivity to the three species must be the fact that these contain a second protonated amine functionality. Aspartic acid is detected after the EOF peak, an indication that the second carboxylic group of this molecule is dissociated at the corresponding pH values and hence the species exists as an anion. In all three background electrolytes, valine, serine, threonine, and proline closely comigrated. Lactic acid buffer, which displayed the lowest conductance, also gave the best detection limits. The detection limits of arginine, histidine, lysine, glycine, glutamic acid, and aspartic acid were determined for the 6 mM lactic acid buffer. The results are listed in Table 1.

Klampfl *et al.* [3] investigated the effect of ion-pairing agents on the separation of some UV-absorbing underivatized amino acids under acidic conditions with direct UV detection. Concentrations as high as 30 mM octanesulfonic acid were found to be suitable for complete resolution of all analyte peaks. In Fig. 2, an electropherogram of 12 amino acids recorded with a 6 mM lactic acid buffer containing 1 mM undecanesulfonic acid is shown. The surfactant led to a reduced electroosmotic flow (EOF), presumably through the resulting increase in ionic strength, and thus improved resolution of the amino acids. However, its presence also caused an unstable baseline, a direct result of increased background conductivity. The use of higher concentrations of the sulfonate would lead to a drastic increase of background conductivity, and thus compromised detection limits.

The effect of the pH value of the buffer on the separation of amino acids was studied with the lactic acid buffer. In Fig. 3, an electropherogram of the 12 amino acids at the lower pH value of 2.36 is shown. A distinct change in the

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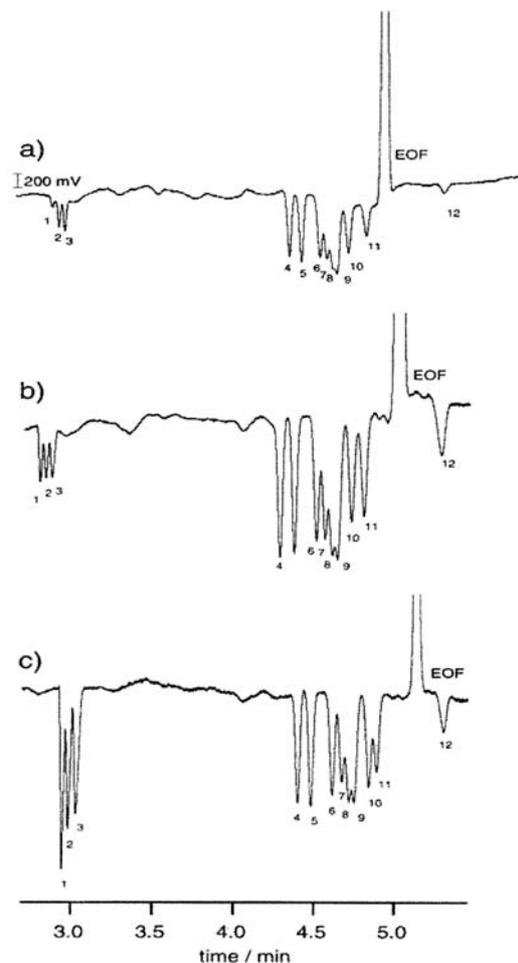


Figure 1. Determination of amino acids at low pH with different background electrolytes: (a) 6 mM α -hydroxyisobutyric acid, pH 2.8; (b) 6 mM glycolic acid, pH 2.7; (c) 6 mM lactic acid, pH 2.9. Analytes: 1, arginine; 2, histidine; 3, lysine (each 10 μ M); 4, glycine; 5, alanine; 6, valine; 7, serine; 8, threonine; 9, phenylalanine; 10, proline; 11, glutamic acid; 12, aspartic acid (each 100 μ M); Injection, electrokinetic (nonstacking conditions), 7 s at 5 kV; separation at +25 kV; capillary, length 60 cm, ID 25 μ m; detection, 450 V_{p-p} , 100 kHz.

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Table 1. Detection limits ($3 \times S/N$, in mol/L) of six amino acids in selected background electrolytes

	6 mM lactic acid	50 mM AMP	50 mM AMP, 10 mM CAPS
Arginine	0.21×10^{-6}	—	—
Histidine	0.33×10^{-6}	1.7×10^{-6}	3.3×10^{-6}
Lysine	0.38×10^{-6}	2.3×10^{-6}	8.0×10^{-6}
Glycine	5.6×10^{-6}	1.1×10^{-6}	1.5×10^{-6}
Glutamic acid	7.2×10^{-6}	0.8×10^{-6}	1.1×10^{-6}
Aspartic acid	10.5×10^{-6}	1.0×10^{-6}	1.2×10^{-6}

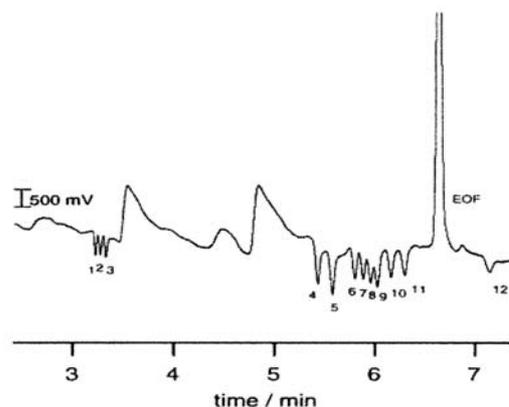


Figure 2. Effect of undecanesulfonic acid additive on the separation of amino acids under acidic conditions. Electrolyte: 6 mM lactic acid, 1 mM undecanesulfonic acid sodium salt, pH 2.9. Other conditions as for Fig. 1.

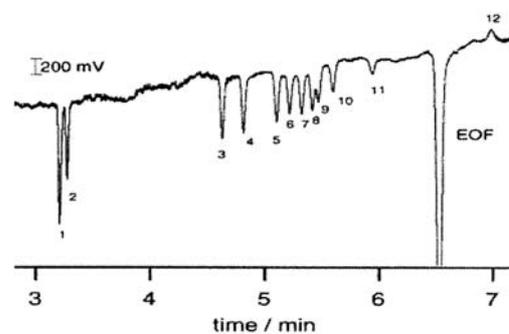


Figure 3. The effect of lowering the pH value on the resolution of amino acids. Electrolyte: 6 mM lactic acid adjusted to pH 2.4. Other conditions as for Fig. 1.

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mobility of lysine was observed (compared to its mobility in lactic acid at pH 2.76). The aspartic acid peak, which previously was negative going, displayed a direct response at pH 2.36. Valine, serine and phenylalanine were baseline-separated at this pH value. However, lowering the pH value of the electrolyte solution resulted in a corresponding increase in background conductance which compromised sensitivity. Also investigated was the suitability of 6 mM citric acid buffer for separation of amino acids at a pH value of 2.4. Complete baseline resolution of all 12 amino acids was achieved, but the baseline was found to be highly unstable.

3.2 Determination at alkaline pH values

In alkaline solutions at pH values above about 10, amino acids exist predominately as anionic deprotonated 2-aminocarboxylate ions. As these anions have relatively low electrophoretic mobilities, but at high pH values a pronounced EOF is obtained, separation may be carried out with positive applied polarity. The separation and detection of a selection of 20 amino acids at 20 μM in two alkaline buffer substances AMP and AMPD at pH values of 10.8 are shown in Figs. 4a and b. All analytes gave an indirect, negative-going response. Arginine, which has its isoelectric point at pH 10.5, comigrates with EOF at pH higher than 10.5. With AMPD as background electrolyte (Fig. 4a), three pairs of comigrating analytes were obtained: leucine and isoleucine, phenylalanine and valine, as well as methionine and glutamine. Leucine and isoleucine are very similar in structure, separation of the two was not attained with any of the buffers used. AMPD was suitable for the baseline separation of 17 amino acids. With AMP, 16 amino acids were baseline-separated, but also leucine, isoleucine, phenylalanine and valine, and methionine and glutamine comigrated. Despite the similarity of the two buffer substances, the analyte migration times were considerably shorter in the AMPD buffer than in AMP. In AMPD, the last peak, assigned to aspartic acid, was detected after 4 min, whereas in AMP it was detected after 7 min. Slight differences in the relative mobility of proline were observed. AMPD led to a somewhat better resolution and slightly higher sensitivity, but AMP yielded more symmetrical peaks.

Metal ion additives in buffers have been known to neutralize the negative charges on the capillary wall. Divalent cations as additives have been used to reduce EOF and improve resolution in previous work [4]. We investigated the effect of magnesium ions on the separation of 11 amino acids in the crowded region of Fig. 4 (peaks 5–15). The insert, Fig. 4b(ii) was recorded with a 50 mM AMP buf-

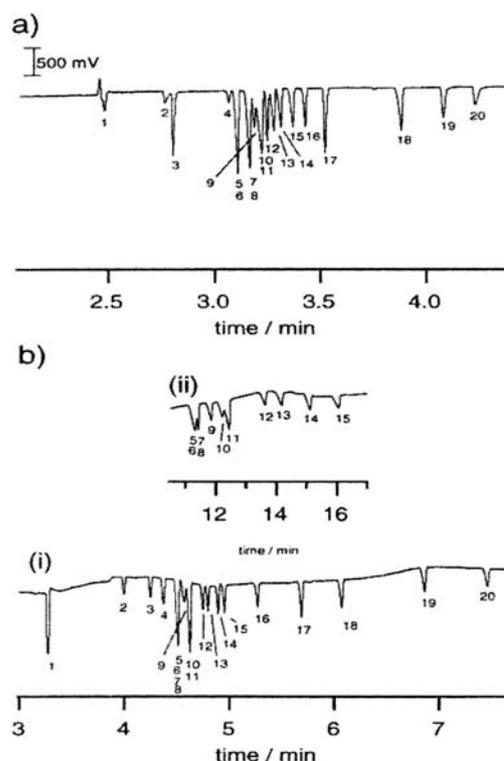


Figure 4. Indirect detection of 20 amino acids (all 20 μM) at alkaline pH. Electrolytes: (a) 50 mM AMPD, pH 10.8; (b) 50 mM AMP, pH 10.8, (bii): 50 mM AMP, 50 μM magnesium, pH 11.4. Peak assignments: 1, arginine (comigrates with EOF); 2, lysine; 3, proline; 4, tryptophan; 5, leucine; 6, isoleucine; 7, phenylalanine; 8, valine; 9, histidine; 10, methionine; 11, glutamine; 12, alanine; 13, threonine; 14, asparagines; 15, serine; 16, glycine; 17, tyrosine; 18, cystine; 19, glutamic acid; 20, aspartic acid. Other conditions as for Fig. 1.

fer containing 50 μM magnesium (first run). The migration times of all analytes more than doubled. The resolution also improved therewith. However, a continuous increase of EOF was recorded from run to run. In the third run the isoleucine and leucine peaks were detected after 20 min. All subsequent peaks were resolved but due to the increase in migration times, smaller peak heights were

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obtained. It appears that magnesium slowly neutralizes the negatively charged capillary wall. In the fifth run an unacceptable increase in migration time was observed. Lower concentrations of magnesium, while still having an effect on the EOF, were not sufficient to resolve comigrating peaks. Although a magnesium additive in AMP buffer improves resolution of amino acids, it failed to give reproducible retention times. The reason for this behavior may be precipitation of $Mg(OH)_2$, even though at the low concentration used such a product was not visible.

By modifying the AMP buffer through the addition of CAPS, positive going, direct peaks could be obtained for all species with the exception of arginine and lysine. In Fig. 5, two traces of 20 amino acids at $20 \mu M$ each

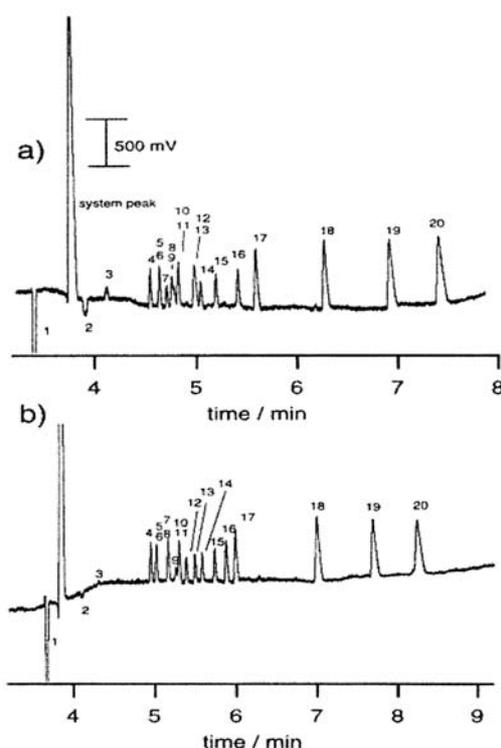


Figure 5. Direct detection of 20 amino acids ($20 \mu M$) each at alkaline pH. Electrolytes: (a) 50 mM AMP/5 mM CAPS, pH 11.1; (b) 50 mM AMP/10 mM CAPS, pH 10.8. Peak assignment as for Fig. 4. Other conditions as for Fig. 1.

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are shown. These were recorded with 50 mM AMP/5 mM CAPS and 50 mM AMP/10 mM CAPS buffers, respectively. A system peak is observed after the EOF/arginine peak. The migration times are comparable to those obtained with 50 mM AMP (Fig. 4b). The inclusion of CAPS in the AMP background electrolyte solution led to low sensitivity for lysine and proline. Increasing the concentration of CAPS from 5 mM to 10 mM also contributed to a decrease in peak heights for both lysine and proline, however, the latter buffer yielded somewhat better reproducibility. Both buffers allowed for 16 baseline-resolved peaks but there were minor changes in migration order. Detection limits for some of the species are given in Table 1. Note, that when working with the buffers of high pH values it was imperative to condition the capillaries frequently with buffer in-between runs. Failure to do so led to diminishing peak heights. Otherwise measurements were highly reproducible.

3.3 Analysis of real samples

The electropherograms of a urine sample is shown in Fig. 6. The sample was analyzed with 50 mM AMP/10 mM CAPS. Nine amino acids could be tentatively identified in the sample by spiking. No attempt was made to identify the other compounds nor to quantify the amino acids. Also analyzed was a diluted and degassed sample of a Swiss lager beer as shown in Fig. 7. Eleven amino acids could be assigned in this case by spiking with standards.

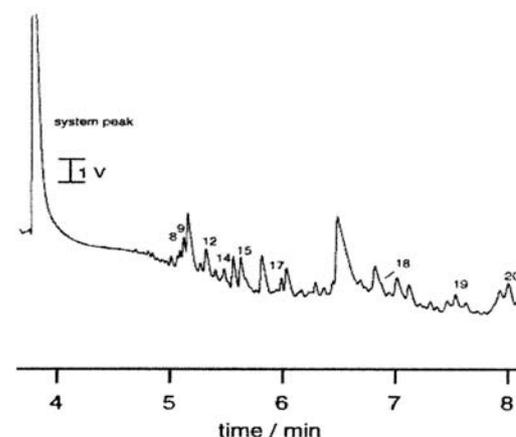


Figure 6. Analysis of a urine sample (1:10 dilution). Buffer: 50 mM AMP/10 mM CAPS, pH 10.8. Peak assignment as for Fig. 4. Other conditions as for Fig. 1.

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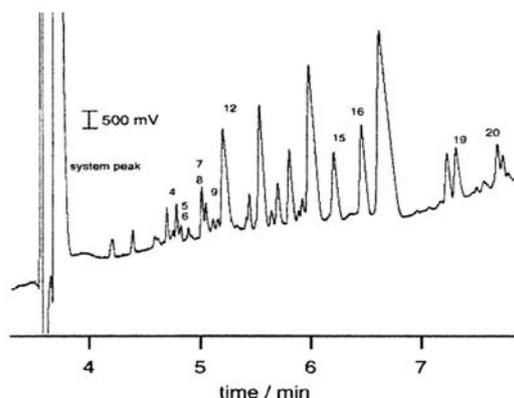


Figure 7. Analysis of a beer sample (1:5 dilution). Buffer: 50 mM AMP/10 mM CAPS, pH 10.8. Peak assignment as for Fig. 4. Other conditions as for Fig. 1.

4 Concluding remarks

It was found possible to determine amino acids by high-voltage contactless conductivity detection with good sensitivity in both acidic and alkaline buffers. The acidic electrolytes are ideal for analysis of arginine, histidine and lysine as very low detection limits were obtained for these three. However, the system was not as sensitive to glycine, alanine, valine, serine, threonine, phenylalanine, proline, glutamine, and aspartic acid. Basic conditions generally led to more stable baselines and indirect and direct detection of 20 amino acids could be achieved with more consistent sensitivity. For the majority of the amino acids lowest detection limits could be achieved using alkaline conditions but arginine requires an acidic buffer as the species is neutral at the alkaline pH value of 10.5 employed. Further optimization with regard to sensitivity and resolution may be required for specific analytical tasks at hand. The large number of unidentified species detected in the two complex samples, urine and beer, demonstrates the power and universality of the method for detection in capillary electrophoresis.

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5 References

- [1] Issaq, H. J., Chan, K. C., *Electrophoresis* 1995, 16, 467–480.
- [2] Smith, J. T., *Electrophoresis* 1999, 20, 3078–3083.
- [3] Klampfl, C. W., Buchberger, W., Turner, M., Fritz, J. S., *J. Chromatogr. A* 1998, 804, 349–355.
- [4] Lee, Y.-H., Lin, T.-I., *J. Chromatogr. A* 1994, 680, 287–297.
- [5] Guo, Y., Colon, L. A., Dadoo, R., Zare, R. N., *Electrophoresis* 1995, 16, 493–497.
- [6] Zhou, J., Lunte, S. M., *Electrophoresis* 1995, 16, 498–503.
- [7] Ye, J., Baldwin, R. P., *Anal. Chem.* 1994, 66, 2669–2674.
- [8] Kappes, T., Hauser, P. C., *J. Chromatogr. A* 1999, 834, 89–101.
- [9] Huang, X., Pang, T. J., Gordon, M. J., Zare, R. N., *Anal. Chem.* 1987, 2747–2749.
- [10] Zemmann, A. J., Schnell, E., Volgger, D., Bonn, G. K., *Anal. Chem.* 1998, 70, 563–567.
- [11] Fracassi da Silva, J. A., do Lago, C., *Anal. Chem.* 1998, 70, 4339–4343.
- [12] Kuban, P., Kuban, P., Kuban, V., *Electrophoresis* 2002, 23, 3725–3734.
- [13] Tuma, P., Opekar, F., Jelinek, I., *Electroanalysis* 2001, 13, 989–992.
- [14] Muzikar, J., Van de Goor, T., Gaš, B., Kenndler, E., *J. Chromatogr. A* 2001, 924, 147–154.
- [15] Pumera, M., Jelinek, I., Jindrich, J., *Fresenius' J. Anal. Chem.* 2001, 369, 666–669.
- [16] Chvojka, T., Jelinek, I., Opekar, F., Stulik, K., *Anal. Chim. Acta* 2001, 433, 13–21.
- [17] Hilder, F. E., Zemmann, A. J., Macka, M., Haddad, P. R., *Electrophoresis* 2001, 22, 1273–1281.
- [18] Gaš, B., Zuská, J., Coufal, P., van de Goor, T., *Electrophoresis* 2002, 23, 3520–3527.
- [19] Baltussen, E., Guijt, R. M., van der Steen, G., Laugere, F., Baltussen, S., van Dedem, G. W. K., *Electrophoresis* 2002, 23, 2888–2893.
- [20] Kuban, P., Karlberg, B., Kuban, P., Kuban, V., *J. Chromatogr. A* 2002, 964, 227–241.
- [21] Tanyanyiwa, J., Galliker, B., Schwarz, M. A., Hauser, P. C., *Analyst* 2002, 127, 214–218.
- [22] Tanyanyiwa, J., Leuthardt, S., Hauser, P. C., *J. Chromatogr. A* 2002, 978, 205–211.
- [23] Tanyanyiwa, J., Hauser, P. C., *Electrophoresis* 2002, 23, 3781–3786.
- [24] Tanyanyiwa, J., Hauser, P. C., *Anal. Chem.* 2002, 74, 6378–6382.



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Electrophoretic separations with polyether ether ketone capillaries and capacitively coupled contactless conductivity detection

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Abstract

Polyether ether ketone (PEEK) capillaries were found to be compatible with capacitively-coupled contactless-conductivity detection (C⁴D). Detection limits in the order of 10^{-7} M were obtained with C⁴D employing a high excitation voltage (HV-C⁴D) for inorganic cations and anions. The organic polymer capillary shows a relatively low electroosmotic flow of $2.6 \cdot 10^{-4}$ cm² V⁻¹ s⁻¹. Thus inorganic anions and slower organic anions can be separated with a PEEK capillary in a single run without flow modifier. This feature also enables the analysis of both, cations and (fast and slow) anions, in a sample in two subsequent runs just by reversing the polarity or in a single run if dual opposite end injection is employed. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Capillary columns; Capacitively coupled contactless conductivity detection; Conductivity detection; Detection, electrophoresis; Polyether ether ketone; Inorganic cations; Inorganic anions; Organic acids

1. Introduction

Capillary electrophoresis is commonly carried out in capillaries made from fused-silica, and it might be even argued that the commercial introduction of these capillaries enabled electrophoresis in its modern form. Fused silica capillaries however, possess charged silanol groups on the surface which causes a pronounced electroosmotic flow (EOF) in the cathodic direction. Therefore only anions with sufficiently high mobility to overcome the electroosmotic flow can be determined by migration to the anode. For anionic species with low mobility the separation in untreated fused-silica capillaries is only possible

by allowing them to be swept towards the cathode by the electroosmotic flow. The charged surface sites can also lead to unwanted interactions with the analytes (e.g. with proteins). The degree of deprotonation of the silanol groups is dependent on the pH-value and this poses a particular problem when analytes are to be determined which require basic conditions.

In order to reduce, remove or reverse the surface charge on fused-silica, different methods to modify the capillary permanently via in situ polymerization procedures have been described [1]. These modifications are fairly elaborate and therefore costly and avoided if possible. For the determination of anions, dynamic coating procedures are routinely employed in order to obtain conditions useful for a broad range of species with different mobilities. These methods rely on the inclusion of a surface active compound

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(such as a quaternary amine) in the running buffer to reverse the polarity of the surface charge and hence the direction of the electroosmotic flow.

This approach has the consequence that if in a single sample both cations and anions are to be determined an intervening change of buffer solutions in the capillary is necessary. In practice this not only requires flushing of the capillary and all containers but also a prolonged conditioning period. Several approaches have been described to address this limitation of fused-silica. These include the use of complexing agents to form negatively charged chelates with metal ions which are separated with other anions (see for example Refs. [2,3]). Bächman and coworkers achieved the concurrent detection of anions and cations by loading the sample simultaneously into two capillaries, each of which is fitted with its own detector [4]. A related, but instrumentally simpler, approach is the use of dual injection into a single capillary from both ends with a single detector located at the centre of the capillary [5–9]. Detection was carried out by indirect UV-absorption and in order to make this method suitable for more than just a few anions with the highest mobilities, it was necessary to suppress the electroosmotic flow.

Unlike fused-silica, polymer capillaries do not possess intrinsically charged surface groups. In an early publication Lukacs and Jorgenson reported that the electroosmotic flow is indeed much reduced, albeit not eliminated, in capillaries made from polytetrafluoroethylene [10]. The remaining electroosmotic flow in the polymer was attributed to the establishment of a zeta-potential by adsorption of anionic species on the surface. More recently, capillaries made from poly(methyl methacrylate) (PMMA) [11–13] and polyether ether ketone (PEEK) [13,14] and other materials [11,12] have been investigated. These capillaries also show a relatively low electroosmotic flow, but detection by the usual method of UV-absorption is impaired because of the lack of optical transparency at short wavelengths. In any case, polymeric capillaries have not been widely available and have rarely been used.

In a different development, capacitively-coupled contactless-conductivity detection (C^4D) has recently been introduced to capillary electrophoresis [15,16]. In comparison to conventional conductivity detection, the arrangement has the advantage of easier

alignment with the capillary and the inherent prevention of electrode fouling [17]. It has been shown that by the use of a high a.c. voltage (several hundred volt) for excitation (HV- C^4D), detection limits in the order of $10^{-7} M$ can be achieved for small ions [18]. Unterholzner et al. [19], as well as Kuban et al. [20] have very recently also demonstrated that contactless conductivity detection may be used for concurrent cation and anion determination when using the dual opposite end injection approach.

The performance and ease of use establishes C^4D as a serious contender to the now prevalent optical detection methods, in particular for the determination of small inorganic ions. Also, PEEK capillaries of dimensions suitable for capillary electrophoresis have recently become readily available. Lack of optical transparency is not a hindrance in the use of this material with conductivity detection. The aim of the work presented herein was therefore to investigate the performance of the combination of the HV- C^4D system with PEEK capillaries. It was hoped that it would be possible to carry out anion separations without an EOF modifier. This then would allow to use the same set of conditions for the determination of cations and anions by simply changing the polarity of the separation voltage.

2. Experimental

2.1. Instrumentation

Separations were performed on a purpose made capillary electrophoresis set-up comprising of an injection compartment made of Perspex and a detection section housed in a Faraday cage in order to minimise electrical interferences. A high-voltage power supply with reversible polarity (CZE1000R, Start Spellman, Pulborough, UK) was used for the electrophoretic separations. The detector cell is based on two steel tubes of 4 mm length which form the excitation and pick-up electrodes on the separation capillary. These are housed in a small metal case and are separated from each other by a gap of 2 mm. An internal Faraday shield is mounted between the two electrodes in order to prevent direct capacitive coupling. An operational amplifier (OPA 627, Texas Instruments, Dallas, TX, USA) in the current-to-

voltage configuration (feedback resistor: 820 k Ω) is mounted in close proximity to the pick-up electrode. The external electronics consist of a conventional stand-alone function generator and a high voltage operational amplifier (PA94, Apex Microtechnology, Tucson, AZ, USA), to obtain a sine-wave with a peak-to-peak voltage of 450 V at 100 kHz for excitation, and a synchronous detector as well as gain and offset stage at the pick-up side. The detector arrangement is a slight modification of an earlier design employed in our laboratory and more details may be found in our previous report [18].

2.2. Materials and methods

All chemicals were of analytical reagent grade and were supplied by Fluka (Buchs, Switzerland) with the exception of potassium bromide, potassium fluoride and tri-sodium phosphate, which were purchased from Merck (Darmstadt, Germany). The electrolyte solution was composed of 2-morpholinoethanesulfonic acid (MES), DL-histidine (His) in equimolar portions and 18-crown-6, which was required to resolve the ammonium and potassium peaks. This buffer was used for all separations. A fresh solution was prepared immediately before use and was used without adjustment at its natural pH value of 6. Stock solutions of the cations were prepared from their chloride salts. Those of bromide, chloride, nitrate and oxalate were prepared from potassium salts. Sulphate, acetate and phosphate stock solutions were prepared from magnesium, lithium and sodium salts respectively. Samples were diluted 1:20 in the buffer solution. Volumetric flasks made of poly(methylpentene) (PMP), supplied by Semadeni (Ostermundigen, Switzerland), were used for the preparation and dilution of working standards. The dilutions were carried out immediately before use. All solutions were degassed in an ultrasonic bath and filtered through 0.2 μm nylon filters before analysis. All capillaries were of 75 μm I.D. \times 360 μm O.D. except where stated otherwise. The lengths quoted refer to the total and the detector cell was placed approximately 5 cm from the capillary end except where stated otherwise. The fused-silica capillaries were purchased from Polymicro Technologies (Phoenix, AZ, USA). PEEK polymer capillaries were supplied by Ercatech (Berne, Switzerland), a dis-

tributor for Upchurch Scientific (Oak Harbor, WA, USA). Capillaries were preconditioned with a 0.1 M sodium hydroxide solution before flushing with deionised water followed by running buffer. All standards were contained in the background buffer in order to carry out electrokinetic injections under non-stacking conditions.

3. Results and discussion

Electropherograms for a standard cation mixture obtained in fused-silica and PEEK capillaries are shown in Fig. 1. As evidenced by the two traces, no

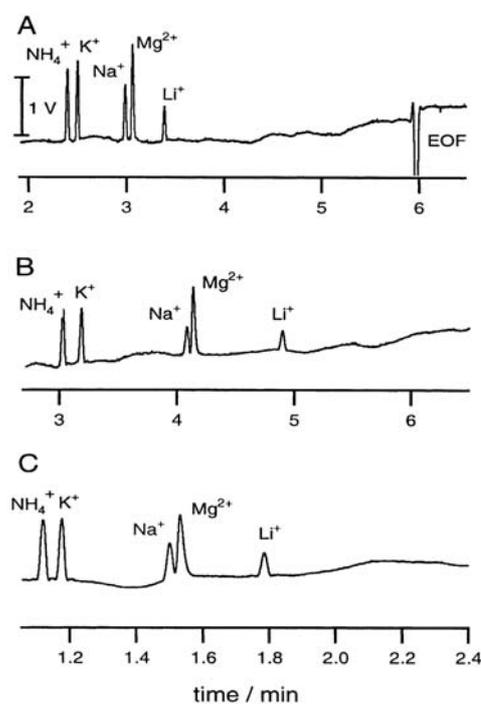


Fig. 1. Electropherograms of cations at 10 μM . (A) Fused silica 75 cm; (B) PEEK 75 cm; (C) PEEK 50 cm. Buffer: 10 mM MES/His, 1 mM 18-crown-6 (pH 6.0). Injection: electrokinetic (non-stacking conditions), 7 s at +5 kV. Separation: +25 kV.

significant differences are apparent except for the migration times. Clearly both types of capillaries are suitable for the separation of the cations and for use with contactless conductivity detection. The indirect peaks obtained for methanol as an EOF marker shows that this is due to the fact that the electroosmotic flow is indeed lower in PEEK. The values were determined to be $2.6 \cdot 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ and $5.8 \cdot 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$, for PEEK and fused-silica respectively (for the 10 mM MES/His-buffer at pH 6). In order to compensate for the effect of the reduced EOF in the PEEK capillary, the same separation was then carried out in a capillary of 50 cm length. As illustrated in Fig. 1C the ions can also be successfully resolved in the shorter capillary in less than 2 min.

It was noted that the EOF in PEEK increased by about 7% over the first 2 days of use. Constant values were obtained afterwards and the value quoted above was determined after stabilization. The peak heights and retention times were found to be highly reproducible. However, the baseline recorded with the PEEK capillary was found to be somewhat less stable than that obtained with the silica capillary. More frequent conditioning of the PEEK capillary was therefore necessary.

In Fig. 2, the electropherograms for anions separated in PEEK capillaries of different lengths are presented. A common buffer, i.e. 10 mM MES/His, 1 mM 18-crown-6, was used for the analysis of both cations and anions. Separation of anions without electroosmotic flow modifier was successful. However, as seen in Fig. 2A, the retention times were found to be fairly long for the 75 cm capillary. The shorter capillary proved to be more suitable. Separation of the same anions was accomplished within 5.5 min in the 50 cm capillary and better resolution of the nitrate and sulphate peaks was even achieved with this capillary (Fig. 2B). As evidenced by Fig. 2C the slow anions fluoride, citrate, succinate and acetate could also successfully separated with the 50 cm PEEK capillary. The EOF in PEEK was sufficiently low to enable, in particular, the determination of the acetate ion. This species cannot be detected in an unmodified silica capillary with a positive applied separation voltage since the EOF is larger and in the wrong direction. The ionic mobility of acetate has been reported as $4.2 \cdot 10^{-4} \text{ cm}^2 \text{ V}^{-1}$

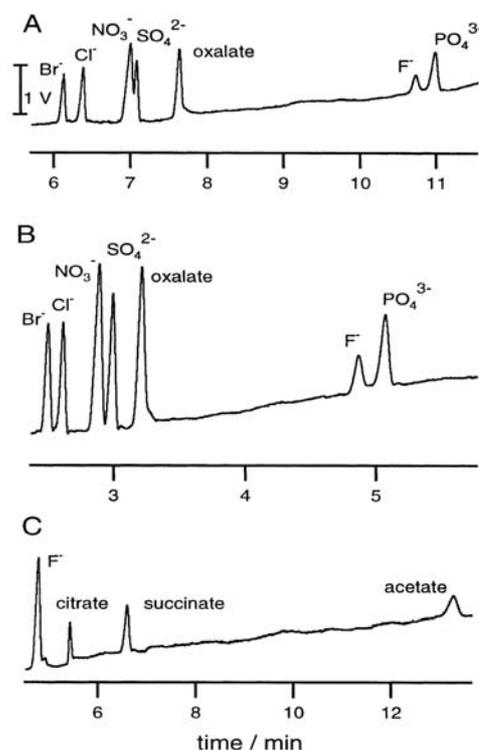


Fig. 2. Electropherograms of anions at 20 μM (A and B) or 30 μM (C) in PEEK capillaries: (A) 75 cm, (B) and (C) 50 cm. Buffer: 10 mM MES/His, 1 mM 18-crown-6 (pH 6.0). Injection: electrokinetic (non-stacking conditions), 7 s at -5 kV . Separation: -25 kV .

s^{-1} [21], but is expected to be a fraction lower under the conditions used here because of partial protonation. Note, that in fused-silica the slow anions may be determined together with the cations because the stronger EOF pushes them in the cathodic direction, but concurrent determination with the fast anions in unmodified standard capillaries is not possible.

Electropherograms of inorganic cations and anions at 1 μM recorded with the PEEK capillary are presented in Fig. 3A and B, respectively. As shown,

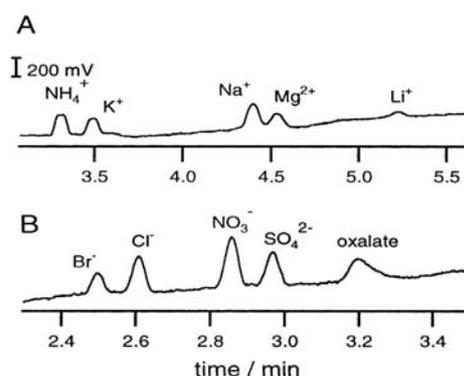


Fig. 3. Electropherograms of $1 \mu\text{M}$ cations and anions in a PEEK capillary of 50 cm. Buffer: 10 mM MES/His, 1 mM 18-crown-6 (pH 6.0). Injection: electrokinetic (non-stacking conditions), 7 s at +5 kV for cations and -5 kV for anions. Separation: +10 kV for cations and -25 kV for anions.

the detector clearly is sensitive to such low concentrations. A slight baseline drift is evident in both electropherograms and for unknown reasons was found to be more pronounced in the cation determination. As this is also dependent on the level of the applied voltage, presumably because of Joule-heating, the separation of the cations at $1 \mu\text{M}$ was carried out at the relatively low voltage of 10 kV. The regression equations for the ammonium, potassium, sodium, magnesium and lithium calibration curves were: $y = 0.2004x + 0.0295$, $y = 0.1597x + 0.1734$, $y = 0.098x + 0.2376$, $y = 0.2455x + 0.0428$ and $y = 0.075x + 0.0515$ ($y = \text{peak area}$, $x = \mu\text{M}$). The corresponding correlation coefficients were determined as 0.9963, 0.9984, 0.981, 0.9987 and 0.985, respectively. The $3 \times S/N$ detection limits for ammonium, potassium, sodium, magnesium and lithium in the 50 cm PEEK capillary were determined to be 1, 1.2, 0.73, 0.84 and $1.6 \cdot 10^{-7} \text{ M}$, respectively. For the anions, detection limits of 3.3, 1.8, 1.8, 2.1, and $5.4 \cdot 10^{-8} \text{ M}$ were obtained for bromide, chloride, nitrate, sulphate and oxalate, respectively. There also appears to be a systematic difference between the detection limits achieved for the cations and anions, which may be correlated to the baseline effect

mentioned above. The limits of detection are comparable to the values obtained with HV-C⁴D for the same ions in fused-silica [18].

As illustrated in Fig. 4, the simultaneous determination of cations and anions with dual opposite end injection is possible with a PEEK capillary by using the 10 mM MES/His buffer without alteration for EOF suppression, as is required when using fused-silica capillaries [19,20]. Note, that such a buffer modification is likely to compromise the sensitivity in conductimetric detection. It was found that PEEK capillaries are well suited for this approach and slower organic anions could be included as well. However, when using opposite end injection it is necessary to establish a position along the capillary length which yields good separation for both, anions and cations, and at the same time avoids an overlap of the interleaving cation and anion bands moving in opposite direction. Although this adjustment is readily carried out when using contactless conductivity detection, as no optical window has to be created on the capillary, it was found to be a fairly tricky and time-consuming procedure. If the method, once developed, is then used for a longer period of time for similar samples, the set-up procedure may be justified. If however, the analytical

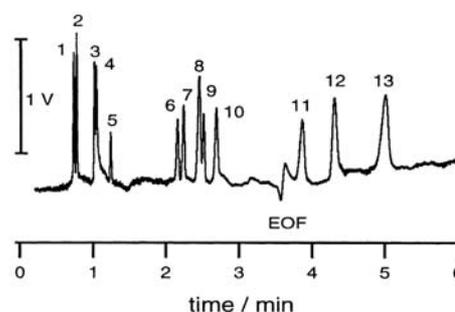


Fig. 4. Simultaneous determination of cations and anions via dual opposite end injection in a PEEK capillary. (1) NH_4^+ , (2) K^+ , (3) Na^+ , (4) Mg^{2+} , (5) Li^+ , (6) Br^- , (7) Cl^- , (8) NO_3^- , (9) SO_4^{2-} , (10) oxalate, (11) tartrate, (12) citrate and (13) succinate. Concentrations: $20 \mu\text{M}$ (except citrate: $50 \mu\text{M}$). Buffer: 10 mM MES/His, 1 mM 18-crown-6 (pH 6.0). Injection: electrokinetic (non-stacking conditions), 5 s at $\pm 5 \text{ kV}$. Separation: $\pm 25 \text{ kV}$. Capillary: 60 cm (detection 35 cm from cathode) $\times 50 \mu\text{m}$ I.D.

task changes frequently it is considered that it is more convenient to carry out the analysis in two subsequent runs. As the buffer is identical for both cations and anions, all that is required is a switch of polarity, and the additional instrumental constraint and method development effort is avoided entirely.

As an example for a potential application, the analysis of inorganic ions in a tap water sample was carried out with the single PEEK capillary and the one common buffer. Cations were analysed first. The polarity was reversed after detection of the EOF peak, followed immediately by a second injection for the determination of the anions. In Fig. 5A and B the two electropherograms for the cations and anions in the single sample are illustrated. Potassium, calcium, sodium and magnesium ions were detected within 3 min and the separation of anions took 4 min, so that the complete analysis could be carried out in approximately 8 min. The tap water sample contained approximately 120, 0.4, 4, 9, 8, 10 and 12 mg l⁻¹ of calcium, potassium, sodium, magnesium,

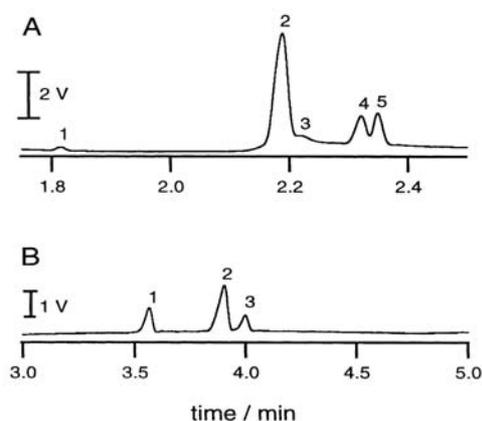


Fig. 5. Subsequent determination of cations and anions in tap water with a PEEK capillary of 60 cm. The sample was diluted 1:20 with buffer. (A) (1) K⁺, (2) Ca²⁺, (3) unknown, (4) Na⁺ and (5) Mg²⁺. (B) (1) Cl⁻, (2) NO₃⁻ and (3) SO₄²⁻. Buffer: 10 mM MES/His, 1 mM 18-crown-6 (pH 6.0). Injection: electrokinetic (non-stacking conditions), 7 s at +5 kV for cations and at -5 kV for anions. Separation: +25 kV for cations and -25 kV for anions.

chloride, nitrate and sulphate ions, respectively. The analysis of wine is perhaps somewhat more challenging and the rapid subsequent determination of cations and anions is shown in Fig. 6. The detection of the organic anions in the sample illustrates very nicely that contactless conductivity detection is useful not only for the determination of inorganic ions. A number of additional, smaller but quantifiable, peaks were detected in both the cation and anion run, but no effort was made to identify the species.

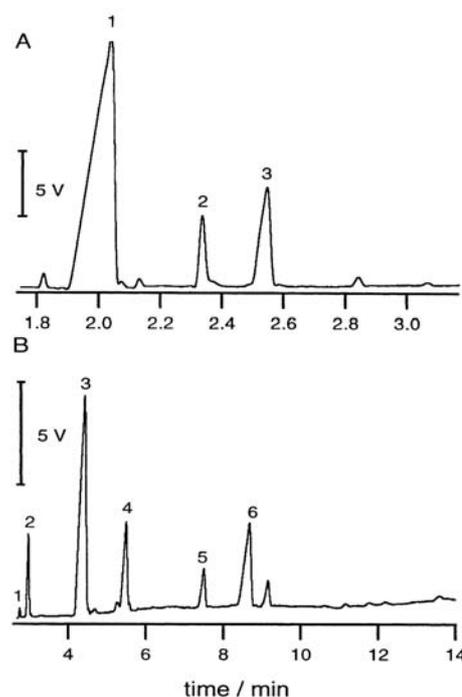


Fig. 6. Subsequent determination of cations and anions in a red wine sample with a PEEK capillary of 60 cm. The sample was diluted 1:20 with buffer. (A) (1) K⁺, (2) Ca²⁺ and (3) Na⁺. (B) (1) Cl⁻, (2) SO₄²⁻, (3) tartrate, (4) succinate, (5) acetate and (6) lactate. Buffer: 10 mM MES/His, 1 mM 18-crown-6 (pH 6.0). Injection: electrokinetic (non-stacking conditions), 5 s at +5 V for cations and at -5 kV for anions. Separation: +25 kV for cations and -25 kV for anions.

4. Conclusions

PEEK capillaries were found to be suitable for use with the enhanced capacitively-coupled contactless-conductivity detector. The EOF was found to be sufficiently low to enable the analysis of anions with intermediate mobility without having to add an EOF-modifier to the electrolyte solution. The absence of an EOF modifier and the low buffer concentration allow a low background conductivity of the buffer and hence detection limits for anions are not compromised. Detection of oppositely charged species in a single sample can be accomplished in a straightforward manner with two subsequent runs by simply switching the polarity of the separation voltage. This is considered an attractive alternative to the method of simultaneous detection by dual injection, since it does not require instrumental modifications, nor optimization with regard to detector positioning and buffer composition.

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References

- [1] J.P. Landers, *Handbook of Capillary Electrophoresis*, CRC Press, Boca Raton, FL, 1997.
- [2] T. Soga, G.A. Ross, *J. Chromatogr. A* 834 (1999) 65.
- [3] P. Kuban, P. Kuban, V. Kuban, *J. Chromatogr. A* 836 (1999) 75.
- [4] K. Bächmann, I. Haumann, T. Groh, *Fresenius J. Anal. Chem.* 343 (1992) 901.
- [5] P. Kuban, B. Karlberg, *Anal. Chem.* 70 (1998) 360.
- [6] A. Padaruskas, V. Olsauskaite, G. Schwedt, *J. Chromatogr. A* 800 (1998) 369.
- [7] A. Padaruskas, V. Olsauskaite, V. Paliulionyte, *J. Chromatogr. A* 829 (1998) 359.
- [8] D. Durkin, J.P. Foley, *Electrophoresis* 21 (2000) 1997.
- [9] I. Haumann, J. Boden, A. Mainka, U. Jegle, *J. Chromatogr. A* 895 (2000) 269.
- [10] K.D. Lukacs, J.W. Jorgenson, *J. High Resolut. Chromatogr.* 8 (1985) 407.
- [11] S. Chen, M.L. Lee, *J. Microcol. Sep.* 9 (1997) 57.
- [12] H. Bayer, H. Engelhardt, *J. Microcol. Sep.* 8 (1996) 479.
- [13] P.J. Schneider, H. Engelhardt, *J. Chromatogr. A* 802 (1998) 17.
- [14] C. Fujimoto, M. Sakurai, Y. Muranaka, *J. Microcol. Sep.* 11 (1999) 693.
- [15] A.J. Zemann, E. Schnell, D. Volgger, G.K. Bonn, *Anal. Chem.* 70 (1998) 563.
- [16] J.A. Fracassi da Silva, C.L. do Lago, *Anal. Chem.* 70 (1998) 4339.
- [17] A.J. Zemann, *Trends Anal. Chem.* 20 (2001) 346.
- [18] J. Tanyanyiwa, B. Galliker, M.A. Schwarz, P.C. Hauser, *Analyst* 127 (2002) 214.
- [19] V. Unterholzner, M. Macka, P.R. Haddad, A. Zemann, *Analyst* 127 (2002) 715.
- [20] P. Kuban, B. Karlberg, P. Kuban, V. Kuban, *J. Chromatogr. A* 964 (2002) 227.
- [21] P.W. Atkins, *Physical Chemistry*, Oxford University Press, Oxford, 1998.

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Contactless conductivity detection of selected organic ions in on-chip electrophoresis

The detection of underivatized anionic sulfonates, carboxylates, amino acids, sugars, and artificial sweeteners, and of cationic dopamine, ephedrine, and metanephrine in microfabricated electrophoresis devices is demonstrated. This was achieved by high-voltage contactless conductivity measurements with external electrodes. Poly(methyl methacrylate) chips with thin covers to enable sensitive contactless detection were used for most determinations but glass microchips had to be employed for amino acids and sugars. The plastic chips were found not to be stable in the alkaline media required to render those two classes of species in the ionic form amenable for separation and detection. The reproducibility of peak area measurements was about 1% or better and the detection limits ranged between 1 and 30 μM for the different compounds examined.

Keywords: Contactless conductivity / Electrophoresis / Miniaturization / Lab-on-chip / Organic ion
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1 Introduction

Contactless conductivity detection was introduced to standard capillary zone electrophoresis simultaneously and independently by two groups in 1998 [1, 2]. The technique relies on a pair of tubular electrodes which encompass the capillary side by side. This allows the capacitive coupling of an AC voltage into the solution contained in the capillary and the read-out of the resulting current in dependence of the conductance. The main benefits of conductometric detection in capillary electrophoresis are its universality and its simplicity. The analytes do not have to be UV-absorbing and derivatization, which is usually imperative for fluorescence detection, is not needed. Except for the electrodes, no physical parts are needed, all the other components consist of inexpensive electronic circuitry. The contactless approach features also unprecedented ease of the cell arrangement and inherent prevention of electrode fouling. For these reasons, contactless conductivity detection has been adopted since 1998 by a number of research groups for conventional capillary electrophoresis (for a recent review see [3]).

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Abbreviations: AMP, 2-amino-2-methyl propanol; DL, detection limit; MOPSO, 3-morpholino-2-hydroxypropanesulfonic acid; PMMA, poly(methyl methacrylate)

For microfabricated devices the use of conductivity detection is even more compelling since direct or indirect UV detection, the most common method for conventional capillaries, is not well suited for the chip format. In order to enable the use of the micromachined platform for analytes other than the common fluorescently tagged life-science related species, a more comprehensive universal approach is needed [4]. Conductivity detection on-chip using embedded electrodes has therefore also been studied by a number of groups [5–12].

Wang and co-workers [13–17] have shown that it is also possible to carry out conductometric detection on planar-microfabricated devices in the contactless mode by using external electrodes placed on top of the chip. This leads to a substantial simplification in the manufacturing process as embedding of electrodes is not required and bare chips as used for optical detection can be readily adopted. Tanyanyiwa and co-workers [18–21] subsequently demonstrated that in this approach the sensitivity can be greatly enhanced by using an AC voltage with an elevated peak-to-peak amplitude of several hundred volts [18], a mode previously demonstrated to be beneficial in conventional CE as well [19–21].

Most early work with conductometric detection in CE was concerned with inorganic species as these can be expected to give the most sensitive signal because of their high mobility in the electric field. Nevertheless, indications that the method may also be employed successfully for small organic species can be found in the literature. Chvojka *et al.* [22] have detected benzoate and related compounds as well as drugs in conventional capillaries

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and Muzikar *et al.* [23] investigated the determination of amines. Coufal *et al.* [24] described the determination of amino acids in an acidic electrolyte solution and Tanyanyiwa *et al.* studied the use of both acidic and alkaline buffers for these species [25]. Lopez-Avila *et al.* [26] reported the detection of haloacetic acids and Vuorinen *et al.* [27] the detection of catecholamines. Tanyanyiwa *et al.* [20] demonstrated the detection of carboxylic acids and their determination in wine. Guijt *et al.* [5] and Laugere *et al.* [8] have shown that carboxylic acids can be detected conductometrically also on microfabricated electrophoresis devices. Wang and co-workers demonstrated the detection of phenols [16], phosphonic acids [15] and amines [17] on microchips and Galloway *et al.* [9] the determination of selected amino acids in a neutral pH buffer. In our own work we found that certain anionic analgesics could be detected with good sensitivity when using the high-voltage detector [18]. This promising preliminary result prompted us to carry out this further investigation into the detection of organic ions reported on herein. The analysis of several straightforward species (aliphatic acids) was tested first, followed by species of higher practical relevance (artificial sweeteners and neurotransmitters) as well as analytes requiring conditions (high pH) which are challenging in CE (carbohydrates, amino acids). With the exception of the amino acids, these species are not readily accessible via fluorescence detection, the established detection technique for on-chip analysis, nor by UV absorption.

2 Materials and methods

2.1 Instrumentation

Glass microchips were purchased from Micralyne (model MC-BF4-TT100, Edmonton, Canada). These were modified in order to get closer access to the separation channel by milling two trenches of 1 mm width across the top of the separation channel and near its end by using a cutting wheel, attached to a high-frequency spindle. The remaining gap to the top of the channel was about 200 μm . Two thin adhesive copper strips, which functioned as electrodes were placed into these wells [18]. Poly(methyl methacrylate) (PMMA) microchips were supplied by the Microfluidic Chip Shop (Jena, Germany). The copper electrodes of 1 mm width were placed on the holder underneath the 175 μm thick bottom cover; it was not necessary to alter the chip before measurements could be carried out [28]. The separation channels on both types of chips were of identical length, *i.e.*, 8 cm. The chips were mounted on a specially made holder which features a vertical earth plane to separate the excitation and pickup electrodes. A sketch of the arrangement is given in Fig. 1. The detector circuitry comprised

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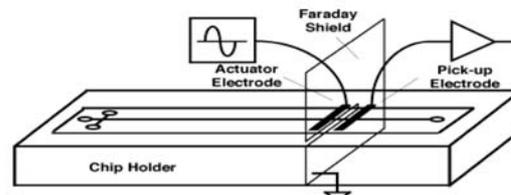


Figure 1. Sketch of the detector arrangement for the electrophoresis chip. For clarity, the clamp holding the chip in place and accessories needed for the electrophoresis are not shown.

a function generator, a high-voltage operational amplifier, a current-to-voltage converter on the pickup side, a rectifier, and offset circuitry. The electronics have been described in detail elsewhere [18]. All measurements were carried out at 400 V_{pp} and 100 kHz. Two high-voltage power supplies (model CZE 1000R; Start-Spellman, Pulborough, England) were used for injection and separation. These were controlled by a purpose-built interface connected to a multifunctional I/O card (model PCI-MIO-16XE-50; National Instruments, Austin, TX, USA) located in a personal computer. Separation parameters were set in a program written in LabVIEW (National Instruments). Data acquisition was carried out with a MacLab/4e system (AD Instruments, Castle Hill, Australia).

2.2 Reagents and methods

Ephedrine hydrochloride, acesulfame-K, sulfonic acids, mono- and dicarboxylic acids, amino acids, and sugars were purchased from Fluka (Buchs, Switzerland). Cyclamic acid was supplied by Sigma (Buchs, Switzerland). All chemicals were used as received. The following compounds were used to prepare the background electrolyte substances; 2-(*N*-morpholino)ethanesulfonic acid (MES), histidine (His) in equimolar amounts, *i.e.*, 20 mM each was used for the analysis of mono- and dicarboxylic acids, and artificial sweeteners. A 50 mM AMP (2-amino-2-methyl propanol)/10 mM CAPS (3-(cyclohexylamino)-1-propanesulfonic acid) buffer at pH 10.8 was used for the analysis of amino acids. The sulfonates were analyzed with a neutral 25 mM MOPSO (3-morpholino-2-hydroxypropanesulfonic acid)/15 mM arginine electrolyte solution. Highly alkaline electrolytes, namely sodium hydroxide and triethylamine, were used for the separation of sucrose and fructose. Citric acid was used for the analysis of ephedrine. For some analysis 100 μM CTAB (cetyltrimethylammonium bromide) was added to the buffer in order to modify the EOF. All buffer substances were supplied by Fluka. The glass chips were preconditioned with 0.1 M NaOH before

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washing with water and filling with buffer. The plastic chips were washed with water prior to measurements. Fresh buffers and standards were prepared on daily bases prior to measurements. The standards were diluted in the running buffer to ensure injection under nonstacking conditions. All solutions were degassed and filtered through 0.2 μm nylon filters before measurements and introduced into the reservoirs with hypodermic needles.

3 Results and discussion

3.1 Alkyl sulfonates

An electropherogram for a mixture of the two organic surfactants ethane sulfonate and heptane sulfonate is illustrated in Fig. 2. It was found that the PMMA chips used showed an EOF in the cathodic direction of about half the magnitude obtained in glass chips. For the separation of these anions, 100 μM CTAB was therefore included in the background buffer in order to obtain an EOF in the anionic direction and the analysis carried out in the reversed-polarity mode. The two sulfonates could be baseline-separated within 25 s. A linear calibration curve with a correlation coefficient of 0.966 was obtained for concentrations between 100 μM and 1.2 mM (correlation coefficient, $r^2 = 0.966$; detection limit, DL ($3 \times S/N$) = 6 μM).

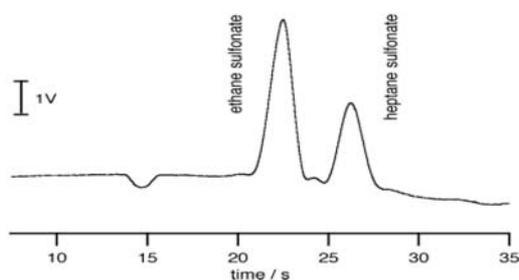


Figure 2. Electropherogram of ethane sulfonate and heptane sulfonate at 250 μM each. Buffer, 25 mM MOPSO/15 mM Arg, 100 μM CTAB (pH 7); injection, -1 kV for 3 s; separation: -3 kV.

3.2 Carboxylic acids

An electropherogram of a four-component mixture of monocarboxylic acids at 100 μM each is shown in Fig. 3. Again, dynamic coating with CTAB was employed for EOF reversal. All analytes could be baseline-resolved and separated within less than 25 s with reversed polarity. The fact that the peak heights for species at identical molar concentrations are decreasing with increasing

CCD of organic ions 905

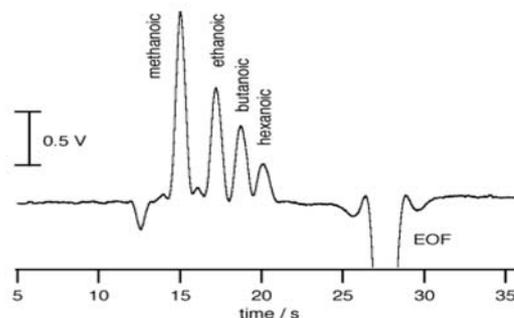


Figure 3. Electropherogram of 100 μM each of monocarboxylic acids. Buffer, 20 mM MES/His, 200 μM CTAB (pH 6); injection, -1 kV for 3 s, separation, -3.5 kV.

migration time is to be expected as both features are related. The effect of separation voltage on the separation of the monocarboxylic acids was investigated. Increasing the separation voltage from -1 kV to -5 kV led to reduced migration times and yielded sharper peaks. However, the higher voltages led to increasingly unstable baselines. The best compromise was found at a separation voltage of -3.5 kV (DLs: 3.3, 5, 10, and 30 μM for methanoic, ethanoic, butanoic, and hexanoic acid, respectively). In Fig. 4

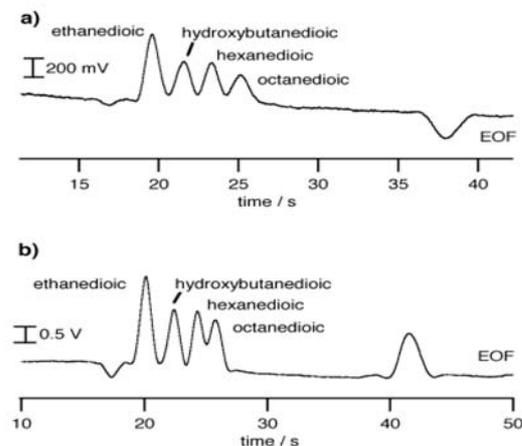


Figure 4. Electropherograms of dicarboxylic acids. (a) 50 μM (b) 200 μM . Buffer, 20 mM MES/His, 100 μM CTAB (pH 6); injection: -1 kV; separation, -3 kV.

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the electropherograms of dicarboxylates are illustrated. The two traces differ only in analyte concentration. As evident from the figure, at the higher concentrations hexanedioic and octanedioic acids are not baseline-resolved. The DLs (1.5, 2.7 and 12 μM for ethanedioic, malic and hexanedioic acids, respectively) are lower than those of the monocarboxylic acids as a result of the higher sensitivity for the doubly charged ions.

3.3 Amino acids

Because of the presence of both amino and carboxy functional groups, amino acids are amphoteric and may be determined as cations in a buffer of low pH value and as anions at high pH value. At neutral pH values only a few amino acids can be determined [9] as most species will be present as zwitterions which cannot be separated in electrophoresis. Coufal and co-workers [24] detected amino acids in an acidic buffer in conventional silica capillaries *via* contactless conductivity detection. In our own work with silica capillaries, the detection of amino acids in both acidic and basic media was investigated [25]. Under alkaline conditions generally better baseline stability and higher sensitivity was obtained. For this reason, this mode was adopted here. However, the PMMA chips employed thus far are not chemically stable at alkaline pH conditions and could only be used for a short period of time. Therefore, glass microchips were employed for the separation of the amino acids. In Fig. 5 an electropherogram of the amino acids tryptophan, glutamine and aspartic acid in a buffer of pH 10.8 is shown (DLs: 10, 7.5 and 5 μM for tryptophane, glutamic acid, and aspartic acid, respectively).

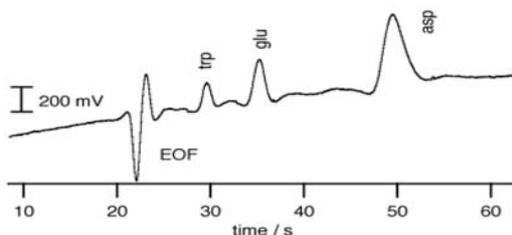


Figure 5. Electropherogram of underivatized amino acids, 100 μM each of tryptophane, glutamic acid and aspartic acid. Buffer, 50 mM AMP/10 mM CAPS (pH 10.8); injection, +1 kV for 3 s; separation, +3 kV.

3.4 Artificial sweeteners

The electropherogram of the two artificial sweeteners acesulfame-K and cyclamate is shown in Fig. 6. These species are difficult to determine in conventional CE as

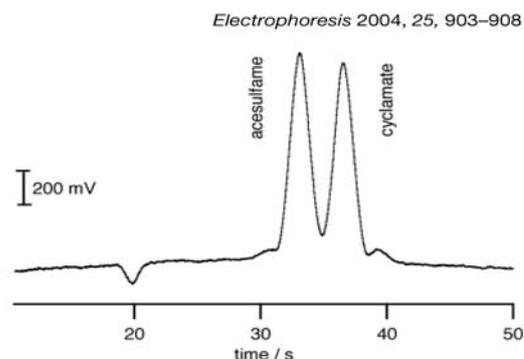


Figure 6. Electropherogram of two artificial sweeteners at 250 μM . Buffer, 20 mM MES/His, 100 μM CTAB (pH 6); injection, -1 kV for 3 s; separation, -3 kV.

the lack of UV-absorbing moieties precludes the standard UV-detection method. For this reason, potentiometric detection with a membrane electrode responding to lipophilic ions had been described [29]. Conductometric detection is another option for these species which is found here to work well on the chip platform. A calibration curve was plotted for acesulfame-K in the concentration range from 100 μM to 1 mM. Linearity was found between 200 μM and 1 mM. At concentrations below 200 μM a slight curvature was observed. The calibration equation in the linear range yielded a correlation coefficient of 0.9975. The reproducibility of the peak area was determined for 0.5 mM concentration and amounted to 1% for $n = 5$ (DL: 9 μM).

3.5 Sugars

In Fig. 7, two electropherograms of sucrose and fructose in two different background buffers are shown. Carbohydrates in general are neutral under normal conditions. The hydroxyl groups in carbohydrates have small dissociation constants and only dissociate at pH values greater than 12. Under those pH conditions they become anionic and can be separated by CE. A glass device was employed again because of the strongly alkaline medium. The first trace was recorded with a 10 mM sodium hydroxide electrolyte solution of pH 12, whilst the second was recorded with a 100 mM triethylamine buffer at identical pH value. The normal-polarity mode was used for the determination in both traces. Better sensitivity was obtained with a sodium hydroxide electrolyte; the area under each peak is larger with sodium hydroxide. However, in this solution a pronounced EOF peak is found which is not completely resolved from the sucrose peak. In both background elec-

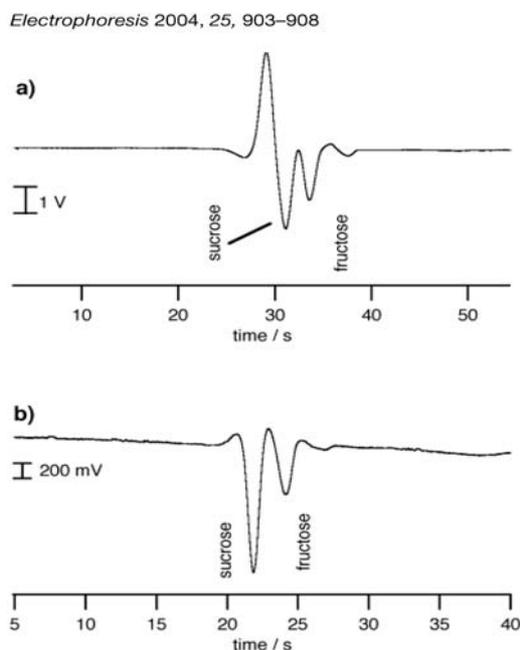


Figure 7. Electropherograms of sucrose and fructose in (a) 10 mM NaOH; (b) 100 mM triethylamine. Concentration, 500 μM each; injection, +0.5 kV for 3 s; separation, +4 kV.

tolyle solutions the sugars showed a negative going response. (DLs: 10 μM and 30 μM for sucrose and fructose, respectively.)

3.6 Pharmaceutical drug substances

An electropherogram of the basic drug ephedrine in an acidic buffer is shown in Fig. 8a. A calibration curve was acquired for concentrations ranging from 50 μM to 2 mM ($r^2 = 0.998$, DL: 4.6 μM). The lower trace (Fig. 8b) shows the electropherogram of the two catecholamines dopamine and metanephrine in a MES buffer containing 0.2% hydroxyethylcellulose (HEC). The additive leads to a reduction in the EOF and thus to a better resolution. In its absence the two analytes comigrate. A gradual increase in resolution is observed upon the addition of HEC up to a concentration of 0.2%. A further increase however had no impact on resolution, it led to increase in background noise instead.

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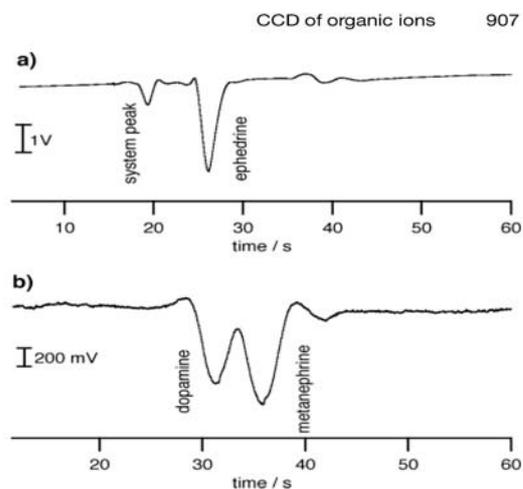


Figure 8. Electropherograms of basic drugs. Injection, +1 kV for 3 s; separation, +3 kV. (a) 250 μM ephedrine; buffer, 5 mM citric acid (pH 2.3). (b) 100 μM each of dopamine and metanephrine; buffer, 10 mM MES, 0.2% HEC.

4 Concluding remarks

High-voltage contactless conductivity measurement was found suitable for the sensitive detection of a wide range of anionic and cationic organic species on lab-on-chip devices. Note, that most of the substances chosen as examples in this study are not amenable to UV detection, which is the standard technique in conventional CE, nor to fluorescence detection, the most often used technique on chips. In comparison to conventional CE, separations on chip are much faster. On the other hand, at least in the present configuration, the peak capacity is generally lower, which will lead to a higher likelihood of selectivity problems when complex samples are to be analyzed. PMMA, the preferred material from a technological point of view because the channels can be produced more cheaply by hot embossing, was found to give a less stable EOF than glass. This leads to poorer reproducibility in migration times. Furthermore, measurements in alkaline buffers, required for sugars and amino acids, are not possible in this material.

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- [1] Zemmann, A. J., Schnell, E., Volgger, D., Bonn, G. K., *Anal. Chem.* 1998, 70, 563–567.
- [2] Fracassi da Silva, J. A., do Lago, C., *Anal. Chem.* 1998, 70, 4339–4343.
- [3] Tanyanyiwa, J., Leuthardt, S., Hauser, P. C., *Electrophoresis* 2002, 23, 3659–3666.
- [4] Schwarz, M. A., Hauser, P. C., *Lab Chip* 2001, 1, 1–6.
- [5] Guijt, R. M., Baltussen, E., van der Steen, G., Schasfoort, R. B. M., Schlautmann, S., Billiet, H. A. H., Frank, J., van Dedem, G. W. K., van den Berg, A., *Electrophoresis* 2001, 22, 235–241.
- [6] Guijt, R. M., Baltussen, E., van der Steen, G., Frank, H., Billiet, H., Schalkhammer, T., Laugere, F., Vellekoop, M., Berthold, A., Sarro, L., van Dedem, G. W. K., *Electrophoresis* 2001, 22, 2537–2541.
- [7] Berthold, A., Laugere, F., Schellevis, H., de Boer, C. R., Laros, M., Guijt, R. M., Sarro, P. M., Vellekoop, M. J., *Electrophoresis* 2002, 23, 3511–3519.
- [8] Laugere, F., Guijt, R. M., Bastemeijer, J., van der Steen, G., Berthold, A., Baltussen, E., Sarro, P., van Dedem, G. W. K., Vellekoop, M., Bossche, A., *Anal. Chem.* 2003, 75, 306–312.
- [9] Galloway, M., Stryjewski, W., Henry, A., Ford, S. M., Llopis, S., McCarley, R. L., Soper, S. A., *Anal. Chem.* 2002, 74, 2407–2415.
- [10] Zuborova, M., Masar, M., Kaniansky, D., Jöhnck, M., Stanislawski, B., *Electrophoresis* 2002, 23, 774–781.
- [11] Zuborova, M., Demianova, Z., Kaniansky, D., Masar, M., Stanislawski, B., *J. Chromatogr. A* 2003, 990, 179–188.
- [12] Lichtenberg, J., de Rooij, N. F., Verpoorte, E., *Electrophoresis* 2002, 23, 3769–3780.
- [13] Pumera, M., Wang, J., Opekar, F., Jelinek, I., Feldman, J., Loewe, H., Hardt, S., *Anal. Chem.* 2002, 74, 1968–1971.
- [14] Wang, J., Pumera, M., Collins, G., Opekar, F., Jelinek, I., *Analyst* 2002, 127, 719–723.
- [15] Wang, J., Pumera, M., Collins, G. E., Mulchandani, A., *Anal. Chem.* 2002, 74, 6121–6125.
- [16] Wang, J., Pumera, M., *Anal. Chem.* 2002, 74, 5919–5923.
- [17] Wang, J., Pumera, M., *Anal. Chem.* 2003, 75, 341–345.
- [18] Tanyanyiwa, J., Hauser, P. C., *Anal. Chem.* 2002, 74, 6378–6382.
- [19] Tanyanyiwa, J., Galliker, B., Schwarz, M. A., Hauser, P. C., *Analyst* 2002, 127, 214–218.
- [20] Tanyanyiwa, J., Leuthardt, S., Hauser, P. C., *J. Chromatogr. A* 2002, 978, 205–211.
- [21] Tanyanyiwa, J., Hauser, P. C., *Electrophoresis* 2002, 23, 3781–3786.
- [22] Chvojka, T., Jelinek, I., Opekar, F., Stulik, K., *Anal. Chim. Acta* 2001, 433, 13–21.
- [23] Muzikar, J., Van de Goor, T., Gaš, B., Kenndler, E., *J. Chromatogr. A* 2001, 924, 147–154.
- [24] Coufal, P., Zuska, J., van de Goor, T., Gaš, B., *Electrophoresis* 2003, 24, 671–677.
- [25] Tanyanyiwa, J., Schweizer, K., Hauser, P. C., *Electrophoresis* 2003, 24, 2119–2124.
- [26] Lopez-Avila, V., van de Goor, T., Gaš, B., Coufal, P., *J. Chromatogr. A* 2003, 993, 143–152.
- [27] Vuorinen, P. S., Jussila, M., Siren, H., Palonen, S., Riekkola, M.-L., *J. Chromatogr. A* 2003, 990, 45–52.
- [28] Tanyanyiwa, J., Abad-Villar, E. M., Fernández-Abedul, M. T., Costa-García, A., Hoffmann, W., Guber, A. E., Herrmann, D., Gerlach, A., Gottschlich, N., Hauser, P. C., *Analyst* 2003, 128, 1019–1022.
- [29] Schnierle, P., Kappes, T., Hauser, P. C., *Anal. Chem.* 1998, 70, 3585–3589.

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Detection of Human Immunoglobulin in Microchip and Conventional Capillary Electrophoresis with Contactless Conductivity Measurements

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The detection of human immunoglobulin M (IgM) was performed using capacitively coupled contactless conductivity detection (CCD) in electrophoresis carried out in conventional capillaries as well as on glass and poly(methyl methacrylate) (PMMA) microdevices. Also achieved was the analyses of IgG (an anti-human IgM) and the complex formed in the reaction between the two immunoreagents. It is demonstrated that CCD is a powerful tool suitable not only for the detection of antibodies but also for monitoring an immunological interaction. Conductivity measurements allow the direct determination of immunoreagents, and it is advantageous, since no labels are required. The immunoglobulin IgM has been taken as model analyte. The reproducibility of the analytical signal (RSD = 1%), sensitivity and limits of detection obtained for IgM (0.15 ng/mL in conventional capillaries and 34 ng/mL in microchips) are comparable to those previously obtained with amperometric detection. The immunological reaction was performed either in conventional microtiter plates as used in ELISA or in situ on the glass chip.

Immunoassays are very powerful techniques in clinical, pharmaceutical, and environmental chemistry because of their unique selectivity and applicability to a wide variety of compounds of interest.^{1–3} Capillary electrophoresis (CE), on the other hand, has demonstrated tremendous power in the separation of macromolecules, such as proteins, and in the characterization of antibodies. The combination, CE-based immunoassays, has emerged as a new analytical technique and has been termed capillary electrophoretic immunoassays (CEIA).⁴ It has been used for separating whole antibodies from their fragments and other impurities, in the monitoring of the process of antibody (Ab) production, and in determining the binding constant between an

antibody and its antigen (Ag). By taking advantage of the specific recognition between the two immunological reagents, it is also possible to use one of them as a sample treatment tool for enhancing the sensitivity and selectivity of analysis. Many potential applications of CEIA in both research and clinical diagnostics have been demonstrated.⁵

CEIA, like conventional immunoassays, can be performed in either the noncompetitive or the competitive format. In most cases, CE was used as a separation tool to remove the Ab–Ag complexes from free Ag or Ab after the immunoreaction was carried out off-line, but performing both the immunoreaction and sample analysis on-line is also possible.⁶

The most common detection methods employed for CEIA involve the use of labeled reagents for fluorescence⁷ or electrochemical detection.⁸ Nonlabeled detection techniques, such as UV–vis have been demonstrated;⁶ however, the major disadvantage in this case is the lack of sensitivity.

In electrophoresis, conductivity detection methods are increasingly being used not only for conventional capillaries but also for on-chip methods.^{9,10} The presence of analyte ions is detected by a change in the conductivity of the electrolyte solution. Most recent reports have been based on a contactless conductivity detector (CCD), which was introduced in its present form to standard capillary electrophoresis concurrently by Zemann and co-workers¹¹ and do Lago and co-workers¹² in 1998. This arrangement relies on external electrodes, an approach which greatly simplifies the construction of the detector and prevents its fouling. An ac-excitation voltage is applied through the capillary wall as the tubular electrode fitted on the outside forms a capacitor with the solution in the capillary. The resulting cell current is picked up with a second electrode downstream. Wang and co-workers demonstrated in 2002 that contactless conductivity detection is also possible in microfabricated electrophoresis devices simply by

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(1) Rowe, C. A.; Scruggs, S. B.; Feldstein, M. J.; Golden, J. P.; Ligler, F. S. *Anal. Chem.* 1999, 71, 433–439.

(2) Mallat, E.; Barceló, D.; Barzen, C.; Gauglitz, G.; Abuknesha, R. *Trends Anal. Chem.* 2001, 20, 124–132.

(3) Besler, M. *Trends Anal. Chem.* 2001, 20, 662–672.

(4) Schmalzing, D.; Buonocore, S.; Piggee, C. *Electrophoresis* 2000, 21, 3919–3930.

(5) Heegaard, N. H. H.; Kennedy, R. T. *J. Chromatogr., B* 2002, 768, 93–103.

(6) Little, M. K.; Crawley, C. D. *Anal. Chim. Acta* 2002, 464, 25–35.

(7) Ban, E.; Nam, H. S.; Yoo, Y. S. *J. Chromatogr., A* 2001, 924, 337–344.

(8) He, Z. H.; Jin, W. R. *Anal. Biochem.* 2003, 313, 34–40.

(9) Zemann, A. J. *Trends Anal. Chem.* 2001, 20, 346–354.

(10) Guijt, R. M.; Baltussen, E.; van der Steen, G.; Frank, H.; Billiet, H.; Schalkhammer, T.; Laugere, F.; Vellekoop, M.; Berthold, A.; Sarro, L.; van Dedem, G. W. K. *Electrophoresis* 2001, 22, 2537–2541.

(11) Zemann, A. J.; Schnell, E.; Volgger, D.; Bonn, G. K. *Anal. Chem.* 1998, 70, 563–567.

(12) Fracassi da Silva, J. A.; do Lago, C. *Anal. Chem.* 1998, 70, 4339–4343.

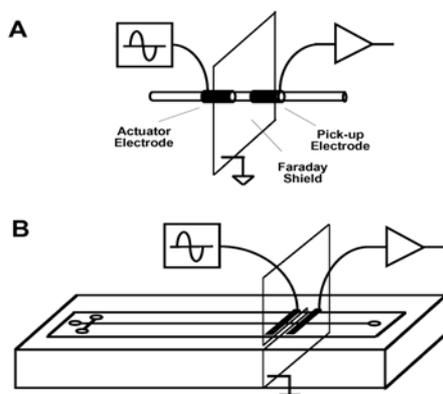


Figure 1. Schematic drawings of the cell arrangements for the conventional capillary (A) and the lab-on-chip devices (B).

placing planar electrodes externally on top of the chip.¹³ Hauser and co-workers showed that the sensitivity is enhanced for capillaries as well as chip devices by using an elevated voltage for cell excitation (high voltage-CCD, HV-CCD).^{14–18} Zemmann has recently presented a review of contactless conductivity detection,¹⁹ as have Tanayanywa et al.²⁰

In this paper, we demonstrate for the first time HV-CCD as a new and suitable detection method for CEIA. The detector has been employed for the determination of the human IgM and for the complex between IgM and anti-IgM after performing the immunological interaction in microtiter plates, as in conventional ELISA, and also on a glass microchip.

EXPERIMENTAL SECTION

Instruments. Schematic drawings of the detector arrangements for conventional capillaries and for the chip format are given in Figure 1, and further detail can be found in references 14 and 16 and 15 and 18, respectively. The detector cell for capillaries is based on two steel tubes of 4-mm length with an internal diameter of $\sim 400 \mu\text{m}$, which matches the outer diameter of the capillary ($365 \mu\text{m}$) pushed through both of these electrodes.¹⁴ A gap of 1 mm between the electrodes defines the detection volume, and a Faraday shield (earth plane) is mounted between the two electrodes in order to prevent direct capacitive coupling. Excitation is carried out by applying a sine voltage with a peak-to-peak amplitude of 300–400 V and a frequency of typically 50 or 100

kHz to the actuator electrode. The resultant cell current is picked up with the second electrode.

On the chips, the electrodes consisted of two parallel self-adhesive copper strips of 1-mm width and 5-mm length placed in a 90° angle across the microchannel and separated by a gap of 1 mm.¹⁵ When using microdevices made from glass, the electrodes were placed on the bottom of two trenches of 1-mm width milled across the top of the separation channel by using a high-frequency cutting wheel. The remaining distance from the electrodes to the top of the channel was $\sim 200 \mu\text{m}$. When using the PMMA chip, these were mounted on the holder (rather than on the chip itself) so that they came to lie directly underneath the $175\text{-}\mu\text{m}$ -thick bottom cover.¹⁸ Both chips were placed on a specially made holder, which bears a Faraday shield to separate the excitation and pick-up electrodes.¹⁵

For both platforms, a function generator (GFG-8019G; Goodwill Instruments, Tapei, Taiwan) was used to create the sinusoidal excitation signal, boosted by a high-voltage amplification stage (PA94, Apex Microtechnology Corporation, Tucson, AZ). An OPA627 (Texas Instruments, Dallas, TX) operational amplifier was used as the pick-up amplifier to convert the cell current to an ac voltage which was followed by rectification, amplification, and offset circuitry.^{14,15} Visualization of wave parameters was achieved with an oscilloscope supplied by Tektronix (TDS360, Beaverton, OR). Data acquisition was performed using a MacLab/4e system (AD Instruments, Hastings, U.K.).

The measurements on conventional capillaries were performed on a purpose-made electrophoretic instrument. It consists of a Perspex box divided into two compartments, the injection cage and the detector cell housed in a Faraday cage in order to minimize electrical interferences. The Perspex box is fitted with a microswitch, which interrupts the power supply on opening. Two power supplies (model CZE 1000R, Start-Spellman Ltd., Pulborough, England) provided the voltages for injection and separation and were controlled by a purpose-built interface connected to a multifunctional I/O card (model PCI-MIO-16XE-50, National Instruments, Austin, Texas) located in a personal computer. The voltages and time sequences were controlled with a program written in LabVIEW (National Instruments).

Both electrophoretic and detector voltages are hazardous, and appropriate precautions to avoid accidental contact must be taken.

Materials. Microtiter plates Immulon 2HB (flat bottom, high binding, polystyrene-based) were purchased from DYNEX Technologies (Chantilly, VA 20151-1683). Fused-silica capillaries of $50\text{-}\mu\text{m}$ i.d. and 50-cm length were obtained from Polymicro Technologies (Phoenix, AZ). A poly(methyl methacrylate) (PMMA) chip containing a manifold in the double-T configuration was purchased from the Microfluidic Chip Shop (Jena, Germany) and the glass microchip was from Micralyne (model MC-BF4-TT100, Edmonton, AB, Canada). The separation channel on both chips was 8 cm long. Hypodermic needles were used to introduce the buffer and solutions into the reservoirs of the microchip.

Reagents. 2-Amino-2-methyl-1,3-propanediol (AMPD) and *N*-tris(hydroxymethyl)-methyl-3-aminopropanesulfonic acid (TAPS) were supplied by Fluka (Buchs, Switzerland). Tris(hydroxymethyl)aminomethane (Tris) and Tween 20 (polyoxyethylene sorbitan monolaurate, 70% in water) were supplied by Sigma (Buchs, Switzerland). The following immunological reagents were also

(13) Pumera, M.; Wang, J.; Opekar, F.; Jelinek, I.; Feldman, J.; Loewe, H.; Hardt, S. *Anal. Chem.* **2002**, *74*, 1968–1971.

(14) Tanayanywa, J.; Galliker, B.; Schwarz, M. A.; Hauser, P. C. *Analyst* **2002**, *127*, 214–218.

(15) Tanayanywa, J.; Hauser, P. C. *Anal. Chem.* **2002**, *74*, 6378–6382.

(16) Tanayanywa, J.; Hauser, P. C. *Electrophoresis* **2002**, *23*, 3781–3786.

(17) Tanayanywa, J.; Schweizer, K.; Hauser, P. C. *Electrophoresis* **2003**, *24*, in press.

(18) Tanayanywa, J.; Abad-Villar, E. M.; Fernández-Abedul, M. T.; Costa-García, A.; Hoffmann, W.; Guber, A. E.; Herrmann, D.; Gerlach, A.; Gottschlich, N.; Hauser, P. C. *Analyst* **2003**, *128*, 1019–1022.

(19) Zemmann, A. J. *Electrophoresis* **2003**, *24*, 2125–2137.

(20) Tanayanywa, J.; Leuthardt, S.; Hauser, P. C. *Electrophoresis* **2002**, *23*, 3659–3666.

purchased from Sigma: human IgM (purified immunoglobulin, 1 mg/mL) and monoclonal anti-human IgM (IgG fraction of mouse ascites fluid, clone MB-11, 2 mg/mL). Human IgM was stored at 4 °C, and working aliquots of anti-IgM were frozen (-20 °C). Dilutions were made with 0.1 M Tris/HCl buffer, pH 7.4, to perform the immunological interaction and with 20 mM TAPS/AMPD buffer, pH 8.7, containing 0.01% Tween 20 for the detection in the capillary and 0.05% for the PMMA chip. Water was purified employing a Milli-Q Plus 185 system from Millipore (Bedford, MA). Fresh buffer solutions were prepared daily before use, degassed in an ultrasonic bath, and filtered with 0.2- μ m syringe filters supplied by BGB-Analytik (Anwil, Switzerland).

Procedures. IgM Detection. Capillaries were washed with 0.1 M sodium hydroxide and water and then flushed with running buffer prior to measurements. The chip was also preconditioned with 0.1 M sodium hydroxide before thoroughly rinsing with water and subsequently flushing with buffer. For the direct determination of IgM, aliquots of different concentrations (varying from 0.05 to 5 ng/mL in the capillary and from 10 to 500 ng/mL in the chip) were diluted with 20 mM TAPS/AMPD buffer, pH 8.7, containing 0.01% Tween 20 for the capillary and 0.05% for the PMMA chip. Electrokinetic injections were performed at 5.7 kV for 5 s in the capillary and at 1 kV for 3 s for the chip. Separations were achieved at 20 and 4 kV, respectively. Washing steps with buffer between runs ensured reproducibility and maintained a stable baseline. After use, the capillary and the chip were rinsed with water to prevent clogging of the channel.

Immunoassay. When the immunoassay was carried out in conventional ELISA microtiter plates, the first step was the incubation with 200 μ L of a 2% BSA solution overnight. After washing thoroughly with 0.1 M Tris/HCl buffer, pH 7.4, 100 μ L of an IgM solution (with concentrations ranging from 0.5 to 2 μ g/mL) and 100 μ L of IgG (10 μ g/mL) was placed in each well and allowed to react for 15 min. A 50- μ L portion of the contents of each well was then placed in the reservoir of the capillary system and diluted with 20 mM TAPS/AMPD buffer, pH 8.7, containing 0.01% Tween 20. When using the chip, 25 μ L of the contents of each well was diluted to 1000 μ L with the same buffer but containing 0.05% of Tween 20, and this solution was placed in the microchip reservoir. Electrokinetic injection was performed at 5.7 kV for 5 s in the capillary and at 1 kV for 3 s in the PMMA chip. Separations were achieved at 20 and 4 kV, respectively.

When the immunoassay was performed inside the glass chip, this device was preconditioned with 0.1 M sodium hydroxide before being thoroughly rinsed with water and subsequently flushed with 20 mM TAPS/AMPD buffer, pH 8.7, containing 0.01% Tween 20. One of the reservoirs was then filled with a 10 μ g/mL IgM solution. Injection was performed by applying a voltage of 2 kV for 5 s. The contents of this reservoir was removed and replaced by a solution of IgG (10 μ g/mL). Again, 2 kV was applied for 5 s. Separation of the mixture along the channel was achieved by applying 4 kV.

RESULTS AND DISCUSSION

Selection of Conditions for CE. Knowledge of the isoelectric point (pI) of the compounds to be examined is very important in determining the optimum conditions for an electrophoretic separation of macromolecules, such as immunoglobulins.²¹ In the case

of IgM, the pI lies between 5.1 and 7.8, and for IgG, between 5.8 and 7.3.²² Thus, a buffer should be chosen with a pH value which provides maximum separation without destroying the properties of the sample.

A further consideration in the analysis of proteins is wall-adsorption, which can present a problem in capillary electrophoresis. The analytes may be retarded in their migration through adsorption which also causes band-broadening. There are different options to overcome this problem. One is to reduce the pH value of the running buffer to a value higher than the pI of the antibody, which results in minimum interaction between protein and capillary surface because both would be negatively charged. In this approach, a buffer with pH above 8 should be used. A further aid in controlling and preventing protein adsorption is the coating of the surface, for simplicity, preferentially in a dynamic rather than a permanent approach with a surfactant (such as Tween 20). Such substances are commonly present in immunological reagent preparations and do not negatively affect the reagents or the immunoreaction.

Since Tris buffer had been successfully used before for IgM determination,^{23,24} First tests were carried out with 0.1 M Tris (natural pH 10.6) containing 0.01% of Tween 20, which was also found to exhibit low conductivity, a desirable feature when using conductometric detection. However, the electropherograms with this buffer showed several not understood system peaks after the EOF (the region of interest), so the immunoglobulins could, in fact, not be analyzed using this buffer (data not shown). AMPD is a zwitterionic buffer employed normally for the electrophoretic separation of polypeptides and macromolecules. For a 20 mM TAPS/AMPD buffer, the natural pH value is 8.7, which is also appropriate, and this electrolyte solution was indeed found suitable because it also showed low conductivity and none of the interfering system peaks, even on inclusion of the surfactant Tween 20.

Determination of IgM. IgM was chosen as the model analyte because it possesses important significance from a clinical point of view.^{25,26} It predominates in some primary viral infections and bloodstream infections, such as malaria. Abnormal levels of human IgM indicate the presence or the stage of an infection, and its level is markedly increased at birth and in intrauterine infections as well as in all phases of HIV infection. The interaction between this immunoglobulin and IgG (anti IgM) was also taken as a model and will be described in more detail in the subsequent section.

The detection of IgM was performed using the TAPS/AMPD buffer, including 0.01% Tween 20. In Figure 2, the electrophero-

(21) Kaplan, L. A.; Pesce, A. J.; Kazmierczak, S. C. *Clinical Chemistry: Theory, Analysis, Correlation*, 4th ed.; Mosby: St. Louis, 2003.

(22) www.ncbi.nlm.nih.gov.

(23) Abad-Villar, E. M.; Fernandez-Abedul, M. T.; Costa-Garcia, A. *Biosens. Bioelectron.* **2002**, *17*, 797–802.

(24) Abad-Villar, E. M.; Fernandez-Abedul, M. T.; Costa-Garcia, A. *Anal. Bioanal. Chem.* **2003**, *377*, 267–272.

(25) Burtis, C. A.; Ashwood, W. R. *The Tietz Textbook of Clinical Chemistry*; Saunders: Philadelphia, 1994.

(26) Mellanen, L.; Sorsa, T.; Lahdevirta, J.; Helenius, M.; Kari, K.; Meurman, J. *H. J. Oral Pathol. Med.* **2001**, *30*, 553–559.

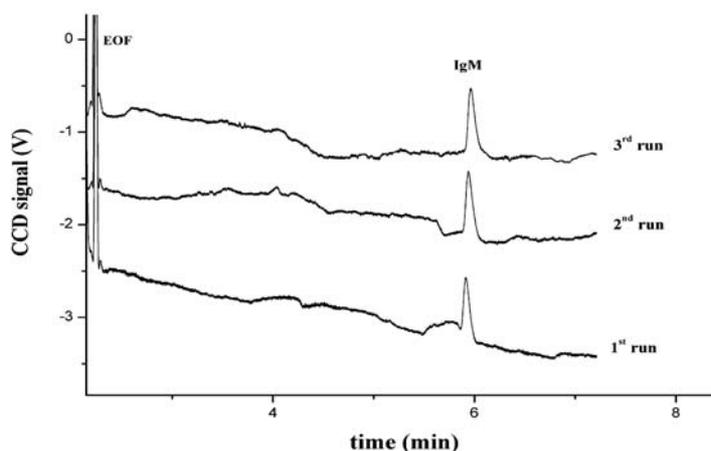


Figure 2. Successive electropherograms for 0.05 ng/mL of IgM obtained with the conventional capillary. Buffer 20 mM TAPS/AMPD containing 0.01% of Tween 20; injection 5.7 kV for 5 s; actuator voltage 300 V_{p-p} , frequency 100 kHz; separation voltage 20 kV, capillary 50- μ m i.d., and 50-cm length.

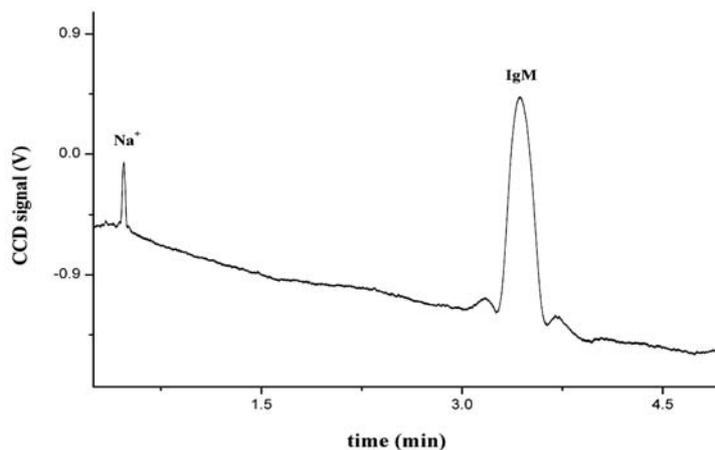


Figure 3. Electropherogram for 100 ng/mL IgM obtained with the PMMA chip. Buffer 20 mM TAPS/AMPD containing 0.05% of Tween 20; injection 1 kV for 3 s; actuator voltage 400 V_{p-p} , frequency 100 kHz; separation voltage 4 kV, 8-cm length.

grams for three successive injections of 0.05 ng/mL IgM are presented. Considering the peak area, the relative standard deviation (RSD) for these successive injections was 1%. Reconditioning the capillary with 20 mM TAPS/AMPD buffer pH 8.7 containing 0.01% of Tween 20 between runs was essential for reproducible results. A calibration curve was acquired under these conditions in order to determine the dynamic range of the assay. Concentrations of IgM between 0.05 and 5 ng/mL were analyzed. The peak area was found to increase linearly until it reached a plateau at 1 ng/mL. The calibration curve was linear between 0.05 and 1 ng/mL according to the following equation: peak area (Vs) = 5.7 [IgM] (ng/mL) + 1.4 ($r = 0.997$, $n = 3$). The limit of

detection, calculated as the concentration corresponding to three times the standard deviation of the intercept, was determined as 0.15 ng/mL of human IgM. This value is comparable to results obtained for enzyme-labeled immunoassays for IgM employing amperometric detection in a flow system as reported previously.^{23,24}

Also investigated was the detection of IgM on an electrophoresis-chip made from PMMA. In this case, the TAPS/AMPD buffer contained a higher level of Tween 20 (0.05%) in order to better reduce adsorption of proteins on the walls of the plastic device. Washing steps with buffer between runs were essential to warrant reproducibility. A representative electropherogram obtained for

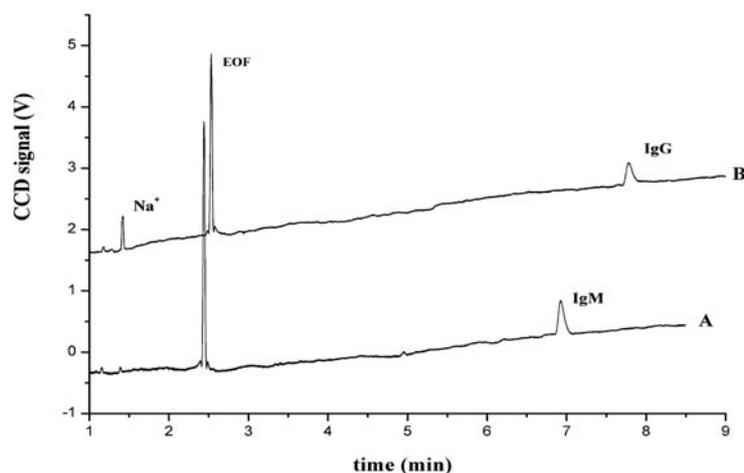


Figure 4. Electropherograms obtained with the conventional capillary for 0.05 ng/mL IgM (A) and 0.5 $\mu\text{g/mL}$ IgG (B) in 20mM TAPS/AMPD buffer of pH 8.7, containing 0.01% of Tween 20. Conditions as for Figure 2.

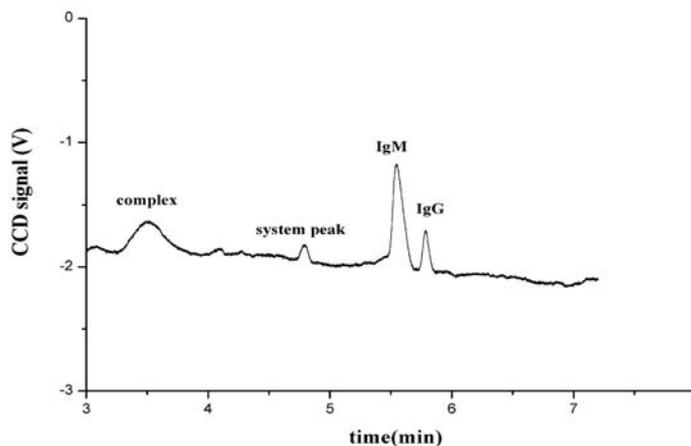


Figure 5. Electropherogram obtained with the conventional capillary for a mixture of 10 $\mu\text{g/mL}$ IgG and 10 $\mu\text{g/mL}$ IgM after 15 min of immunological reaction in 0.1 M Tris buffer, pH 7.4. Conditions as for Figure 2.

an IgM concentration of 100 ng/mL is shown in Figure 3. Because IgM is provided in a solution containing sodium azide as a preservative, its cation was observed early in the electropherogram. Compared to the results on the standard capillary (Figure 2), the analysis time was reduced almost to one-half. A linear calibration curve was obtained for concentrations from 10 to 500 ng/mL (peak area (Vs) = 0.15 [IgM] (ng/mL) + 3.07, $r = 0.998$, $n = 4$). The limit of detection calculated in the same way as before was 34 ng/mL. The higher value, in comparison to the conventional capillary, was mainly caused by a loss of sensitivity, as evidenced by the lower slope factor in the regression equation. Even though the sensitivity of our detector in the chip configuration is one of the best reported for conductometric detection in

any arrangement (i.e., also in comparison to buried contacted as well as buried contactless electrodes), the difference in sensitivity between the two formats is evident for all classes of ions.²⁰ This is thought to be due to the less efficient cell arrangement in comparison to the conventional capillary. The electrodes cannot fully encompass the channel in the chip, as is the case with the tubular electrodes used on the capillary.

Immunoassay. To further investigate the usefulness of the detection approach, the immunological interaction between IgM and IgG (anti IgM) was then studied in the CEIA-format. Initially, each antibody was evaluated separately. In Figure 4 the electropherograms obtained for separate solutions of 0.05 ng/mL IgM (Figure 4A) and 0.5 $\mu\text{g/mL}$ IgG (Figure 4B) are shown. As seen,

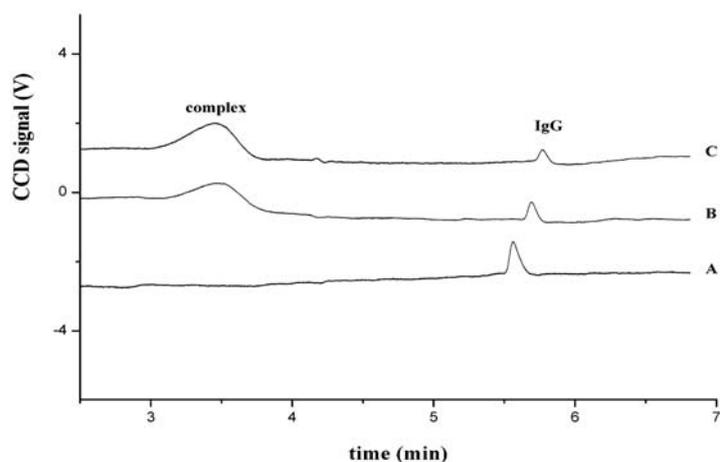


Figure 6. Electropherograms obtained with the conventional capillary for 10 $\mu\text{g/mL}$ IgG (A) and mixtures of 10 $\mu\text{g/mL}$ IgG with 0.5 $\mu\text{g/mL}$ IgM (B) and with 2 $\mu\text{g/mL}$ IgM (C). Conditions as for Figure 2.

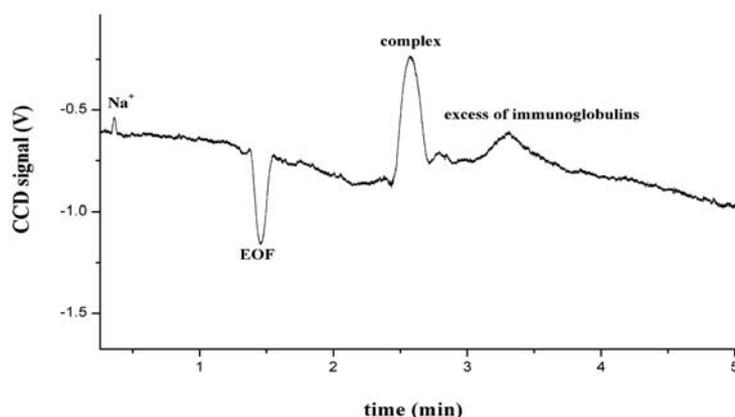


Figure 7. Electropherogram obtained with the PMMA chip for a mixture of 10 $\mu\text{g/mL}$ IgG and 10 $\mu\text{g/mL}$ IgM after reaction for 15 min in the 0.1 M Tris buffer, pH 7.4. Conditions as for Figure 3.

IgM and IgG possess different migrations times. The electropherogram obtained for a mixture of the two species is shown in Figure 5. A 100- μL portion of 10 $\mu\text{g/mL}$ IgG and 100 μL of 10 $\mu\text{g/mL}$ IgM were mixed in a conventional microtiter plate as used for ELISA, and after 15 min were diluted with the 20 mM TAPS/AMPD buffer containing 0.01% of Tween 20 and injected into the capillary. A new peak due to the complex formed in the immunoreaction appears early in the electropherogram while still unreacted IgM and IgG are clearly identifiable.

When the same concentration of IgG (10 $\mu\text{g/mL}$) is incubated with lower concentrations of IgM (0.5 and 2 $\mu\text{g/mL}$) the behavior was found to be slightly different, as illustrated in Figure 6. The peak in the electropherogram of Figure 6A corresponds to the injection of a solution of 10 $\mu\text{g/mL}$ IgG alone and serves as a reference. On the addition of 0.5 $\mu\text{g/mL}$ IgM, the complex is

formed, as seen in Figure 6B, but unreacted IgM is not detected. When increasing the concentration of IgM to 2 $\mu\text{g/mL}$, the peak for the complex gains in area, as evidenced in trace C of the figure. Thus, the higher the concentration of IgM incubated was, the higher the resulting peak area for the complex and also the lower the area for the IgG peak area.

The products of the immunological reaction between IgG and IgM were also analyzed on the PMMA chip. Again, 100 μL of 10 $\mu\text{g/mL}$ IgG and 100 μL of 10 $\mu\text{g/mL}$ IgM were mixed and separated after 15 min. The result is shown in Figure 7. The peak of the complex arrives at the detector in ~ 2 min, and an unresolved mixture of free immunoglobulins appears just after. Clearly, a reduction in analysis time results from the use of the planar device, as compared to the standard capillary. However, the lower sensitivity found in this chip made it difficult to evaluate

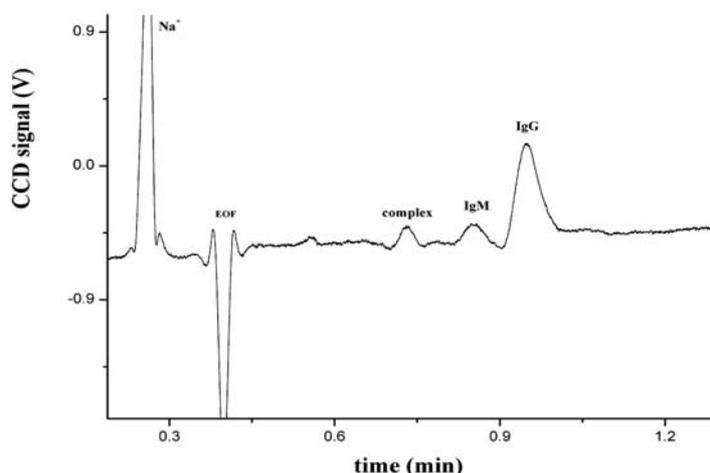


Figure 8. Electropherogram obtained with the glass chip for 10 $\mu\text{g/mL}$ IgG and 10 $\mu\text{g/mL}$ IgM in 20 mM TAPS/AMPD buffer containing 0.01% of Tween 20; injection 1 kV for 5 s; actuator voltage 400 V_{p-p} , frequency 50 kHz; separation potential 4 kV, 8-cm length.

mixtures of lower concentrations as reported above for the conventional capillary. In addition, it was found that the EOF is not very stable on plastic devices, as has been reported previously,²⁷ presumably due to unspecified adsorption effects.

Glass chips should give performance similar to that of fused-silica capillaries; thus, the study of the immunological reaction was also carried out on such a device. In this case, the reaction was performed on the chip itself using the access hole on one of the short sidearms as reaction vessel. Initially, a 0.1 M Tris/HCl buffer of pH 7.4 containing 0.01% of Tween 20 was placed there for the reaction of the antibodies while the separation channel was filled with the 20 mM TAPS/AMPD buffer of pH 8.7 containing 0.01% of Tween 20. However, the combination of both buffers on the chip negatively affected the stability of the baseline. Therefore, the 20 mM TAPS/AMPD buffer pH 8.7 containing 0.01% of Tween 20 was also employed in the reaction compartment. The immunoassay was performed on the glass chip, as described in the Immunoassay portion of the Experimental Section. In Figure 8, a representative electropherogram is shown. The peaks for complex and free immunoglobulins are clearly distinguished, as compared to the results with the PMMA chip. In addition, the analysis time was reduced to just under a minute, and the baseline was found to be more stable. The findings regarding the difference

in performance in different materials incidentally corroborate well the results obtained by Lunte and co-workers, who compared polymeric chips made from poly(dimethylsiloxane) (PDMS) and chips made from glass in the separation of peptides.²⁸

CONCLUSIONS

The demand for the analytical determination of large biomolecules is steadily growing. The present need for optical labeling to obtain acceptable detection levels may be considered an obstacle. However, the use of a sensitive detector based on a universal principle, such as conductivity, in combination with electrophoretic separation allows the development of direct determination methods and immunoassays without labeling molecules. IgM has been presented as a model analyte, and the good detection limits obtained using a conventional capillary (0.15 ng/mL) or a chip (34 ng/mL) demonstrate a promising future of this approach.

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AC0346656

(27) Bianchi, F.; Wagner, F.; Hoffmann, P.; Girault, H. H. *Anal. Chem.* **2001**, *73*, 829–836.

(28) Lacher, N. A.; de Rooij, N. F.; Verpoorte, E.; Lunte, S. M. *J. Chromatogr., A* **2003**, *1004*, 225–235.

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Capillary and microchip electrophoresis of basic drugs with contactless conductivity detection

The extension of contactless conductivity detection in electrophoresis to the determination of basic drugs is demonstrated using β -adrenergic blocking agents (β -blockers) and other physiologically active amines as examples. The high-voltage approach to conductivity detection was employed for conventional capillaries as well as microchip devices. Acidic buffers were used in all cases. A buffer consisting of 100 mM acetic acid and 1 mM histidine was deemed most optimal for the separation of six β -blockers and best results for the analysis of the other amines were achieved with a 20 mM lactic acid buffer at low pH-value. The detection limits ranged from 0.06 to 5 μ M. To demonstrate potential practical applications, a main component assay was conducted for three pharmaceutical formulations. On-chip, five pharmaceutical amines could be baseline-resolved in a 8 cm long microchannel in 90 s, albeit a reduced sensitivity and peak capacity compared to conventional capillary electrophoresis.

Keywords: β -Blockers / Buffer additive / Contactless conductivity detection / Microchip / Miniaturation / Pharmaceutical amines
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1 Introduction

Capillary electrophoresis (CE) may be employed as an alternative to high-performance liquid chromatography (HPLC) for the analysis of basic pharmaceutical drug substances. Its main advantages over HPLC are low reagent consumption, rapid method development, and simplicity of use. Applications of CE for the determination of basic drugs have been described, for example, by Altria *et al.* [1], Lin *et al.* [2], and Lukkari *et al.* [3, 4] who employed micellar electrokinetic chromatography (MEKC). These workers used UV-absorption as detection technique, which together with fluorescence are the standard methods and these are commercially available. Electrochemical techniques, which include amperometry, potentiometry, and conductimetry, are not as widely used but have the advantage of simplicity. Potentiometric and amperometric detection methods may only be applied to the detection of certain classes of analytes. Conductivity detection on the other hand is in principle universal and may in theory be applied to all charged analytes. Conductivity detection has previously been used in the analysis of pharmaceutical preparations for small amines, cationic and anionic counter ions, and inorganic ions [5–7].

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Abbreviation: PMMA, poly(methylmethacrylate)

Relatively recently a contactless, capacitively coupled configuration for conductivity detection in capillary and microchip electrophoresis has been introduced [8–13]. In this arrangement the cell construction is much simplified and as the electrodes are isolated from the solution by the capillary wall they cannot deteriorate. Tanyanyiwa and Hauser [14, 15] demonstrated that the signal-to-noise ratio is improved by using elevated ac-voltages of several hundred volt for cell excitation. When applied to conventional capillaries this leads to detection limits which are about one order of magnitude lower than with standard voltages. With microchip devices it enables detection through the cover of the chip using electrodes which are part of the holder without sacrificing sensitivity; this means that simple chips without built-in electrodes can be used [11, 16].

It was initially anticipated that conductivity works best for small inorganic species, which can only be measured using indirect techniques when employing commercial instruments equipped with the usual absorption detector. However, it is now increasingly becoming evident that also different organic ions can be detected with high sensitivity. Lopez-Avila *et al.* [17] showed the detection of haloacetic acids, Vuorinen *et al.* [18] the analysis of catecholamines, and Baltussen *et al.* [19] the analysis of peptides. Tanyanyiwa *et al.* [20] demonstrated the determination of organic acids in wine. Amino acids, most of which do not possess chromophore moieties, are usually determined by fluorescence after derivatization. Indeed this was the standard method used in all the early work on electrophoresis chips. The universal nature of conducto-

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metric detection allows also the direct determination of these important species in conventional capillaries [21, 22] and on chips [16]. Reviews on contactless conductivity detection are available [23, 24].

In this project, we investigated the analysis of basic pharmaceutical drugs coupled with high-voltage contactless conductivity detection. Two classes of basic drugs were chosen as model analytes, β -adrenergic blocking agents (β -blockers) and physiologically active amines. β -Blockers possess an aromatic group and have an alkanolamine side chain terminating into a secondary amino group and are used for the treatment of cardiac arrhythmia, angina pectoris, and anxiety attacks. The physiologically active amines dealt with in this publication have an aromatic functional group and an amine group in the side chain. These structurally similar compounds possess different pharmacological properties. For instance, doxylamine is an antihistamine whilst adrenaline and noradrenaline are stimulants for the central nervous system. Ephedrine causes loss of appetite, which is the reason for its use in the treatment of obesity and inclusion in diet formulations.

2 Materials and methods

2.1 Instrumentation

Conventional and on-chip separations were performed on purpose-built instruments. For capillary separations, a perspex box subdivided into injector and detector compartments was employed. Injection and separation voltages were produced by a high-voltage power supply (model CZE1000R; Start-Spellman, Pulborough, England) with interchangeable polarities. Fused-silica capillaries of 60 cm length and 25 μm internal diameter were used for all separations (Polymicro Technologies, Phoenix, AZ, USA). For microchip-analysis poly(methylmethacrylate) (PMMA) microchips with a channel length of 80 mm and a cover plate of 175 μm thickness, purchased from Chip-Shop (Jena, Germany), were utilized. These were mounted on a holder also constructed from PMMA. The chip holder has been described in detail in our previous work [16]. Two high-voltage power supply units were required for injection and separation purposes. These were driven by a specially built interface connected to a multifunctional I/O-card (model PCI-MIO-16XE-50; National Instruments, Austin, TX, USA) located in a personal computer which was programmed to control the injection and separation sequence. The detectors consist of a pair of identical tubular electrodes of 4 mm length in the case of the conventional capillaries and of strips of copper tape of 1 mm width separated by 1 mm and shielded from each other in both cases. The detector circuitry is identical for the two arrangements and consists of a func-

tion generator with voltage booster on the actuator side and current-to-voltage converter on the pick-up side followed by rectifier and offset circuitry. Detection parameters for both modes, capillary and microchip electrophoresis were 300 V_{pp} actuation at 100 kHz. MacLab 4e systems (ADInstruments, Hastings, UK) connected to a Macintosh PowerPC (Apple, Cupertino, CA, USA) were used for data acquisition. More details on the detector arrangements have been given previously [11, 14–16].

2.2 Reagents and methods

All chemicals were of reagent grade and were used as received. All β -blockers were supplied by Sigma (Buchs, Switzerland). DL-Octopamine hydrochloride, doxylamine succinate, (+)-ephedrine, DL-noradrenaline hydrochloride, isoproterenol, and L-adrenaline were supplied by Fluka (Buchs, Switzerland). The electrolyte substances, acetic acid, citric acid, lactic acid, and L-histidine were also obtained from Fluka. Fresh lactic acid and citric acid buffers were prepared prior to use. The required standards were prepared by diluting stock solutions directly in buffer. All solutions were degassed by ultrasonication and filtered through membrane filters before use. Ternomin[®], Sanalepsin[®], and Vick Medi-Nite were obtained from a local pharmacy. The tablets were pulverized in a mortar before being dissolved in the run buffer. The capillaries were preconditioned with 1 M sodium hydroxide for 5 min, washed with deionized water for 10 min, and then flushed with buffer. Before use, the PMMA chip was preconditioned with 0.1 M hydrochloric acid prior to thorough rinsing with deionized water and flushing with buffer.

3 Results and discussion

3.1 Determination of physiologically active amines using conventional capillaries

Six physiologically active amines chosen as representative examples, namely doxylamine, octopamine, ephedrine, noradrenaline, adrenaline and isoproterenol, were investigated. For conductivity detection most commonly a 10 mM MES/His buffer at its natural pH-value of 6.1 has been employed because its low background conductivity gives generally good sensitivity. At this pH-value most amine groups will be present in the protonated, and thus positively charged form. The species may therefore be electrophoretically separated as cations. An electropherogram resulting from the use of this buffer is illustrated in Fig. 1. All peaks displayed a positive going response and represent concentrations of 100 μM analyte. Lower concentrations could be detected but the resulting peaks were distorted. It was also found that the peaks for iso-

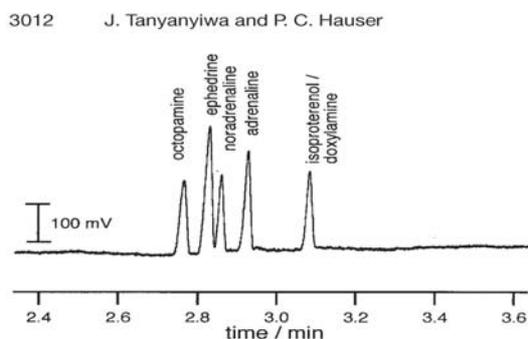


Figure 1. Electropherogram for six pharmaceutical amines separated in conventional capillaries. Buffer, 10 mM MES/His (pH 6.2); concentrations, 100 μM ; injection, 5 kV, 7 s; separation voltage, 25 kV.

proterenol and doxylamine strongly overlap. The latter molecule has two basic groups, the pyridine moiety has a pK_a of 5.29. Thus, at pH 6 this group is predominantly deprotonated and the molecule singly charged. The use of buffers with lower pH-value is therefore preferable for doxylamine and molecules with other weakly basic groups. A further advantage of operating at lower pH-values is that the ionization of the silanol groups on the surface of the fused-silica capillaries suppressed and thus the electroosmotic flow reduced. This results in longer residence times of the analytes and therefore improved separation.

Using an acidic buffer solution consisting of 10 mM citric acid, complete separation of the six amines could be achieved as shown in Fig. 2. At the pH-value of 3 employed doxylamine is doubly protonated, it therefore exhibits the highest mobility in this buffer. Also the sensitivity is improved in this buffer. The calibration data for adrenaline, noradrenaline, octopamine, and isoproterenol was determined for the concentration range from 20 μM to 500 μM . The calibration curves were linear and the following regression equations were obtained (y = peak area in arbitrary units, x = concentration in μM): $y = 0.0829x - 0.7235$; $y = 0.0368x + 0.0384$; $y = 0.0402x - 0.1239$, and $y = 0.0759x - 0.2483$ for adrenaline, noradrenaline, octopamine, and isoproterenol, respectively (correlation coefficients: 0.9951, 0.9948, 0.9997, and 0.9991). The detection limits were calculated as 5.2, 2.6, 4.8, and 2.2 μM . Due to the high conductance of citric acid, however, a relatively low separation potential of 12 kV had to be employed in order to avoid excessive Joule heating, which would have caused poor baseline stability. This resulted in a fairly long analysis time as evident from the electropherogram.

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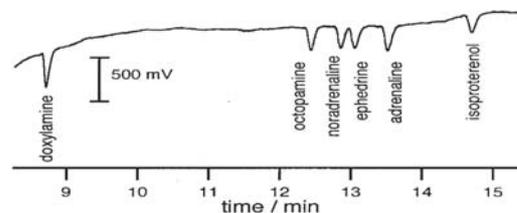


Figure 2. Electropherogram for six pharmaceutical amines separated in conventional capillaries. Buffer, 10 mM citric acid (pH 3.0); concentrations, 20 μM ; injection, 5 kV, 7 s; separation voltage, 12 kV.

A buffer consisting of 20 mM lactic acid (pH 2.8) possesses a lower background conductance and therefore it was possible to employ much higher separation voltages. The electropherogram shown in Fig. 3 was acquired with a voltage of 25 kV and thus the separation could be achieved in less than half the time needed in the citrate buffer. On the other hand, noradrenaline and ephedrine were not completely baseline-resolved. A not understood small system peak was observed between the fast migrating doxylamine and octopamine when using the lactic acid buffer. The calibration data with lactic acid buffer was determined for the concentration range from 10 μM to 500 μM . The calibration curves were linear and the following regression equations were obtained: $y = 0.9829x + 0.0554$; $y = 0.0464x - 0.062$; $y = 0.0416x - 0.1035$ and $y = 0.0331x + 0.2137$ for adrenaline, noradrenaline, octopamine, and isoproterenol, respectively (correlation coefficients: 0.9999, 0.9997, 0.9952, and 0.9984). The following detection limits were obtained for adrenaline, noradrenaline, octopamine, and isoproterenol, respectively: 0.28, 0.44, 0.52, and 0.42 μM . Thus, the lower background conductivity of the lactic acid buffer also translates into improved detection limits when compared to the citric acid buffer.

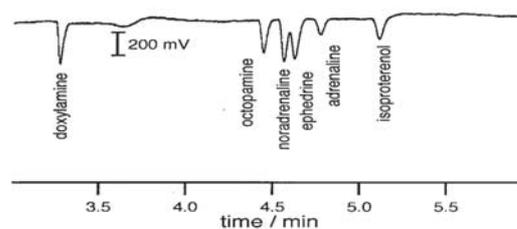


Figure 3. Electropherogram for six pharmaceutical amines separated in conventional capillaries. Buffer, 20 mM lactic acid (pH 2.83); concentrations, 20 μM ; injection, 5 kV, 7 s; separation voltage, 25 kV.

3.2 Determination of β -blockers using conventional capillaries

The suitability of the lactic acid buffer was also investigated for the determination of the β -blockers pindolol, propranolol, atenolol, metoprolol, labetalol, and acebutolol. As illustrated in Fig. 4a, peak overlaps occur for these species when using this buffer. In order to improve this situation histidine was added to the buffer. As evident from Fig. 4b, the inclusion of histidine in the background buffer leads to a pronounced increase in migration times and therefore improvement of peak resolution. Presumably the higher ionic strength resulting from the addition of histidine (only a small fraction of the lactic acid is dissociated) leads to a stronger suppression of any residual EOF at this pH-value. Baseline resolution of the two pairs is complete upon the addition of 2 mM histidine in the background electrolyte solution (see Fig. 4b) and the buffer is thus suitable for the separation of the six β -blockers. However, a further increase in histidine concentration is accompanied by a decrease in peak heights. Histidine does not have any effect on selectivity; the migration sequence of the analytes is identical in its presence or absence. The regression data for pindolol, atenolol, and labetalol were determined for the 10–500 μM concentration range. The following regression equations were obtained: $y = 0.0529x + 0.0167$; $y = 0.0466x + 0.0564$, and $y = 0.0424x + 0.1736$, with the correlation coefficients 0.9990, 0.9998, and 0.9995 for pindolol, atenolol, and labetalol, respectively. The $3 \times S/N$ ratio detection limits were calculated to be 1.3, 0.89, and 1.6 μM for pindolol, atenolol, and labetalol, respectively.

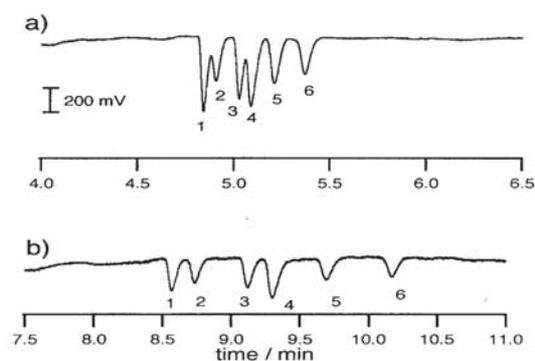


Figure 4. Electropherograms for six β -blockers separated in conventional capillaries. Buffers: (a) 20 mM lactic acid (pH 2.83), (b) 20 mM lactic acid, 2 mM histidine; concentrations, 20 μM ; injection, 5 kV, 7 s; separation voltage, 25 kV. Peak assignments: (1) pindolol, (2) propranolol, (3) atenolol, (4) metoprolol, (5) labetalol, (6) acebutolol.

Also investigated was the suitability of acetic acid buffers. The results are illustrated in Fig. 5. A poor separation is obtained with 100 mM acetic acid although sensitivity is at its highest. Increasing the background electrolyte concentration to 250 mM leads to longer migration times and better, but not complete, resolution. With 500 mM acetic acid, pindolol and propranolol are baseline-resolved, but the baseline stability is compromised. For this reason, the effect of the addition of histidine at concentrations between 0.5 and 2 mM was investigated also for this buffer. The migration times for all six analytes were found to increase sharply as the histidine concentration was increased from 0.5–1 mM. All six analytes were baseline-separated upon the addition of 1 mM histidine to the run buffer. Increasing the histidine concentration to 2 mM led to prolonged migration times with no significant improvement in resolution and caused decreased peak heights. Thus, a buffer composed of 100 mM acetic acid and 1 mM histidine was deemed most suitable. The corresponding electropherogram is illustrated in Fig. 6. The calibration data for pindolol, atenolol, and labetalol were determined for the 5 μM –500 μM concentration range. The equations: $y = 0.05x + 0.0373$; $y = 0.0422x - 0.0256$; $y = 0.0363x + 0.0286$ with correlation coefficients of 0.9979, 0.9996, and 0.9999 were obtained, respectively. The following $3 \times S/N$ detection limits were obtained for pindolol, atenolol, and labetalol, respectively: 0.06 μM , 0.48 μM , and 0.96 μM . As it provided the highest sensitivity, the buffer composed of 100 mM acetic acid and 1 mM histidine is therefore the most suitable electrolyte solution for the analysis of the six β -blockers.

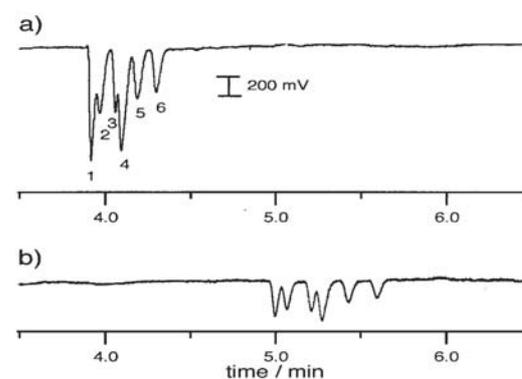


Figure 5. Electropherograms for six β -blockers separated in conventional capillaries. Buffers: (a) 100 mM acetic acid (pH 2.91), (b) 250 mM acetic acid (pH 2.71); concentrations, 20 μM each; injection, 5 kV, 7 s; separation voltage, 25 kV. Peak assignments: (1) pindolol, (2) propranolol, (3) atenolol, (4) metoprolol, (5) labetalol, (6) acebutolol.

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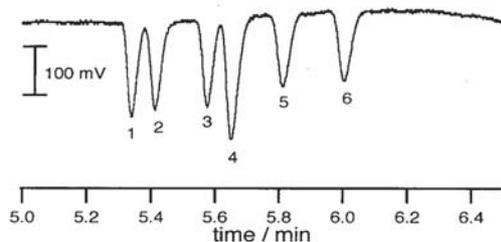


Figure 6. Electropherogram for six β -blockers separated in conventional capillaries. Buffer: 100 mM acetic acid, 1 mM histidine; concentrations, 20 μM each; injection: 5 kV, 7 s; separation voltage, 25 kV. Peak assignments: (1) pindolol, (2) propranolol, (3) atenolol, (4) metoprolol, (5) labetalol, (6) acebutolol.

3.3 Analysis of real samples using conventional capillaries

To demonstrate its potential in real sample analysis, three different pharmaceutical preparations were analyzed with contactless conductivity detection. Ternormin[®], a formulation containing 100 mg of atenolol per tablet, was dissolved to give a final concentration of approximately 750 μM in the run buffer. The resulting electropherogram is illustrated in Fig. 7. Peak identification was accomplished by spiking. In addition to atenolol, magnesium was detected. This demonstrates the universal nature of the detection mode. Inorganic anions and organic components in pharmaceutical preparations can be detected simultaneously, a feature not possible with direct UV-detection as this method is not suitable for most inorganic ions.

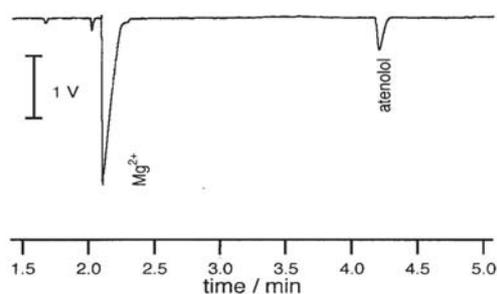


Figure 7. Analysis of a solution of a tablet of Ternormin[®] containing atenolol (final concentration appr. 750 μM) using conventional capillaries. Buffer, 100 mM acetic acid; injection, 5 kV, 7 s; separation voltage, 25 kV.

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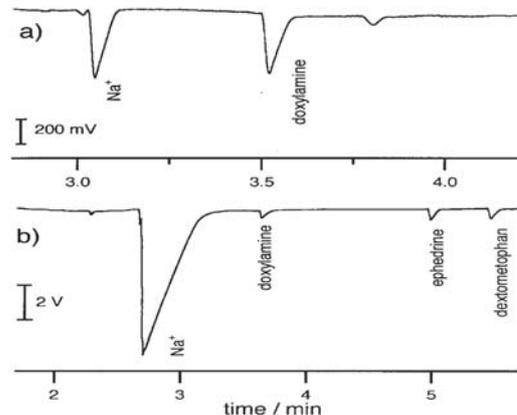


Figure 8. Analysis of two drug formulations using conventional capillaries: (a) Sanalepsi[®] N (Roche) (1:1000 dilution); (b) Vick Medi-Nite (1:10 dilution). Buffer, 100 mM acetic acid; injection, 5 kV, 7 s; separation voltage, 25 kV.

Sanalepsin[®] N, a pharmaceutical preparation containing the active ingredient doxylamine at 14 mg/mL, was diluted by a factor of 1000 (yielding appr. 52 μM of doxylamine in the final solution) and analyzed by capillary electrophoresis. The resulting electropherogram is illustrated in Fig. 8a. The electropherogram of a 1:10 dilution of Vick Medi-Nite is given in Fig. 8b. The active ingredients of Vick Medi-Nite include doxylamine, ephedrine, and dextrometorphan. The final concentrations of doxylamine and ephedrine are appr. 185 μM and 150 μM respectively. Dextrometorphan had not been investigated previously in this study. A large amount of sodium and traces of other inorganic cations are also detected.

3.4 Microchip capillary electrophoresis of β -blockers and physiologically active amines

In our previous work, the on-chip detection of inorganic as well as organic ion species by high-voltage contactless conductivity measurements has been demonstrated successfully [11, 16]. Note that in our approach to on-chip contactless conductivity detection, the electrodes are contained in the holder, which means that bare chips as used for optical detection can be employed. This approach leads to a significant reduction in the fabrication cost in comparison to chips with embedded electrodes. An electropherogram of the three β -blockers pindolol, atenolol, and acebutolol at 100 μM each is shown in Fig. 9.

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Basic drugs and contactless conductivity detection

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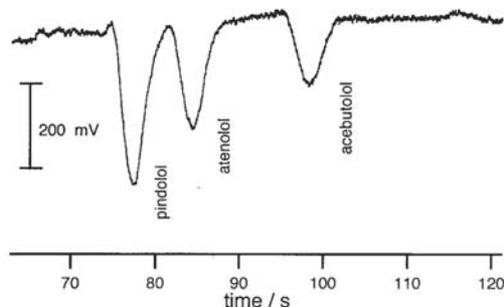


Figure 9. Microchip separation of pindolol, atenolol, and acebutolol at $100\ \mu\text{m}$ each. Buffer, 100 mM acetic acid, 1 mM histidine; injection, 1 kV, 3 s; separation, voltage, 3 kV.

Baseline resolution could be achieved in the 8 cm long microchannel within less than 2 min. The separation of five pharmaceutical amines could also be carried out rapidly on the chip (Fig. 10). Note, however, that the peak resolution is not as good as achieved on the longer conventional capillaries. The separation efficiency is thought to be limited by the length of the separation channel and of the dimensions of the detection cell on the chip. The sensitivity was also found to be lower on-chip than what could be achieved with the standard capillaries. This is thought to be related to the fact that the chip geometry does not allow a coupling as efficient as that possible with conventional capillaries. Generally, however, the sensitivity achieved on-chip with our approach belongs to the best reported for conductivity detection in this format.

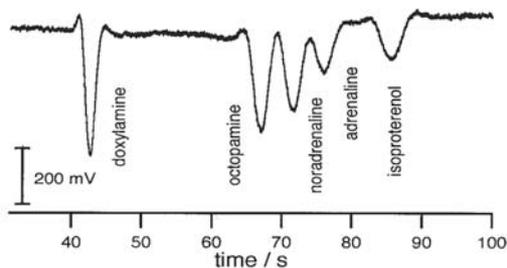


Figure 10. Microchip separation of doxylamine, octopamine, noradrenaline, adrenaline, and isoproterenol at $100\ \mu\text{m}$ each. Buffer, 10 mM citric acid; injection, 1 kV, 3 s; separation voltage, 3 kV.

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4 Concluding remarks

The results for the exemplary substances demonstrate that in capillary electrophoresis contactless conductometric detection is a suitable alternative to UV-absorbance for the determination of pharmaceutical amines. High sensitivity is assured by the use of acidic buffers of low conductivity and the detection limits obtained in standard capillaries are generally comparable to direct UV- or amperometric detection. The universal nature of conductometric measurements allows the concurrent detection of the amines with other species such as inorganic ions. The method could also be successfully implemented on the microfabricated lab-on-chip format, which has the advantage of faster analysis times, but a compromise has to be made with regard to sensitivity and peak resolution.

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5 References

- [1] Altria, K. D., Frake, P., Gill, I., Hadgett, T., Kelly, M. A., Rudd, D. R., *J. Pharm. Biomed. Anal.* 1995, 13, 951–957.
- [2] Lin, C.-E., Chang, C.-C., Lin, W.-C., Lin, E. C., *J. Chromatogr. A* 1995, 753, 133–138.
- [3] Lukkari, P., Nyman, T., Riekkola, M.-L., *J. Chromatogr. A* 1994, 674, 241–246.
- [4] Lukkari, P., Vuorela, H., Riekkola, M.-L., *J. Chromatogr.* 1993, 652, 451–457.
- [5] Williams, R. C., Boucher, R. J., *J. Pharm. Biomed. Anal.* 2000, 22, 115–122.
- [6] Williams, R. C., Boucher, R., Brown, J., Scull, J. R., Walker, J., Paolini, D., *J. Pharm. Biomed. Anal.* 1997, 16, 469–479.
- [7] Bowman, J., Tang, L., Silverman, C. E., *J. Pharm. Biomed. Anal.* 2000, 23, 663–669.
- [8] Zemann, A. J., Schnell, E., Volgger, D., Bonn, G. K., *Anal. Chem.* 1998, 70, 563–567.
- [9] Fracassi da Silva, J. A., do Lago, C., *Anal. Chem.* 1998, 70, 4339–4343.
- [10] Pumera, M., Wang, J., Opekar, F., Jelínek, I., Feldman, J., Loewe, H., Hardt, S., *Anal. Chem.* 2002, 74, 1968–1971.
- [11] Tanyanyiwa, J., Hauser, P. C., *Anal. Chem.* 2002, 74, 6378–6382.
- [12] Laugere, F., Guijt, R. M., Bastemeijer, J., van der Steen, G., Berthold, A., Baltussen, E., Sarro, P., van Dedem, G. W. K., Vellekoop, M., Bossche, A., *Anal. Chem.* 2003, 75, 306–312.
- [13] Lichtenberg, J., de Rooij, N. F., Verpoorte, E., *Electrophoresis* 2002, 23, 3769–3780.
- [14] Tanyanyiwa, J., Galliker, B., Schwarz, M. A., Hauser, P. C., *Analyst* 2002, 127, 214–218.
- [15] Tanyanyiwa, J., Hauser, P. C., *Electrophoresis* 2002, 23, 3781–3786.

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Electrophoresis 2004, 25, 3010–3016

- [16] Tanyanyiwa, J., Abad-Villar, E. M., Fernandez-Abedul, M. T., Costa-Garcia, A., Hoffmann, W., Guber, A. E., Hermann, D., Gerlach, A., Gottschlich, N., Hauser, P. C., *Analyst* 2003, 128, 1019–1022.
- [17] Lopez-Avila, V., van de Goor, T., Gaš, B., Coufal, P., *J. Chromatogr. A* 2003, 993, 143–152.
- [18] Vuorinen, P. S., Jussila, M., Siren, H., Palonen, S., Riekkola, M. L., *J. Chromatogr. A* 2003, 990, 45–52.
- [19] Baltussen, E., Guijt, R. M., van der Steen, G., Baltussen, S., van Dedem, G. W. K., *Electrophoresis* 2002, 23, 2888–2893.
- [20] Tanyanyiwa, J., Leuthardt, S., Hauser, P. C., *J. Chromatogr. A* 2002, 978, 205–211.
- [21] Coufal, P., Zuska, J., van de Goor, T., Gaš, B., *Electrophoresis* 2003, 24, 671–677.
- [22] Tanyanyiwa, J., Schweizer, K., Hauser, P. C., *Electrophoresis* 2003, 24, 2119–2124.
- [23] Zemann, A. J., *Electrophoresis* 2003, 24, 2125–2137.
- [24] Tanyanyiwa, J., Leuthardt, S., Hauser, P. C., *Electrophoresis* 2002, 23, 3659–3666.

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Review

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Conductimetric and potentiometric detection in conventional and microchip capillary electrophoresis

Potentiometric detection is rarely used in separation methods but is promising for certain classes of analytes which can only with difficulty be quantified by more standard methods. Conductimetric detection of ions is very versatile and has recently received renewed interest spurred by the introduction of the capacitively coupled contactless configuration. Both are useful and complementary alternatives to the established optical detection methods, and to the more widely known electrochemical method of amperometry. The simplicity of the electrochemical methods makes them particularly attractive for microfabricated devices, but relatively little work has to date been carried out with regard to potentiometric and conductimetric detection.

Keywords: Conductimetry / Microchip capillary electrophoresis / Potentiometry / Review
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1 Introduction

The standard and currently commercially available detection methods in capillary electrophoresis are UV-absorbance and laser induced fluorescence (LIF). Absorbance measurements are well suited for UV-absorbing organic species, but its sensitivity is limited because of the short optical pathlengths available. Fluorescence is mainly used for its very high sensitivity for certain biochemically important species (amino acids and larger entities) which can be rendered fluorescent by derivatization. The two methods are therefore well established for these groups of analytes. For other species, namely metal ions, inorganic anions and nonaromatic organic compounds (such as surfactants and carboxylic acids) indirect absorbance

methods are often employed. These rely on the displacement of charged dye molecules by the analyte, leading to peaks going negative from a constant background baseline. The detection limits are generally relatively high for these methods because of the reliance on the stability of the baseline and values between 50 and 500 ppb (corresponding to appr. 10^{-6} – 10^{-5} M) are obtained for inorganic ions [1]. For electrophoretic separations on the newer micromachined chip devices with narrow channels, LIF has been predominant and absorbance measurements have rarely been reported. However, most groups of analytes are not suitable for LIF as they cannot be rendered fluorescent and therefore the use of micromachined devices has to date largely been restricted to those biochemical species which are amenable to fluorescence tagging.

Alternative detection methods with high sensitivity are therefore desirable in order to better match capillary electrophoresis, in the conventional and the chip format, to a wider range of analytes. This is particularly true for inorganic species of relevance in industrial and environmental applications of analytical chemistry. Guidance may be obtained by examining the status of the more mature chromatographic methods of HPLC and ion-chromatography. The most widely used detection method in HPLC is optical absorbance, but amperometric detection is employed for electroactive species when high sensitivity is desired. In ion-chromatography, conductivity detection is the standard method because it can be almost universally used for ionic species. Despite the success of these two electrochemical methods in chromatography, they have been relatively little used in capillary electrophoresis. It is

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Abbreviations: C⁴D, capacitively coupled contactless conductivity detection; HV-C⁴D, high-voltage capacitively coupled contactless conductivity detection; ISE, ion-sensitive electrode

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thought that the reason is twofold. Firstly, the early capillary electrophoresis instruments were built for combination with existing UV-detectors. This in turn created a precedence and established usage patterns as many practical procedures were developed for this detection method. Secondly, electrochemical detectors have often been considered incompatible with electrophoresis because of the high voltage used for separation.

Amperometric detection in electrophoresis has over the last few years been studied more widely by a number of research groups for both conventional capillaries and the chip platform and thus gained some level of acceptance. Impressive results have also been reported with conductimetric detection. However, because of the limited commercial availability, the method had been somewhat lingering until the more recent introduction of capacitively coupled contactless conductivity detection. Potentiometric detection, the third electrochemical method, has been relatively little explored for detection in any separation method, but has some unique features which may be particularly useful in capillary electrophoresis.

Several recent reviews on different aspects of electrochemical detection methods for conventional and chip-based electrophoretic separations are available. Baldwin [2] has reviewed amperometric and potentiometric detection. Lacher *et al.* [3] and Wang [4] have focused on amperometric detection for microchips. Zemmann [5] has discussed conductivity detection on capillaries and Nagels and Poels [6] have reviewed potentiometric detection. A discussion on the development of all three electrochemical methods was written by Kappes and Hauser [7]. The subjects of the present survey are the conductimetric and potentiometric methods, with particular consideration of the latest developments.

2 Potentiometric detection

Potentiometric detection is based on electrodes bearing membranes which are semipermeable to certain ions only, leading to a charge separation and thus the build-up of a measurable potential which follows the Nernst equation. Such electrodes are of course well known as ion-selective electrodes (ISE) and used for single ion determinations. In order to be useful for detection in separation methods, the selectivity should extend to a range of ions, a requirement which is unusual for an ISE. The desired response can be given by the following special form of the Nicolsky-Eisenman equation:

$$E = E^{\circ} + \frac{RT}{F} \ln \left(\sum_i K_i^{\text{pot}} c_i^{j/z_i} \right) \quad (1)$$

where E is the electrode potential, E° is a constant, R , T and F are the universal gas constant, temperature and Faraday's constant, respectively, and K and c are the selectivity coefficients and concentrations of species i of charge z_i , present. From an examination of the equation it is evident that the electrode should not show any response to the ion used in the background buffer as this would otherwise dominate the signal and lead to poor detection limits for the analyte ions. On the other hand, for a clear definition of the baseline, at least in principle, a background ion to which the electrode is showing a slight response is necessary. For optimum response it is therefore necessary to carefully match buffer composition to the response of the particular ion-selective membrane. Unique to this detector is also its logarithmic response. This means that in practice a very wide dynamic range is usually obtained, but this may also lead to limited precision of the concentration measurement. A special feature of potentiometric detection is the fact that the detector signal does not scale with the geometrical size of the electrode. This may be of particular advantage in miniaturized systems. Note, that potential gradient detection, a seldom used method, is fundamentally different from potentiometric detection with membrane-electrodes [8].

In the early studies on the use of ISEs for potentiometric detection in capillary electrophoresis, carried out in the first half of the 1990s, micropipette electrodes were placed into the end of the capillary itself with the aid of micromanipulators [9–13]. Micropipette electrodes consist of glass capillaries with drawn-out tips of a few micrometer diameter and have been used in physiological studies for potentiometric ion determination in small compartments or even in single cells [14]. The tips are filled with a viscous lipophilic solvent containing an ionophore which forms the ion-selective membrane and is in contact with a silver/silver chloride internal reference electrode *via* an aqueous filling solution. Two fundamentally different types of ionophores may be employed. Charged ionophores, *i.e.*, highly lipophilic anions or cations, contained in the membrane will lead to the uptake of ions of opposite charge with a relatively flat selectivity pattern solely dependent on the lipophilicity of the analyte ions (the so-called Hofmeister sequence). The lipophilicity is largely governed by the charge and the size of the ion, which, incidentally, are the same parameters responsible for the electrophoretic separation. Electrically neutral ionophores usually lead to a response which is much more selective. Both types of membranes have been used in capillary electrophoresis. Detection limits between about 10^{-5} and 10^{-7} M can be achieved depending on the nature of the analyte.

For these studies, the capillary ends had been internally widened in a conical fashion by etching with hydrofluoric acid. As the cross section is widened, the electrophoretic current is experiencing a lower resistance and thus the

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electric field (or voltage drop) between the detector electrode and the electrophoretic ground is minimized. Thus, electrical decoupling (by grounding the electrolyte solution ahead of the detector electrode) as was commonly employed in amperometric detection, was not found to be necessary and has never been used in potentiometric detection. The conical etch was considered a compromise between maximizing end-column electrical decoupling and minimizing extracolumn band-broadening.

The micropipette-based arrangement was useful to demonstrate the possibility of potentiometric detection but is not suitable for routine measurements because the electrodes are fragile and cannot be stored over extended periods of time. A more practical approach was adopted by the use of more robust so-called coated-wire electrodes which are constructed using a solid poly(vinyl chloride) (PVC)-membrane and without internal filling solution [15, 16]. This configuration is illustrated schematically in Fig. 1. In this case the electrodes are of approximately the same diameter as the outside of the capillary and located at a distance of about 50 μm from the capillary end. The arrangement does not lead to a significant deterioration of the performance compared to the on-column approach with the micropipette electrodes. Bias by the electrophoretic voltage is minimized by the drastic expansion of the cross section between capillary end and the detector electrode. It was also found that the precision of the alignment in this configuration is not very critical and a relatively simple machined holder, rather than a micromanipulator, is adequate [17]. Furthermore, the use of a reference electrode is also not mandatory as the electrophoretic ground electrode can serve as a sufficiently stable pseudoreference. The current passing through this electrode leads to a stable potential and this simplification may, incidentally, also be employed in amperometric detection [18].

Reported applications of coated-wire electrodes with PVC-membranes encompass alkali and alkaline earth metals [16, 17], small inorganic anions [16] and lipophilic organic ions such as quaternary amines, aliphatic sulfonates and carboxylates [15, 19]. The detection of inor-

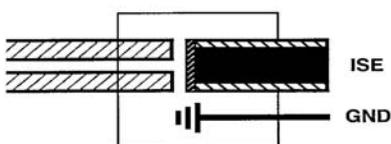


Figure 1. Schematic drawing of a potentiometric detector cell in the wall-jet arrangement with a coated wire ISE.

Conductimetric and potentiometric detection in CE

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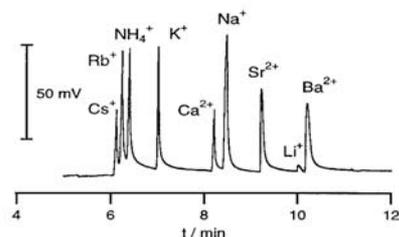


Figure 2. Potentiometric detection of inorganic cations at 10^{-4} and 10^{-5} M with a coated-wire ISE.

ganic cations is illustrated in Fig. 2, and an electropherogram for amines is given in Fig. 3. The differences in peak height observed in Fig. 3 is a feature of the Hofmeister electrode. Clearly, this type of electrode is best suited for the determination of the more lipophilic ions. The utility of the method has also been demonstrated for other species which cannot be detected well by other means such as certain artificial sweeteners and analgesics [19]. Poels and Nagels [20, 21] have subsequently demonstrated the potentiometric detection of carboxylates and certain inorganic anions with electrodes employing conducting polymers as the membrane. These electrodes have the advantage of simpler manufacture than the PVC-based ones, as the membrane does not have to be placed mechanically but can be created *in situ* by electrochemical means.

Certain anionic species are also detectable potentiometrically with bare copper wire electrodes. This approach relies on the complexation of free copper ions on the electrode surface by the analyte species and, as no mem-

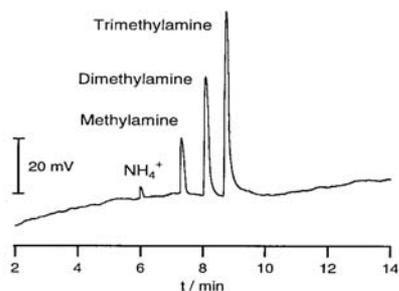


Figure 3. Potentiometric detection of amines at 2.5×10^{-4} M with a coated-wire ISE.

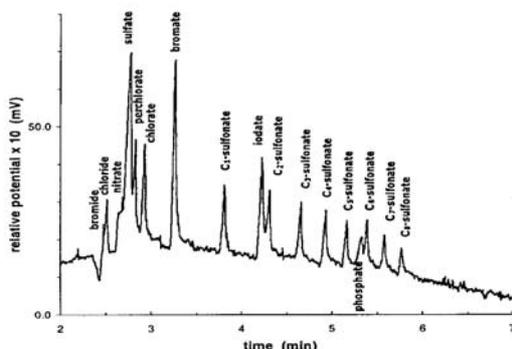
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Figure 4. Potentiometric detection of anions at 1 mM with a copper electrode. Reprinted from [23], with permission.

branes are used, its implementation is easier [22]. The use of these electrodes has been reported for the direct detection of underivatized amino acids [22], of inorganic anions and sulfonates [23] and of organic chelating agents [24]. Detection limits in the low μM -range were obtained. The detection of anions with a copper electrode is illustrated in Fig. 4. Note, that potentiometric detection with metallic copper electrodes is related to the use of the same electrode metal in the amperometric mode, which has been reported more frequently (for a review, see, for example, [7]). Zakaria *et al.* [25] have shown that potentiometric detection of anions is also possible at inert platinum electrodes if these are operated under a certain pulsing regime. Good sensitivities, with detection limits reaching sub- μM values, were obtained, but the response mechanism is not well understood.

From an instrumental point of view potentiometric detection is the most simple of all methods and for this reason it has been implemented in a portable battery powered CE-instrument for analysis in the field [26–28]. Although Tantra and Manz [29] have described a membrane-based potentiometric detector implemented on a microfabricated glass device, the use of potentiometric detection on electrophoresis chips has, at least to our knowledge, not been reported.

3 Conductimetric detection

Conductimetric detection is in principle a universal detection method for most modes of capillary electrophoresis, as all ions will give a measurable response. The conductance of a solution (L) is dependent on the electrode area

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(A), their distance (l), the concentrations (c) of all the charge carriers present and their molar conductivity (λ) according to Eq. (2):

$$L = \frac{A}{l} \sum \lambda_i c_i \quad (2)$$

A limitation is caused by the buffer ions, which give rise to a background signal. This means that small changes on this background signal have to be monitored, which affects the signal-to-noise ratio (S/N) and the lower limit of detection. Also, as the analyte ions displace buffer ions of the same charge in order to maintain electroneutrality, the observed peak arises from the difference in equivalent conductivity between the two types of ions. The peaks might be going positive or negative from the baseline depending on the respective conductivities and either mode is acceptable. It is therefore desirable to choose buffer species with molar conductivities which are very different from the analytes. This is in conflict with the need to match the mobility of analyte and buffer (in order to avoid skewed peaks and therefore poor resolution) as mobility and conductivity are, of course, simply two different manifestations of the same ionic property. In practice, the buffer composition has to be carefully optimized to also satisfy the requirements for a stable high ionic strength and, on the other hand, low background conductivity, as well as pH-buffer capacity.

Conductimetric detection is carried out by applying an ac-voltage to the cell in order to eliminate Faradaic reactions. The signal arises in the bulk of the solution and not on the electrode surface as is the case in amperometry and potentiometry, and the method is therefore intrinsically less prone to interference effects, a feature shared with the optical absorbance and fluorescence measurements. In an early arrangement, a pair of 25 μm Pt-wires were inserted into the capillary through laser drilled holes [30]. However, it could then be shown that a similar performance can be obtained with a simpler wall jet arrangement [31]. Such a configuration was later used in a commercial instrument for capillary zone electrophoresis (Crystal CE from Thermo CE). The availability of this instrument prompted a number of publications which were concerned with the development of different applications. Haber and co-workers [32, 33] carried out conductimetric detection of metal ions, inorganic anions and smaller aliphatic acids, and have shown that for samples with low electrolyte content, which allow stacking conditions, detection limits in the ppt range are possible when an internal standard is used. Katzmayer and co-workers [34] have presented an optimization of the detection of small anions and Gebauer *et al.* [35] have presented a prediction of peak shapes for conductivity detection. Lucy and Wu [36] have optimized the conditions for sur-

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factants and achieved detection limits of 10^{-6} M for alkyl sulfonates, an application also studied by Gallagher and Danielson [37]. Williams and co-workers [38] and Bowman and co-workers [39] have analyzed the purity of pharmaceuticals by CE with conductivity detection. Govindaraju *et al.* [40] have analyzed lung fluid for inorganic ions. Klampfl *et al.* [41] determined small ions in electrodeposition baths. Hissner *et al.* [42] have analyzed environmental water samples for sulfur-containing anions, Valsecchi *et al.* [43] have demonstrated the determination of anions in rain water and Schlegel *et al.* [44] the speciation of selenium and arsenic compounds. Without stacking, typical detection limits for small inorganics reported by these workers are approximately $1 \mu\text{M}$, which compares well with the 50–500 ppb quoted for the indirect absorbance detection of inorganic ions [1]. These efforts clearly demonstrate the usefulness of conductimetric detection. However, to our knowledge this instrument is not sold anymore, nor are presently any others for capillary zone electrophoresis with conductometric detection.

Alternative electrode arrangements have been reported. Opekar and co-workers [45, 46] as well as Zhao *et al.* [47] have used electrodes directly formed at the capillary end either from small wires or by metal deposition. Tuma *et al.* [48] recently have described a cell which is based on the measurement of the conductivity of a wetted plastic strip attached to the capillary end. Bodor *et al.* [49] have used a measuring cell based on a drilled cross in a Perspex-block and the electrical contact to the column effluent is made *via* a bridging electrolyte solution. In an effort to achieve lower detection limits, suppressed conductivity detection, as known from ion-chromatography, has also been adapted for capillary electrophoresis [50–55]. Good detection limits in the order of 10^{-7} M have been achieved, however, the implementation is not easy because of the small dimensions involved and the approach has not been widely adopted.

A significant contribution to conductivity detection was made independently by Zemmann and co-workers [56, 57] and by Fracassi da Silva and do Lago [58] in 1998 by the introduction of the capacitively coupled contactless conductivity detector (C⁴D). This configuration, which has also been termed oscillometric detection, is illustrated in Fig. 5. Two electrodes of a few millimeter length, consisting either of a conductive varnish or short metallic tubes, which are separated by a gap of several millimeters, are placed side by side around the capillary. As each electrode forms a capacitor with the internal electrolyte solution, it is possible to pass an ac-voltage of sufficiently high frequency (above about 20 kHz) through the cell. By using a carefully designed electronic circuitry with a flat frequency response it could be shown that the electrical

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characteristics of this cell arrangement correspond exactly to what is expected for a serial capacitor-resistor-capacitor combination [59]. The experimentally found cell capacitances are approximately 0.1 pF and the resistance 50 M Ω .

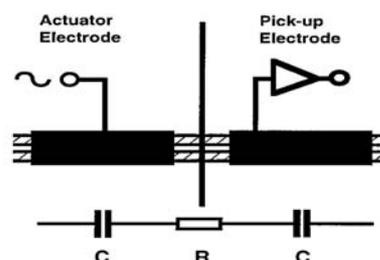


Figure 5. Schematic drawing of a C⁴D.

A different contactless detector arrangement has been reported by Kaniansky and co-workers [60, 61]. This cell is based on four small wire-electrodes of 200 μm diameter placed perpendicularly around the circumference of the capillary. Due to space restriction this cell arrangement is only suitable for capillaries of 300 μm ID and 500 μm OD which are not commonly used in capillary zone electrophoresis. The operating frequency of 4 MHz is much higher than the one used for the cell arrangement discussed above which may be necessitated by smaller capacitive coupling. Detection limits of $1 \mu\text{M}$ have been reported for inorganic anions, which is comparable with those reported for the C⁴D-cell arrangement discussed above.

Both of these arrangements have the important advantages of easy alignment of the electrodes with the capillary and of long-term robustness as prevention of electrode fouling is inherent. Several research groups have therefore taken up the C⁴D-configuration. Tuma and co-workers [62] have presented a modified cell in which two strips of aluminium foil form the electrodes and are clamped onto the capillary with a perspex cell. Pumera *et al.* [63] have demonstrated the determination of cyclodextrins and Fracassi da Silva and do Lago [64] have performed the indirect detection of nonionic alcohols separated by micellar electrokinetic chromatography. Muzikár *et al.* [65] have shown that contactless conductimetric detection is also possible in nonaqueous solvents [65]. This application is illustrated in Fig. 6. Kappes *et al.* [28] have adopted the detection method for a field-portable CE-instrument because of its simplicity. Haddad and co-

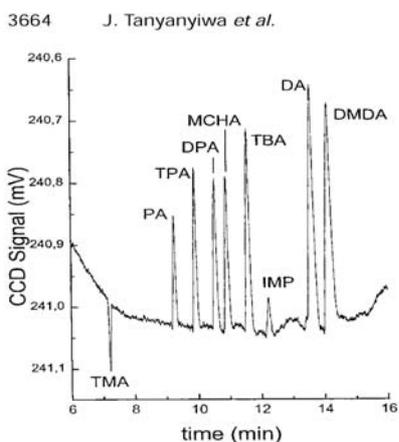


Figure 6. Detection of organic ammonium compounds at 1 mM in an organic solvent using a contactless conductivity detector. Reprinted from [65].

workers [66] have used contactless conductivity detection in capillary electrochromatography [66] and Zemann and co-workers [5, 67] have carried out simultaneous detection of anions and cations by injection from both capillary ends while placing the detector in the center. Chvojka *et al.* [68] have described a dual detector cell which combines contactless conductivity and optical absorption. Fracassi da Silva *et al.* [69] have designed a miniaturized version of the C⁴D in which the detector cell and associated electronic circuitry are all constructed on a printed circuit board. Because of its small size, this detector has the advantage that it may be readily incorporated into existing instruments, but it appears that the performance had to be compromised in order to achieve miniaturization.

Recently, it was reported that the S/N-ratio of the C⁴D, which had been restricting the detection limits, can be improved by using peak-to-peak voltages of several 100 V (high-voltage C⁴D, HV-C⁴D) for excitation rather than the previously typically employed 25 V [59]. An electropherogram for inorganic anions at 5×10^{-7} M obtained with this detector is given in Fig. 7. Better performance may actually be possible in contactless measurements than in the conventional approach because the use of high voltages should only be feasible when the electrodes are isolated from the solution.

Isotachopheresis (ITP) is a mode of separation fundamentally different from capillary zone electrophoresis and has generally not enjoyed as much popularity. However,

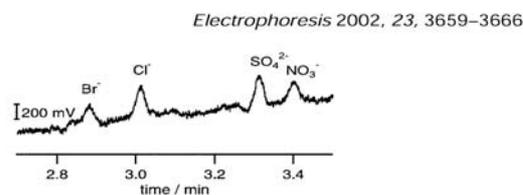


Figure 7. Detection of inorganic anions at 5×10^{-7} M in a fused-silica capillary with an HV-C⁴D.

since in isotachopheresis zones of different conductivity are obtained, conductimetric detection is the method of choice for this mode. Several research groups have used an isotachopheresis setup employing the contactless detector of the four electrode-high frequency design (see, for example, [70–72]). Apparently this instrument is commercially available from at least two different sources. It appears that the detection limits in isotachopheresis are relatively high (in the ppm-range). On the other hand, with this instrument isotachopheresis may also be employed as a preconcentration method ahead of separation by zone electrophoresis, which leads to sub-ppb detection limits [73, 74]. Isotachopheresis with conductimetric detection has recently also been implemented on miniature planar devices. Prest and co-workers [75, 76] have produced isotachopheresis-chips in silicon rubber and demonstrated the determination of metal cations. Kaniansky and Grass [77–84] with co-workers have reported devices manufactured in poly(methylmethacrylate) (PMMA), which may be employed for isotachopheresis and the coupling of isotachopheresis with zone electrophoresis. The determination of different inorganic and organic ions in samples such as natural water, wine, fruit juices and urine has been reported.

Other workers have developed zone electrophoresis on microfabricated devices. Van den Berg and co-workers [85–87] have manufactured glass-chips for zone electrophoresis and demonstrated the detection of inorganic ions and peptides with embedded electrodes. Soper and co-workers [88] have designed a device in PMMA and shown the separation and sensitive detection of amino acids, proteins and DNA fragments with a built-in conventional contacting conductivity detector. Wang and co-workers [89, 90] have also used PMMA chips and demonstrated that contactless detection can be achieved on separation chips by placing the electrodes on top of the cover plate rather than embedding them in the device. This approach allows a significant simplification of the manufacturing process. It has very recently been found in our laboratory that also in this configuration the use of an elevated excitation voltage leads to improved sensitivity, although the performance, in terms of detection limit,

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achieved with the HV-C⁴D on conventional capillaries cannot be matched. The detection of inorganic cations at 20 μm on a microfabricated device using HV-C⁴D is illustrated in Fig. 8.

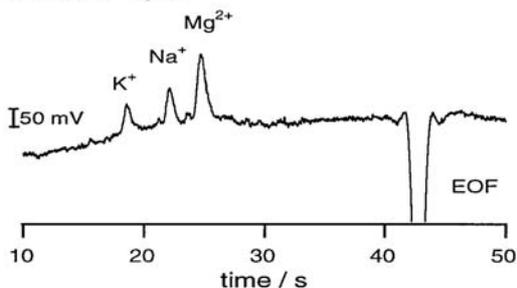


Figure 8. Detection of inorganic cations at 20 μm on-chip with an HV-C⁴D.

4 Conclusions

As has been seen, the usefulness of conductimetric detection has in recent years been amply demonstrated. It is expected that the method will further gain in popularity because of its simplicity, robustness and versatility. Conductimetric detection, in particular in the contactless version, is likely to become the method of choice for the determination of inorganic ions. There are also some early indications that it may prove to be successful for organic analytes and biochemically relevant species as well. Potentiometric detection, on the other hand, is not firmly established. Its strength should lie in the detection of organic lipophilic species which are not accessible via UV-absorption, fluorescence or amperometry. However, more work is required to more clearly define fields of application and perhaps to yet devise still more simple and robust implementations.

The three electrochemical methods (including amperometry) can be considered complementary and cover a wide range of analytes. Common to all is the direct signal transduction into the electronic domain, which means that only limited instrumentation is required. This makes the methods particularly attractive for portable and miniaturized devices. From the point of view of electroanalysis, the combination of electrochemical methods with electrophoretic separation is a great step forward, as it alleviates selectivity problems often encountered otherwise. In particular, conductimetry, the orphan of the electroanalytical methods, turns into a powerful tool with great selectivity and sensitivity when combined with electro-separation.

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5 References

- [1] Jones, W. R., in: Landers, J. P. (Ed.), *Handbook of Capillary Electrophoresis*, CRC Press, Boca Raton, FL 1997.
- [2] Baldwin, R. P., *Electrophoresis* 2000, 21, 4017–4028.
- [3] Lacher, N. A., Garrison, K. E., Martin, R. S., Lunte, S. M., *Electrophoresis* 2001, 22, 2526–2536.
- [4] Wang, J., *Talanta* 2002, 56, 223–231.
- [5] Zemmann, A. J., *Trends Anal. Chem.* 2001, 20, 346–354.
- [6] Nagels, L. J., Poels, I., *Trends Anal. Chem.* 2000, 19, 410–417.
- [7] Kappes, T., Hauser, P. C., *Electroanalysis* 2000, 12, 165–170.
- [8] Landers, J. P. (Ed.), *Handbook of Capillary Electrophoresis*, CRC Press, Boca Raton, FL 1997.
- [9] Haber, C., Silvestri, I., Röösl, S., Simon, W., *Chimia* 1991, 45, 117–121.
- [10] Nann, A., Silvestri, I., Simon, W., *Anal. Chem.* 1993, 65, 1662–1667.
- [11] Nann, A., Simon, W., *J. Chromatogr.* 1993, 633, 207–211.
- [12] Nann, A., Pretsch, E., *J. Chromatogr. A* 1994, 676, 437–442.
- [13] Hauser, P. C., Renner, N. D., Hong, A. P. C., *Anal. Chim. Acta* 1994, 295, 181–186.
- [14] Ammann, D., *Ion-Selective Microelectrodes; Principles, Design and Application*, Springer, Berlin 1986.
- [15] De Backer, B. L., Nagels, L. J., *Anal. Chem.* 1996, 68, 4441–4445.
- [16] Kappes, T., Schnierle, P., Hauser, P. C., *Anal. Chim. Acta* 1997, 350, 141–147.
- [17] Kappes, T., Hauser, P. C., *Anal. Chem.* 1998, 70, 2487–2492.
- [18] Kappes, T., Hauser, P. C., *Analyst* 1999, 124, 1035–1039.
- [19] Schnierle, P., Kappes, T., Hauser, P. C., *Anal. Chem.* 1998, 70, 3585–3589.
- [20] Poels, I., Nagels, L. J., *Anal. Chim. Acta* 1999, 385, 417–422.
- [21] Poels, I., Nagels, L. J., *Anal. Chim. Acta* 1999, 401, 21–27.
- [22] Kappes, T., Hauser, P. C., *Anal. Chim. Acta* 1997, 354, 129–134.
- [23] Macka, M., Gerhardt, G., Andersson, P., Bogan, D., Cassidy, R. M., Haddad, P. R., *Electrophoresis* 1999, 20, 2539–2546.
- [24] Buchberger, W., Aichhorn, D., Niessner, G., Haddad, P. R., Bogan, D., *Monatshfte für Chemie* 1998, 129, 811–816.
- [25] Zakaria, P., Macka, M., Gerhardt, G., Haddad, P. R., *Analyst* 2000, 125, 1519–1523.
- [26] Kappes, T., Hauser, P. C., *Anal. Commun.* 1998, 35, 325–329.
- [27] Kappes, T., Schnierle, P., Hauser, P. C., *Anal. Chim. Acta* 1999, 393, 77–82.
- [28] Kappes, T., Galliker, B., Schwarz, M. A., Hauser, P. C., *Trends Anal. Chem.* 2001, 20, 133–139.
- [29] Tantra, R., Manz, A., *Anal. Chem.* 2000, 72, 2875–2878.
- [30] Huang, X., Pang, T. J., Gordon, M. J., Zare, R. N., *Anal. Chem.* 1987, 59, 2747–2749.
- [31] Huang, X., Zare, R. N., *Anal. Chem.* 1991, 63, 2193–2196.

- 3666 J. Tanyanyiwa *et al.* *Electrophoresis* 2002, 23, 3659–3666
- [32] Haber, C., Jones, W. R., Soglia, J., Surve, M. A., McGlynn, M., Caplan, A., Reineck, J. R., Krstanovic, C., *J. Capil. Electrophor.* 1996, 3, 1–11.
- [33] Haber, C., van Saun, R. J., Jones, W. R., *Anal. Chem.* 1998, 70, 2261–2267.
- [34] Katzmayer, M. U., Klampfl, C. W., Buchberger, W., *J. Chromatogr. A* 1999, 850, 355–362.
- [35] Gebauer, P., Caslavská, J., Thormann, W., Boček, P., *J. Chromatogr. A* 1997, 772, 63–71.
- [36] Lucy, C. A., Wu, Q., *J. Chromatogr. Sci.* 1998, 36, 33–36.
- [37] Gallagher, P. A., Danielson, N. D., *J. Chromatogr. A* 1997, 781, 533–540.
- [38] Williams, R. C., Boucher, R., Brown, J., Scull, J. R., Walker, J., Paolini, D., *J. Pharm. Biomed. Anal.* 1997, 16, 469–479.
- [39] Bowman, J., Tang, L., Silverman, C. E., *J. Pharm. Biomed. Anal.* 2000, 23, 663–669.
- [40] Govindaraju, K., Cowley, E. A., Eidelman, D. H., Lloyd, D. K., *Anal. Chem.* 1997, 69, 2793–2797.
- [41] Klampfl, C. W., Katzmayer, M. U., Buchberger, W., Basener, N., *J. Chromatogr. A* 1998, 804, 357–362.
- [42] Hissner, F., Mattusch, J., Heinig, K., *J. Chromatogr. A* 1999, 848, 503–513.
- [43] Valsecci, S., Tartari, G., Polesello, S., *J. Chromatogr. A* 1997, 760, 326–332.
- [44] Schlegel, D., Mattusch, J., Wennrich, R., *Fresenius' J. Anal. Chem.* 1996, 354, 535–539.
- [45] Müller, D., Jelinek, I., Opekar, F., Stulik, K., *Electroanalysis* 1996, 8, 722–725.
- [46] Opekar, F., Jelinek, I., Trojanek, A., *Anal. Chim. Acta* 1999, 390, 101–106.
- [47] Zhao, H., Dadoo, R., Reay, R. J., Kovacs, G. R. A., Zare, R. N., *J. Chromatogr. A* 1998, 813, 205–208.
- [48] Tuma, P., Opekar, F., Jelinek, I., Stulik, K., *Electroanalysis* 1999, 11, 1022–1026.
- [49] Bodor, R., Kaniansky, D., Masar, M., *J. Chromatogr. A* 2001, 916, 31–40.
- [50] Dasgupta, P. K., Bao, L., *Anal. Chem.* 1993, 65, 1003–1011.
- [51] Kar, S., Dasgupta, P. K., Liu, H., Hwang, H., *Anal. Chem.* 1994, 66, 2537–2543.
- [52] Dasgupta, P. K., Kar, S., *Anal. Chem.* 1995, 67, 3853–3860.
- [53] Dasgupta, P. K., Kar, S., *Anal. Chim. Acta* 1999, 394, 1–12.
- [54] Avdalovic, N., Pohl, A., Rocklin, R. D., Stillian, J. R., *Anal. Chem.* 1993, 65, 1470–1475.
- [55] Harrold, M., Stillian, J., Bao, L., Rocklin, R., Avdalovic, N., *J. Chromatogr. A* 1995, 717, 371–383.
- [56] Zemann, A. J., Schnell, E., Volgger, D., Bonn, G., *Anal. Chem.* 1998, 70, 563–567.
- [57] Mayrhofer, K., Zemann, A. J., Schnell, E., Bonn, G. K., *Anal. Chem.* 1999, 71, 3828–3833.
- [58] Fracassi da Silva, J. A., do Lago, C. L., *Anal. Chem.* 1998, 70, 4339–4343.
- [59] Tanyanyiwa, J., Galliker, B., Schwarz, M. A., Hauser, P. C., *Analyst* 2002, 127, 214–218.
- [60] Kaniansky, D., Zelenská, V., Masár, M., Iványi, F., Gazdiková, S., *J. Chromatogr. A* 1999, 844, 349–359.
- [61] Masár, M., Dodor, R., Kaniansky, D., *J. Chromatogr. A* 1999, 834, 179–188.
- [62] Tuma, P., Opekar, F., Jelinek, I., *Electroanalysis* 2001, 13, 989–992.
- [63] Pumera, M., Jelinek, I., Jindrich, J., *Fresenius' J. Anal. Chem.* 2001, 369, 666–669.
- [64] Fracassi da Silva, J. A., do Lago, C. L., *Electrophoresis* 2000, 21, 1405–1408.
- [65] Muzikár, J., van de Goor, T., Gas, B., Kenndler, E., *J. Chromatogr. A* 2001, 924, 147–154.
- [66] Hilder, F. E., Zemann, A. J., Macka, M., Haddad, P. R., *Electrophoresis* 2001, 22, 1273–1281.
- [67] Unterholzner, V., Macka, M., Haddad, P. R., Zemann, A., *Analyst* 2002, 127, 715–718.
- [68] Chvojka, T., Jelinek, I., Opekar, F., Stulik, K., *Anal. Chim. Acta* 2001, 433, 13–21.
- [69] Fracassi da Silva, J. A., Guzman, N., do Lago, C. L., *J. Chromatogr. A* 2001, 942, 249–258.
- [70] Buzinkaiova, T., Polonsky, J., *Electrophoresis* 2000, 21, 2839–2841.
- [71] Goodwin, L., Hanna, M., Startin, J. R., Keely, B. J., Goodall, D. M., *Analyst* 2002, 127, 204–206.
- [72] Prest, J. E., Fielden, P. R., *Anal. Commun.* 1999, 36, 333–335.
- [73] Hanna, M., Simpson, C., Perret, D., *J. Chromatogr. A* 2000, 894, 117–128.
- [74] Kvasnicka, F., Jaros, M., Gaš, B., *J. Chromatogr. A* 2001, 916, 131–142.
- [75] Prest, J. E., Baldock, S. J., Bektas, N., Fielden, P. R., Brown, B. J. T., *J. Chromatogr. A* 1999, 836, 59–65.
- [76] Prest, J. E., Baldock, S. J., Fielden, P. R., Brown, B. J. T., *Analyst* 2001, 126, 433–437.
- [77] Kaniansky, D., Masar, M., Bielikova, J., Ivanyi, F., Eisenbeiss, F., Stanislawski, B., Grass, B., Neyer, A., Jöhnck, M., *Anal. Chem.* 2000, 72, 3596–3604.
- [78] Masar, M., Zuborova, M., Bielikova, J., Kaniansky, D., Jöhnck, M., Stanislawski, B., *J. Chromatogr. A* 2001, 916, 101–111.
- [79] Bodor, R., Madajova, V., Kaniansky, D., Masar, M., Jöhnck, M., Stanislawski, B., *J. Chromatogr. A* 2001, 916, 155–165.
- [80] Masar, M., Kaninansky, D., Bodor, R., Jöhnck, M., Stanislawski, B., *J. Chromatogr. A* 2001, 916, 167–174.
- [81] Bodor, R., Zuborova, M., Olivecka, E., Madajova, V., Masar, M., Kaniansky, D., Stanislawski, B., *J. Sep. Sci.* 2001, 24, 802–809.
- [82] Grass, B., Neyer, A., Jöhnck, M., Siede, D., Eisenbeiss, F., Weber, G., Hergenroder, R., *Sens. Actuators B* 2001, 72, 249–258.
- [83] Grass, B., Siede, D., Neyer, A., Hergenroder, R., *Fresenius' J. Anal. Chem.* 2001, 371, 228–233.
- [84] Zuborova, M., Masar, M., Kaniansky, D., Jöhnck, M., Stanislawski, B., *Electrophoresis* 2002, 23, 774–781.
- [85] Guijt, R. M., Baltussen, E., van der Steen, G., Schasfoort, R. B. M., Schlautmann, S., Billiet, H. A. H., Frank, J., van Dedem, G. W. K., van den Berg, A., *Electrophoresis* 2001, 22, 235–241.
- [86] Schlautmann, S., Wenskink, H., Schasfoort, R., Elwenspoek, M., van den Berg, A., *J. Micromech. Microeng.* 2001, 11, 386–389.
- [87] Guijt, R. M., Baltussen, E., van der Steen, G., Frank, H., Billiet, H., Schalkhammer, T., Laugere, F., Vellekoop, M., Bertiold, A., Sarro, L., van Dedem, G. W. K., *Electrophoresis* 2001, 22, 2537–2541.
- [88] Galloway, M., Stryjewski, W., Henry, A., Ford, S. M., Llopis, S., McCarley, R. L., Soper, S. A., *Anal. Chem.* 2002, 74, 2407–2415.
- [89] Pumera, M., Wang, J., Opekar, F., Jelinek, I., Feldman, J., Löwe, H., Hardt, S., *Anal. Chem.* 2002, 74, 1968–1971.
- [90] Wang, J., Pumera, M., Collins, G., Opekar, F., Jelinek, I., *Analyst* 2002, 127, 719–723.

3 References

- [1] Hjerten, S., *Chromatogr. Rev.* 1967, 9, 122-.
- [2] Mikkers, F. E. P., Evaraerts, F. M., Verheggen, T. P. E. M., *J. Chromatogr.* 1979, 169, 11-20.
- [3] Jorgenson, J. W., Lukacs, K. D., *Anal. Chem.* 1981, 53, 1298-1302.
- [4] Harrison, D. J., Manz, A., Fan, Z., Lüdi, H., Widmer, H. M., *Anal. Chem.* 1992, 64, 1926-1932.
- [5] Manz, A., Harrison, D. J., Verpoorte, E. M. J., Fettinger, J. C., Paulus, A., Lüdi, H., Widmer, H. M., *J. Chromatogr. A.* 1992, 593, 253-258.
- [6] Effenhauser, C. S., Manz, A., Widmer, H. M., *Anal. Chem.* 1993, 65, 2637-2642.
- [7] Henry, C., *Anal. Chem.* 1996, 68, 747A-751A.
- [8] Jacobson, S. C., Hergenröder, R., Koutny, L. B., Ramsey, J. M., *Anal. Chem.* 1994, 1114-1118.
- [9] Jacobson, S. C., Koutny, L. B., Hergenröder, R., Moore, J. A. W., Ramsey, J. M., *Anal. Chem.* 1994, 66, 3472-3476.
- [10] Seiler, K., Fan, Z. H., Fluri, K., Harrison, D. J., *Anal. Chem.* 1994, 66, 3485-3491.
- [11] Effenhauser, C. S., Paulus, A., Manz, A., Widmer, H. M., *Anal. Chem.* 1994, 66, 2949-2953.
- [12] Jacobson, S. C., W., M. A., Ramsey, J. M., *Anal. Chem.* 1995, 67, 2059-2063.
- [13] Nann, A., Silvestri, I., Simon, W., *Anal. Chem.* 1993, 65, 1662-1667.
- [14] Schnierle, P., Kappes, T., Hauser, P. C., *Anal. Chem.* 1998, 70, 3585-3589.
- [15] Nagels, L. J., Poels, I., *Trends in analytical chemistry* 2000, 19, 410-417.
- [16] Baldwin, R. P., *Electrophoresis* 2000, 21, 4017-4028.
- [17] Wallingford, R. A., Ewing, A. G., *Anal. Chem.* 1987, 59, 1762-1766.
- [18] Wallingford, R. A., Ewing, A. G., *J. Chromatogr. A.* 1988, 441, 299-309.
- [19] Colon, L. A., Dadoo, R., Zare, R. N., *Anal. Chem.* 1993, 65, 476-481.
- [20] Kappes, T., Hauser, P. C., *J. Chromatogr. A* 1999, 834, 89-101.
- [21] Polesello, S., Valsecchi, S., *J. Chromatogr. A.* 1999, 834, 103-116.
- [22] Schwarz, M. A., Hauser, P. C., *J. Chromatogr. A.* 2001, 928, 225-232.
- [23] Schwarz, M. A., Hauser, P. C., *Anal. Chem.* 2003, 75, 4691-4695.

-
- [24] Schwarz, M. A., Galliker, B., Fluri, K., Kappes, T., Hauser, P. C., *Analyst* 2001, 126, 147-151.
- [25] Schwarz, M. A., Hauser, P. C., *Lab on a chip* 2001, 1, 1-6.
- [26] Lacher, N. A., Garrison, K. E., Martin, R. S., Lunte, S. M., *Electrophoresis* 2001, 22, 2526-2536.
- [27] Huang, X., Pang, T. J., Gordon, M. J., Zare, R. N., *Anal. Chem.* 1987, 2747-2749.
- [28] Huang, X., Luckey, J. A., Gordon, M. J., Zare, R. N., *Anal. Chem.* 1989, 61, 766-770.
- [29] Huang, X., Zare, R. N., Sloss, S., Ewing, A. G., *Anal. Chem.* 1991, 63, 189-192.
- [30] Huang, X., Zare, R. N., *Anal. Chem.* 1991, 63, 2193-2196.
- [31] Haber, C., Jones, W. R., Soglia, J., Surve, M. A., McGlynn, M., Caplan, A., Reineck, J. R., Krstanovic, C., *J. Cap. elec.* 1996.
- [32] Williams, R. C., Boucher, R., Brown, J., Scull, J. R., Walker, J., Paolini, D., *J. Pharm. Biomed. Anal.* 1997, 16, 469-479.
- [33] Williams, R. C., Boucher, R. J., *J. Pharm. Biomed. Anal.* 2000, 22, 115-122.
- [34] Bowman, J., Tang, L., Silverman, C. E., *J. Pharm. Biomed. Anal.* 2000, 23, 663-669.
- [35] Valsecchi, S., Tartari, G., Polesello, S., *J. Chromatogr. A.* 1997, 760, 326-332.
- [36] Gallagher, P. A., Danielson, N. D., *J. Chromatogr. A* 1997, 781, 533-540.
- [37] Musial, B. A., Martin, M. N., Danielson, N. D., *J. Sep. Sci.* 2002, 25, 311-318.
- [38] Rosso, T. E., Bossle, P. C., *J. Chromatogr. A.* 1998, 824, 125-134.
- [39] Lucy, C. A., Quanji, W., *J. Chromatogr. Sci.* 1998, 36, 33-36.
- [40] Klampfl, C., Katzmayr, M. U., Buchberger, W., Basener, N., *J. Chromatogr. A* 1998, 804, 357-362.
- [41] Katzmayr, M. U., Klampfl, C. W., Buchberger, W., *J. Chromatogr. A.* 1999, 850, 355-362.
- [42] Klampfl, C. W., Katzmayr, M. U., Buchberger, W., Basener, N., *J. High Resol. Chromatogr.* 1999, 22, 297-299.
- [43] Govindaraju, K., Cowley, D. H., Eidelman, D. H., Lloyd, D. K., *Anal. Chem.* 1997, 69, 2793-2797.
- [44] Govindaraju, K., Govindaraju, V., Eidelman, D. H., *J. Chromatogr. B.* 2003, 788, 369-376.
- [45] Mo, J., Liu, W., Mo, S., *Anal. Commu.* 1998, 35, 365-367.
- [46] McWhorter, S., Soper, S. A., *J. Chromatogr. A.* 2000, 883, 1-9.
- [47] Guijit, R. M., Baltussen, E., van der Steen, G., Schasfoort, R. B. M., Schlautmann, S., Billiet, H. A. H., Frank, J., van Dedem, G. W. K., van den Berg, A., *Electrophoresis* 2001, 22, 235-241.

-
- [48] Guijt, R. M., Baltussen, E., van der Steen, G., Frank, H., Billet, H., Schalkhammer, T., Laugere, F., Vellekoop, M., Berthold, A., Sarro, L., van Dedem, G. W. K., *Electrophoresis* 2001, 22, 2537-2541.
- [49] Galloway, M., Stryjewski, W., Henry, A., Ford, S. M., Llopis, S., McCarley, R. L., Soper, S. A., *Anal. Chem.* 2002, 74, 2407-2415.
- [50] Zuborova, M., Masar, M., Kaniansky, D., Jöhnck, M., Stanislawski, B., *Electrophoresis* 2002, 23, 774-781.
- [51] Masar, M., Zuborova, M., Kaniansky, D., Stanislawski, B., *J. Sep. Sci.* 2003, 26, 647-652.
- [52] Zuborova, M., Demianova, Z., Kaniansky, D., Masar, M., Stanislawski, B., *J. Chromatogr. A* 2002.
- [53] Pungor, E., Pal, F., Toth, K., *Anal. Chem.* 1983, 55, 1728-1731.
- [54] Pungor, E., Pal, F., Kovats, E., *Anal. Chem.* 1988, 60, 2254-2258.
- [55] Zemann, A. J., Schnell, E., Volgger, D., Bonn, G. K., *Anal. Chem.* 1998, 70, 563-567.
- [56] Mayrhofer, K., Zemann, A. J., Schnell, E., Bonn, G. K., *Anal. Chem.* 1999, 71, 3828-3833.
- [57] Fracassi da Silva, J. A., do Lago, C., *Anal. Chem.* 1998, 70, 4339-4343.
- [58] Kaniansky, D., Zelenska, V., Masar, M., Ivanyi, F., Gazdikova, S., *J. Chromatogr. A.* 1999, 844, 349-359.
- [59] Masar, M., Bodor, R., Kaniansky, D., *J. Chromatogr. A.* 1999, 834, 179-188.
- [60] Tuma, P., Opekar, F., Jelinek, I., *Electroanalysis* 2000.
- [61] Tuma, P., Opekar, F., Stulik, K., *Electrophoresis* 2002, 23, 3718-3724.
- [62] Pumera, M., Jelinek, I., Jindrich, J., *Fresenius J Anal Chem* 2001, 369, 666-669.
- [63] Fracassi da Silva, J. A., do Lago, C. L., *Electrophoresis* 2000, 21, 1405-1408.
- [64] Muzikar, J., Van de Goor, T., Gas, B., Kenndler, E., *J. Chromatogr. A* 2001, 924, 147-154.
- [65] Kappes, T., Galliker, B., Schwarz, M. A., Hauser, P. C., *Trends in analytical chemistry* 2001, 20, 133-139.
- [66] Hilder, E. F., Zemann, A. J., Macka, M., Haddad, P. R., *Electrophoresis* 2001, 22, 1273-1281.
- [67] Zemann, A. J., *Trends in Anal. Chem.* 2001, 20, 346-354.
- [68] Unterholzner, V., Macka, M., Haddad, P. R., Zemann, A. J., *Analyst* 2002, 127, 715-718.
- [69] Kuban, P., Karlberg, B., Kuban, P., Kuban, V., *J. Chromatogr. A.* 2002, 964, 227-241.
- [70] Kuban, P., Kuban, P., Kuban, V., *Electrophoresis* 2002, 23, 3725-3734.
- [71] Chvojka, T., Jelinek, I., Opekar, F., Stulik, K., *Analytica Chimica Acta* 2001, 433, 13-21.
- [72] Fracassi da Silva, J. A., Guzman, N., do Lago, C. L., *J. Chromatogr. A.* 2002, 942, 249-258.
- [73] Gas, B., Zuska, J., Coufal, P., van de Goor, T., *Electrophoresis* 2002, 23, 3520-3527.
- [74] Macka, M., Hutchinson, J., Zemann, A. J., Shusheng, Z., Haddad, P. R., *Electrophoresis* 2003, 24, 2144-2149.

-
- [75] Carvalho, A. Z., da Silva, J. A., do Lago, C. L., *Electrophoresis* 2003, 24, 2138-2143.
- [76] Kuban, P., Kuban, P., Kuban, V. 2003.
- [77] Vuorinen, P. S., Jussila, M., Siren, H., Palonen, S., Riekkola, M. L., *J. Chromatogr. A.* 2003, 990, 45-52.
- [78] Lopez-Avila, V., van de Goor, T., Gas, B., Coufal, P., *J. Chromatogr. A.* 2003, 993, 143-152.
- [79] Coufal, P., Zuska, J., van de Goor, T., Gas, B., *Electrophoresis* 2003, 24, 671-677.
- [80] Baltussen, E., Guijt, R. M., van der Steen, G., Baltussen, S., van Dedem, G. W. K., *Electrophoresis* 2002, 23, 2888-2893.
- [81] Surowiec, I., Kaml, I., Kenndler, E., *J. Chromatogr. A.* 2004, 1024, 245-254.
- [82] Kuban, P., Kuban, P., Kuban, V., *Electrophoresis* 2003, 24, in press.
- [83] Kuban, P., Kuban, P., Kuban, V., *Anal. Bioanal. Chem.* 2004, 378, 378-382.
- [84] Kuban, P., Kuban, P., Hauser, P. C., Kuban, V., *Electrophoresis* 2004, 25, 35-42.
- [85] Pumera, M., Wang, J., Opekar, F., Jelinek, I., Feldman, J., Loewe, H., Hardt, S., *Anal. Chem.* 2002, 74, 1968-1971.
- [86] Wang, J., Pumera, M., Collins, G., Opekar, F., Jelinek, I., *Analyst* 2002, 127, 719-723.
- [87] Wang, J., Pumera, M., Collins, G. E., Mulchandani, A., *Anal. Chem.* 2002, 74, 6121-6125.
- [88] Wang, J., Pumera, M., *Anal. Chem.* 2003, 75, 341-345.
- [89] Wang, J., Pumera, M., *Anal. Chem.* 2002, 74, 5919-5923.
- [90] Lichtenberg, J., de Rooij, N. F., Verpoorte, E., *Electrophoresis* 2002, 23, 3769-3780.
- [91] Laugere, F., Guijt, R. M., Bastemeijer, J., van der Steen, G., Berthold, A., Baltussen, E., Sarro, P., van Dedem, G. W. K., Vellekoop, M., Bossche, A., *Anal. Chem.* 2003, 75, 306-312.
- [92] Lin, C.-E., Chang, C.-C., Lin, W.-C., Lin, E. C., *J. Chromatogr. A* 1995, 753, 133-138.

4 Contributions and Collaborations

In nine of the ten papers presented in this publication I am the first author as I carried out the majority of the experiments and prepared the manuscripts for publication. Various co-authors contributed to the thesis to different extents.

Benedikt Galliker designed the capillary electrophoresis station on which I performed the measurements for the first paper. Maria Schwarz, also co-author of the first paper, introduced me to practical capillary electrophoresis. The third paper, chapter 2.3, resulted from a collaboration between four institutes; University of Basel, University of Oviedo, Spain, Forschungszentrum Karlsruhe and Greiner Bio-One GmbH in Germany. I carried out all the measurements except for amino acid determinations which were carried out by the exchange PhD student Eva Abad-Villar from the University of Oviedo. Teresa Fernández-Abedul and Augustín Costa-García were her supervisors. The co-authors from the two Institutes in Germany designed and fabricated the microchip on which the measurements were carried out. Karin Schweizer, a former lab-assistant in our group, helped with the preparation of the amino acid stock solutions.

Sandro Leuthardt worked on his diploma project under my supervision. He optimized the dual detection of cations and anions on PEEK capillaries (see Fig. 4, chapter 2.6). He also contributed Fig. 7 in the review article. Eva Abad-Villar, the second author of the organics on chip paper, carried out the work on surfactant analysis. I am the second author of paper eight chapter 2.8, a collaboration between our group and the Spanish University of Oviedo. I introduced Ms Abad-Villar to capillary electrophoresis and also contributed to the buffer optimisation for this paper.

5 List of Publications and Posters

1. Jatisai Tanyanyiwa, Benedikt Galliker, Maria A. Schwarz and Peter C. Hauser
Improved capacitively coupled conductivity detector for capillary electrophoresis
The Analyst , **127**, (2002), 214-218
2. Jatisai Tanyanyiwa, Sandro Leuthardt, Peter C. Hauser
Electrophoretic separations with polyether ether ketone capillaries and
capacitively coupled contactless conductivity detection
Journal of Chromatography A, **978** (2002), 205-211
3. Jatisai Tanyanyiwa, Sandro Leuthardt, Pater C. Hauser
Conductimetric and potentiometric detection in conventional and microchip
capillary electrophoresis
Electrophoresis, **23**, (2002), 3659-3666
4. Jatisai Tanyanyiwa and Peter C. Hauser
High-voltage contactless conductivity detection of metal ions in capillary
electrophoresis
Electrophoresis, **23**, (2002), 3781-3786
5. Jatisai Tanyanyiwa and Peter C. Hauser
High-voltage capacitively coupled contactless conductivity detection for
microchip capillary electrophoresis
Analytical Chemistry, **74**, (2002), 6378-6382
6. Jatisai Tanyanyiwa, Karin Schweizer and Peter C. Hauser
High-voltage contactless conductivity detection of underivatized amino acids in
capillary electrophoresis
Electrophoresis , **24**, (2003) 2119-2124
7. Jatisai Tanyanyiwa, Eva M. Abad-Villar, M. Teresa Fernandez-Abedul, Augustin
Costa-Garcia, Werner Hoffmann, Andreas E. Guber, Dirk Herrmann, Andreas
Gerlach, Norbert Gottschlich and Peter C. Hauser
High-voltage contactless conductivity detection for lab-on-chip devices using
external electrodes on the holder
Analyst, **128**, (2003) 1019-1022
8. Jatisai Tanyanyiwa, Eva M. Abad-Villar and Peter C. Hauser
Contactless conductivity detection of selected organic ions in on-chip
electrophoresis
Electrophoresis, **25**, (2003) 903 - 908

9. Jatisai Tanyanyiwa, Eva M. Abad-Villar, M. Teresa Fernández-Abedul, Agustín Costa-García and Peter C. Hauser
Detection of human immunoglobulin in microchip and conventional capillary electrophoresis with contactless conductivity Measurements
Anal. Chem., **76**, (2004), **1282 - 1288**
10. Jatisai Tanyanyiwa and Peter C. Hauser
Capillary and Microchip Electrophoresis of Basic Drugs with Contactless Conductivity Detection
Electrophoresis, **23**, (2004), **3010 – 3016**
11. Andreas E. guber, Mathias Hecke, Dirk Hermann, Alban Muslija, Volker Saile, Lutz Eichhorn, Thomas Gietzelt, Werner Hoffmann, Peter C. Hauser, Jatisai Tanyanyiwa, Andreas Gerlach, Norbert Gottschlich, Günther Knebel
Microfluidic lab-on-a-chip systems based on polymers – fabrication and application
Chemical Engineering Journal, **101**, (2004), **447 - 453**

Posters Presented at International Meetings

1. Jatisai Tanyanyiwa and Peter C. Hauser
Kontaktlose Leitfähigkeitsdetektion für Elektrophorese in konventionell Kapillaren und auf Chip
Anakon 2003, Konstanz, Germany, April 2003
2. Jatisai Tanyanyiwa and Peter C. Hauser
High-Voltage contactless conductivity detection for conventional and on-chip electrophoresis
HPLC Conference, Nice, France, June 2003
3. Eva M. Abad-Villar, Jatisai Tanyanyiwa, Teresa Fernández-Abedul, Peter C. Hauser and Agustín Costa-García
Universidade da Coruña, Campus de Elvina, Spain, October 2003
4. Jatisai Tanyanyiwa, Eva M. Abad-Villar, Peter C. Hauser
Microanalytical devices for pharmaceutical and bioscience applications with conductivity detection
HPCE 2004, Salzburg, Austria, February 2004
5. Peter C. Hauser, Jatisai Tanyanyiwa, Pavel Kuban, Eva M. Abad-Villat
High-voltage contactless conductivity detection for conventional and on-microchip electrophoresis
HPCE 2004, Salzburg, Austria, February 2004

