Akademie věd České republiky

Teze disertace
k získání vědeckého titulu "doktor věd"
ve skupině věd Chemické vědy

Capacitively coupled contactless conductivity detection in capillary electrophoresis
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název disertace

Komise pro obhajoby doktorských disertací v oboru Analytická chemie

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V Brně dne 15.5.2015

Table of contents

Summary

This dissertation describes development and application of capacitively coupled contactless conductivity detector (C^4D) in capillary electrophoresis (CE). Theoretical modelling and real experiments have proven that the external C^4D in axial arrangement can be routinely applied in CE. The sensing electrodes are positioned on the outside of the separation capillary and are not in direct contact with the separation media, which greatly simplifies the detection cell design and avoids electrodes fouling. The electrodes are directly connected to an excitation source, which is usually a function generator or an oscillator producing an AC-voltage of specific amplitude and frequency, on one side and to a detection circuitry on the other side of the detection cell. When an AC-voltage is applied to the excitation electrode, it leads to an AC-current flowing through the cell, which is picked-up at the second electrode and transformed back to an AC-voltage using an operational amplifier in the detection circuitry. By using this basic principle, conductivity changes of the solution in the separation capillary between the two electrodes can be monitored. A good fit between theoretical and experimental results shows that the C^4D can effectively be described by the simplest equivalent circuitry consisting of a capacitor, resistor and a second capacitor. The sensing electrodes represent the two capacitors and the gap between the electrodes the resistor in this equivalent circuitry. The cell constant is largely defined by the length of the detection gap and the effective electrode size is not related to the dimensions of the real electrodes but more closely to the cross-sectional area of the internal diameter of the separation capillary.

C^4D has proven to be an effective detection method in CE and due to its universality for detection of all charged species has gained a great popularity among analytical chemists. In the time period between 2004 and 2008, two independent manufacturers started to (and continue to) produce C^4Ds, which holds a great promise for its future. C^4D can be applied to bare fused silica capillaries and no detection window has to be created by removing the protective polyimide coating from the outer surface of the capillary. This greatly simplifies manipulation with the separation capillary, which is not fragile due to the exposed fused silica part, and the C^4D can be positioned in virtually any position on the capillary. Its easy adaptation for battery-powered operation ensures an additional interesting feature of C^4D, i.e. applicability in portable CE and other analytical systems. Applications of CE-C^4D have shown that fast and sensitive determination of inorganic ions can be performed in various matrices, such as, in environmental, biological, food and industrial samples. The method is, however, not restricted to analyses of inorganic species only and representative CE-C^4D analyses of organic and biochemical compounds have also been reported. In addition, the C^4D cell can easily be retrofitted to other analytical methods, such as, flow injection analysis, ion chromatography and liquid chromatography and the overall simple design with externally arranged C^4D cell makes its use in microfluidic, microchip and other down-scaled analytical techniques very convenient. Moreover, since only negligible sensitivity decrease is observed for C^4D in miniaturized separation devices, its wider applications in the recently developed micro- and nano-analytical systems can also be expected in the future.

1. List of symbols and abbreviations

AAS	atomic absorption spectrometry
BGE	background electrolyte
CAPS	N-cyclohexyl-3-aminopropanesulfonic acid
C^4D	capacitively coupled contactless conductivity detector/detection
CE	capillary electrophoresis
FIA	flow injection analysis
GC	gas chromatography
His	L-histidine
HPLC	high performance liquid chromatography
HV	high voltage
IC	ion chromatography
ICP	inductively coupled plasma
ID	internal diameter
LOD	limit of detection
MES	2-(N-morpholino)ethanesulfonic acid
OD	outer diameter
OES	optical emission spectrometry
PTFE	polytetrafluoroethylene
S/N	signal-to-noise
UV-Vis	ultraviolet-visible
V_{pp}	voltage (peak-to-peak)

2. Introduction

Analytical chemistry has undergone a dramatic change and has moved into new directions in the last decades. An increasing demand for accurate, precise and fast analytical information has created a need to develop new and improved techniques and methodologies. Analytical chemistry is a unique interdisciplinary science; all other branches of chemistry, such as physical, organic, inorganic and biochemistry as well as pharmacy, medicine, geology, mineralogy, agriculture and food science benefit from the results of analytical chemistry as a tool for producing analytical data of samples from the above mentioned scientific fields.

A significant progress has been observed in research and development of separation techniques such as gas chromatography (GC), high performance liquid chromatography (HPLC) and capillary electrophoresis (CE). These techniques are usually performed in a fully automated fashion, new materials are developed and used for more efficient and reliable analyses and new detection systems are coupled to the separation techniques for more sensitive and selective detection. Miniaturization is to date the hottest topic in analytical chemistry since lower sample and reagent consumption, faster analysis times, portability and disposability of the separation devices are often required. Capillary- and micro-chromatographic devices are therefore often applied in liquid and gas chromatography and microchip CE has been established as a complementary separation technique to conventional CE. Miniaturization, on the other hand, bears also some disadvantages, such as injection of extremely low sample volumes and detection of analytes in separation devices with significantly reduced internal diameters, which necessitates using of specifically designed injection and detection devices. In this dissertation, development and applications of a new detection scheme for CE, a capacitively coupled contactless conductivity detector (C^4D), are described. Fundamental principles of the new detection technique are complemented by selected practical applications of the CE-C^4D instrumentation.

2.1. Capillary electrophoresis

CE has been established as a separation technique in early 1980s in the first pioneering publication by Jorgenson and Lukacs [1] and since then has gained an increasing interest among analytical chemists. The main advantages of CE over other separation techniques (such as HPLC and GC) are short analysis times, high separation efficiencies, negligible sample and reagents consumption and last but not least easy implementation for automation, miniaturization and portability. The term "capillary electrophoresis" describes a group of related analytical techniques, including capillary zone electrophoresis (CZE), isotachophoresis (ITP), micellar electrokinetic chromatography (MEKC) and capillary electrochromatography (CEC) to name the most important ones that rely on separation of analytes in narrow bore capillaries induced by the action of electric field.

Fundamental principles of CE were comprehensively described in several monographs [2-4] and the basic instrumentation of a CE system is depicted in Figure 1. It consists of a high voltage power supply (HV) providing the CE system electric potential between + and − 30 kV, two platinum electrodes (Pt) placed in two electrolyte vials (E1 and E2), fused silica capillary (C, having internal diameter usually from 25 up to 100 μm), and a detector (D). The electrolyte vials and separation capillary are filled with appropriate background electrolyte.
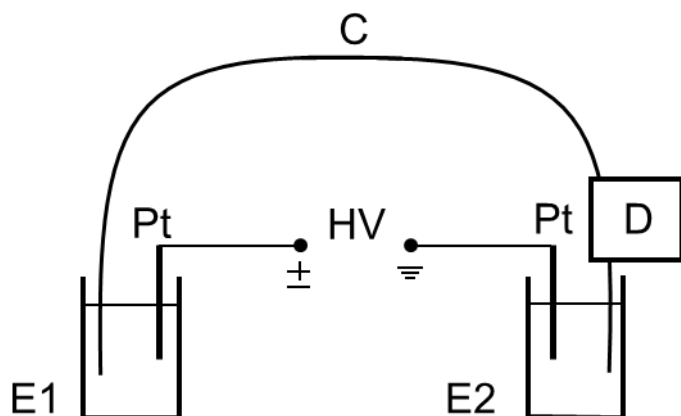
Figure 1. Schematic drawing of a CE system. E1, E2 – electrolyte vial, C – fused silica capillary, HV – high voltage power supply, Pt – platinum electrodes, D – detector.

Detection is one of the most delicate procedures in CE due to the low internal diameters of separation capillaries. The detection volumes in CE are much lower compared to those in standard separation techniques, such as, HPLC, IC and GC, and detection sensitivity in CE is thus significantly compromised. A brief overview of to-date most popular detection methods in CE, along with some less frequently used detection methods, is given in the following Section.

2.2. Detection methods in capillary electrophoresis
2.2.1. UV-Vis absorbance detection
Optical detection methods based on UV-Vis absorbance measurements are the most frequently used detection techniques in CE, partly also due to the fact that UV-Vis is the detection method of choice offered with every commercial CE instrument. UV-Vis detection can be generally subdivided into two categories, direct and indirect detection, of which the former can be applied to compounds that absorb in the wavelength region between 200 and 800 nm and the later can be applied to compounds that absorb weakly in this region or lack any suitable chromophore and do not absorb at all in this region. Organic compounds, such as aromatic hydrocarbons, phenols, benzenes, etc., can be determined using direct UV-Vis detection while inorganic compounds, such as inorganic anions and cations, metal cations, etc., are those that do not absorb and must be determined in indirect mode. Although both UV-absorbing and -transparent compounds can be detected using one common instrumentation, the main drawback of UV-Vis detection represents its low concentration sensitivity. The optical path length through separation capillaries with internal diameters usually below 100 μm is seriously restricted and, as a consequence, considerably higher limits of detection (LODs) are achieved compared to other separation techniques, such as HPLC, using UV-Vis detection. For this reason, specially designed detection cells (Z-shaped, bubble, etc.) [5, 6] have been implemented in CE-UV-Vis detection that increase the detection cell volume and detection path length. Note, however, that satisfactory LODs can be achieved for highly UV-absorbing species only in direct detection, whereas serious lack of sensitivity is encountered for most applications of indirect UV-Vis detection even with the specifically designed detection cells. A number of review articles (see, for example [7-9]) are available describing theoretical and practical aspects of UV-Vis detection in CE.

2.2.2. Laser induced fluorescence detection
Among the commercial detection methods developed for CE, laser induced fluorescence (LIF) is the most sensitive detection mode as in contrast to arc lamps used in conventional

fluorescence detection, laser beams emit highly collimated light, which is adequate for focusing the light onto the small diameter of the capillary. Only a limited number of compounds are native fluorescent agents, i.e. analytes that can be directly detected by LIF detector, and vast majority of analytes has to be derivatized with labelling dyes, which confer fluorescent properties on the analytes. The derivatization step, in which a fluorescent tag is added to the structure of the analyte, is often performed in the analyses of biochemical species and derivatization of amino acids, peptides and proteins for CE-LIF analyses is a commonly used procedure. From the applications of LIF in CE, vast majority deals with determination of biochemical species, as can be evidenced by the following review articles [10-14]. Derivatization of biochemical analytes for LIF detection is performed for two reasons. Firstly, only limited number of biochemical species exhibit native fluorescence [15] and considerable increase in LIF sensitivity can be achieved for other biochemical compounds when these are labelled with fluorescent dyes. CE-LIF is, for example, the method of choice for DNA detection in sequencing and sizing [16]. Secondly, derivatization may also provide a more suitable charge-to-mass ratio of the analytes, which assists their electrophoretic separation. Several comprehensive review articles on LIF detection in CE were published in the past, including detailed description of most often used laser sources [11], describing strategies for ultrasensitive LIF detection [16] and summarizing applications in most commonly used biochemical analyses of proteins, peptides and amino acids [12-14, 17, 18]. Main advantages of LIF detection are low noise, little loss of source light and efficient light focusing in narrow bore capillaries and channels. Note, however, that derivatization is usually a demanding and a time-consuming procedure, the recovery of derivatization processes is not absolute and many groups of analytes are not amenable to this detection technique.

2.2.3. Conductivity detection

Conductivity detection has received considerable attention as an alternative detection method in CE in particular due to its universality for determination of charged species and to the fact that the most commonly used UV-Vis absorbance detection is not suitable for sensitive detection of small ions. Conductivity detectors, which have been adopted from capillary ITP systems, were used in the early developments of CE [19] as one of the easily available detection schemes. These detectors were in contact arrangement, i.e., the sensing electrodes were placed inside or outside the separation capillary and were in direct contact with BGE solutions and samples. The contact conductivity detectors were intensively developed in the following years and different approaches for the construction of on-column and end-column CE conductivity detectors were presented [20-22]. Moreover, a commercial unit for conductivity detection in CE was also introduced [23]. The major problem in contact conductivity detection constituted electrochemical interactions of electrolyte and sample components with the sensing electrodes and their adsorption on the electrode surface, which often resulted in electrodes malfunctioning and low analytical reproducibility. Additionally, low internal diameters of CE separation capillaries caused difficulties in handling the detector cells and purpose-made capillary/cell sets had to be employed, which resulted in higher running costs of CE with contact conductivity detection. These two aspects might explain the fact that contact conductivity detection has never became widely adopted and production of commercial detectors was abandoned. A more robust alternative to contact conductivity detection was obtained by introduction of axial capacitively coupled contactless conductivity detection [24, 25], which will be discussed more in detail in Chapter 3.

2.2.4. Mass spectrometric detection

Mass spectrometry is one of the detection methods, which has been accepted as a suitable detection method for CE relatively recently. Although the first experiments on coupling the two

methods were performed already in late 80s of the last century, the popularity of CE-MS has grown substantially mainly in the last decade [26-36]. This can be attributed to the fact that in the initial stage of the CE-MS development, instrumental aspects of the interfacing part between the CE and MS systems were examined and only home-made units were available at limited number of research institutions at that time. Rapid expansion of commercial CE-MS instrumentation has followed in the beginning of the 21st century and most of the experimental set-ups use commercial systems these days. Fine-tuning of the instruments by analysts is not necessary since it is performed automatically by the commercial CE-MS system and the only analytical task is the method development, which greatly saves costs and time of the whole procedure. From the instrumental point of view, two interfaces are predominantly used in commercial units, sheet-flow and sheet-less interface, for coupling CE to MS. The advantage of the first one is its overall acceptance and high popularity in commercial units and of the second one its improved sensitivity, as no sheet liquid is used that usually dilutes the capillary effluent in the sheet-flow arrangement. Main advantages of CE with MS detection are high selectivity and applicability to a wide range of compounds, moreover at reasonable detection sensitivity. Determination of various compounds can be achieved with CE-MS instrumentation and numerous review articles were published on the topic in the last several years. Applications of CE-MS were reviewed in the field of proteomics and metabolomics [29, 30, 33, 34, 37, 38], drug analysis [39], food analysis [40], biosciences [35, 41-44] and also in other applications [45-48].

2.2.5. Other detection methods

Other detection methods commonly used in CE are electrochemical (amperometric, potentiometric), chemiluminescence, and hyphenated detection techniques such as inductively coupled plasma (ICP), atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS) and optical emission spectrometry (OES) with or without combination with MS.

Electrochemical methods suitable for detection in CE include also conductometry, which has been discussed in the above Section and will therefore not be included in this paragraph. Unlike UV-Vis absorption detection methods, which suffer from severe sensitivity loss when miniaturized, electrochemical methods offer high sensitivity in CE applications. Problems in electrochemical detection arise from the need to isolate the small detection potential from the CE high voltage and current and from the need for precise alignment of the separation capillary with working and reference electrodes, which is often difficult in the separation capillaries with narrow internal diameters. Moreover, amperometric detection can be applied solely to species that can undergo redox reactions, potentiometric detection is selective to several species only and its sensitivity is generally one to two orders of magnitude lower than that of conductometric and amperometric detection [49].

Chemiluminescence (CL) detection uses no light source, which avoids stray light and source instability problems, and results in low background noise and excellent sensitivity [50, 51]. CL is a very sensitive method and in most applications lower LODs are achieved compared to CE with other detection methods. Applications of CE-CL are, however, seriously limited since only a restricted number of compounds can be detected using the CL technique. Other complications in CL detection can be encountered with the construction of the post-capillary reaction cell and due to the fact that limited number of CL reagents can be used in CE. In addition, applications of CE-CL in analysis of real samples are often unsatisfactory, probably due to matrix interferences.

Hyphenated detection methods are often used in determination of metalloid compounds that are not easily and sensitively detected using other detection methods and are also often applied in speciation analysis. Michalke [45] and Alvarez-Llamas *et al*. [46], for example, have addressed

theoretical aspects and applications of ICP-MS in CE for elemental speciation. CE is also often combined with AAS and AFS for speciation analysis of inorganic and organic mercury compounds [52] and with OES and ICP-OES for speciation analysis of other metalloid compounds that can easily generate hydrides [53].

Less frequently, other detection methods are used in CE, such as Raman spectroscopy, infrared, nuclear magnetic resonance, thermal lens and refractive index detection [54]. Note, however, that these methods are species specific, can be used in selected applications only and are rarely used in CE compared to above discussed detection schemes.

3. Capacitively coupled contactless conductivity detection

Contactless conductivity detection, based on AC-voltage capacitively coupled to the detection cell is more easily implemented and a more robust alternative to contact conductivity detection. C^4D was first adapted for electromigration separations in the early 1980s for the ITP determination of small anions by Gaš and coworkers [55, 56], who used a radial C^4D cell. The diagram of the C^4D for ITP and design of the radial C^4D cell is depicted in Figure 2A and 2B, respectively.

A   B
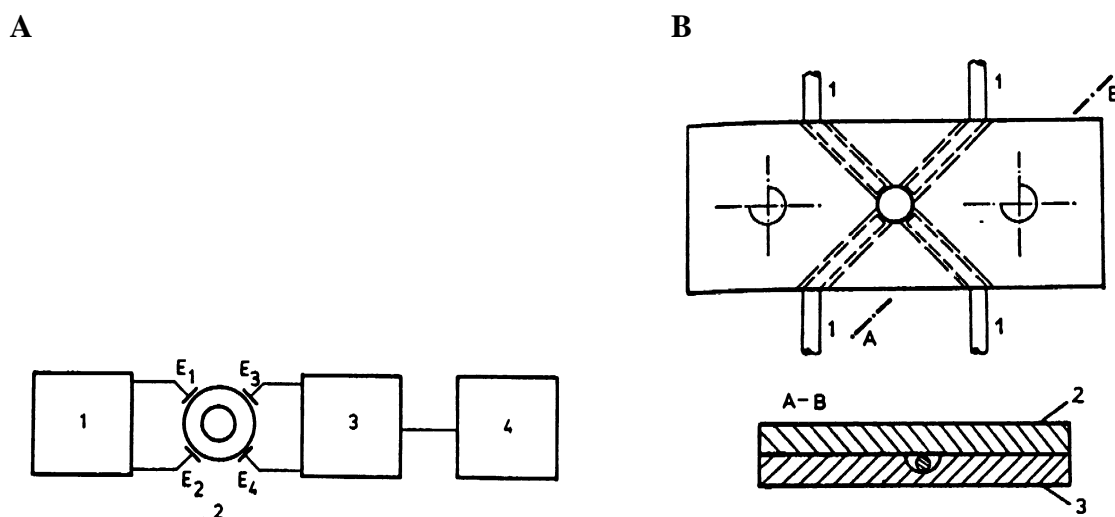

Figure 2. (A) Schematic drawing of the C^4D from Gaš et al. [55]. 1 – Function generator, 2 – detection cell, 3 – receiver, 4 – recorder, E1 and E2 – excitation electrodes, E3 and E4 – pick up electrodes. (B) Arrangement of the detection cell from Gaš et al. [55]. 1 – Cu electrodes, 2 – upper part of a shielding, 3 – lower part of a shielding.

The cell was based on four thin wires that were placed perpendicularly around the circumference of the separation tubing for capacitive coupling of the signal into and out of the solution. The design of the radial C^4D cell was later adopted by several other research groups and was used for various ITP applications, that were reviewed in general (e.g. [57]) and in specialized review papers dealing with contact and contactless conductivity detection [58-63]. A significant contribution to C^4D was made independently by Zemann *et al.* [24] and Fracassi da Silva and do Lago [25] by introduction of axial C^4D. In this arrangement, the C^4D cell was based on two tubular electrodes, which encompassed a standard separation capillary and were positioned side by side along the capillary axis. A gap between the electrodes was formed with dimensions of usually one up to several millimetres, which acted as the detection cell. Nowadays, the two sensing electrodes in C^4D are usually separated by a gap of several hundred μm to 2 mm. Conductivity of the solutions was monitored in that part of the separation capillary, which was positioned between the electrodes in the C^4D cell and the signal was recorded using a conventional analog-to-digital converter. The two designs of the axial C^4D from original papers by Zemann *et al.* and Fracassi da Silva and do Lago are depicted in Figure 3A and 3B, respectively.

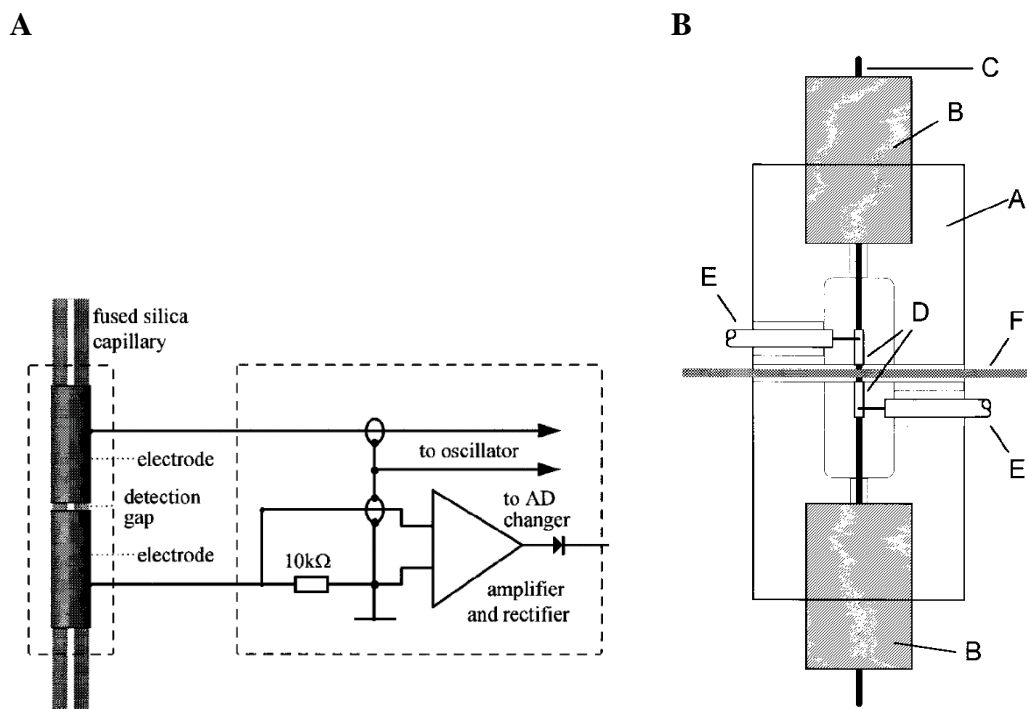**A**   **B**

Figure 3. (A) Original C⁴D schematics by Zemann et al. [24]. (B) Original C⁴D schematics by Fracassi da Silva and do Lago [25], A – Plexiglas base, B – silicon septa, C – separation capillary, D – electrodes, E – electrode terminal, F – Faraday shield.

The fact that C^4D responds to all charged species and can therefore be considered almost universal, that the external electrodes cannot deteriorate and also due to the simplicity of the detection cell design and detector's electronic circuitry has led to a massive popularization of C^4D in the last decade. Principles of C^4D have been addressed in a number of theoretical papers [64-69] and several comprehensive reviews were published in recent years on C^4D in conventional and microchip CE summarizing theoretical aspects as well as practical applications [58, 59, 62, 63, 70-76]. Moreover, other review papers were published on conductivity and electrochemical detection in CE, which included a substantial part on C^4D [60, 61, 77, 78]. The description of basic instrumental set-ups of C^4D are briefly summarized in the following paragraphs. A detailed description of fundamental principles of C^4D supported with theoretical models and experimental measurements is presented in Chapter 5.

Electrodes for axial C^4D in conventional CE were cut from stainless steel syringe needles [24, 79], painted on capillaries with silver varnish [25], made of soldered coils of copper wire [80, 81] and of strips of aluminium foil [82, 83]. The basic C^4D cell configuration in the mentioned designs was, however, almost identical and is illustrated in Figure 4A. A grounded Faraday shield [25, 84], usually made of a thin metal foil (copper or aluminium), has been often positioned between the two sensing electrodes (see Figure 4B) in order to minimize direct capacitive coupling between the two electrodes and to minimize stray capacitance, which affects the C^4D performance [65]. In the simplest possible equivalent circuitry for the detection cell (see Figure 4C), the two electrodes form capacitors (C) with the electrolyte solution inside the separation capillary, which are connected by a resistor (R) formed by the solution in the capillary between the electrodes. C_0 represents a stray capacitance, which originates from direct capacitive coupling between the two sensing electrodes when a Faraday shield is not used or when the shielding is not efficient.
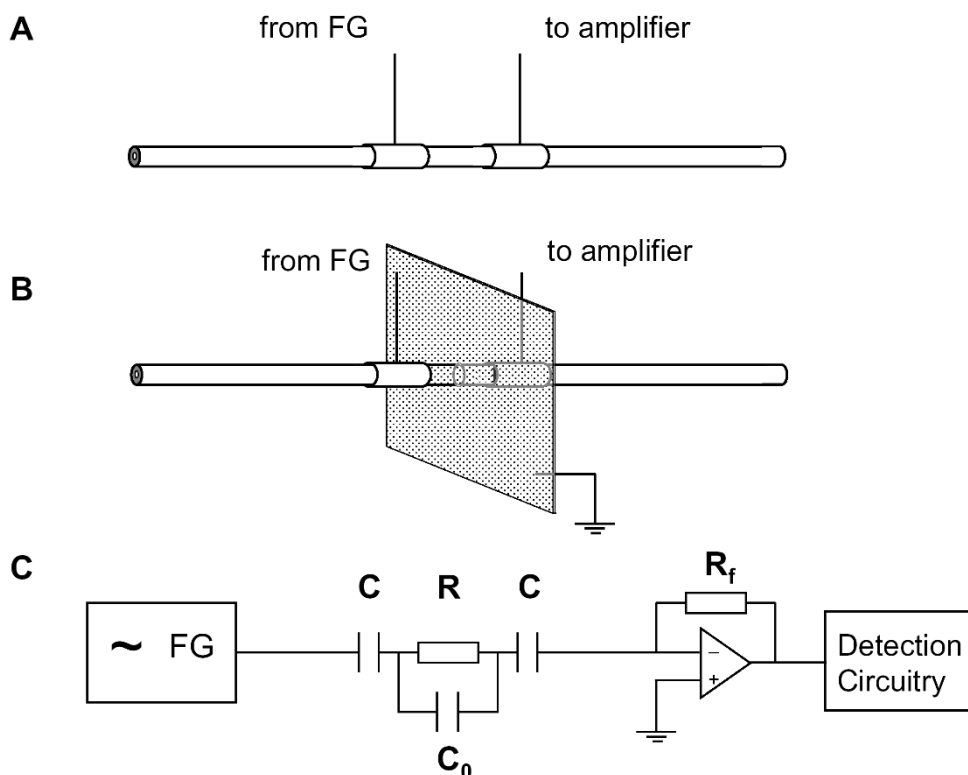
Figure 4. (A) Schematic drawing of the C⁴D cell without shielding; (B) schematic drawing of the C⁴D cell with shielding; (C) equivalent circuitry representing the cell without shielding, FG – function generator, C – cell capacitance, R – cell resistance, C_0 – stray capacitance, R_f – feedback resistor on the operational amplifier [59].

If an AC-voltage is applied to the excitation electrode it leads to an AC-current flowing through the detection cell, which is picked-up at the pick-up electrode and transformed back to AC-voltage using an operational amplifier in an appropriate configuration. Several authors have studied effects of different cell parameters on the C⁴D performance and some alternative designs were also examined. A dual C⁴D-photometric detector was developed for simultaneous conductivity and absorbance determination of inorganic and organic compounds in pharmaceutical solutions [81] and was later followed by a considerable number of publications describing dual detectors based on C⁴D and absorbance [85-87], C⁴D and fluorescence [88] and even three-in-one detector [89] combining C⁴D, absorbance and fluorescence detection. A review on dual detectors in CE is also available [90].

The sensing electrodes for the C⁴D cell construction were also examined and several designs, including tubular, semi-tubular and planar electrodes were applied [69, 82]. It has been shown that the overall performance of the C⁴D has not changed significantly when semi-tubular electrodes were used instead of tubular electrodes and only marginal decrease in sensitivity was observed. A more significant sensitivity decrease was obtained when planar electrodes were used, which was attributed to lower signal coupling due to improper fit between the planar electrodes and tubular separation capillaries [87]. The length of the sensing electrodes was found insignificant with respect to detector performance and detection sensitivity [24, 69, 79] and electrode lengths between 3 and 50 mm were used in most designs published in the literature. Similarly, no effect on detector performance was observed when square wave or triangular wave excitation signal was used instead of sine wave [24, 91-93].

Tanyanyiwa *et al.* [84] proposed cell excitation at several hundred volts for better signal-to-noise (S/N) ratio, while Fracassi da Silva *et al.* [80] suggested that the use of a considerably lower excitation voltage of 2 V_{pp} resulted in the same detector sensitivity as the commonly used 20 V_{pp}. A miniaturized C^4D built on a printed circuit board with dimensions of 18×18 mm was presented by Fracassi da Silva *et al.* [80] and several other authors have designed small C^4D cells that could be incorporated into existing commercial CE instruments [94-96].

In 2004, a commercial C^4D was presented with a detection cell that can be fitted into the cassettes of most commercial CE systems [97], which was followed by a second manufacturer starting to produce C^4Ds in 2008 [98]. Applications of the commercial C^4D units in analytical separations have been continuously published in the last decade (see, for example, the following review papers [70-72]) showing that C^4D was accepted as a detection scheme suitable for use in home-made as well as in commercial CE instruments.

C^4D was originally developed as a detection technique for CE, nevertheless, it was used in other separation techniques in the past, for example in flow based separation techniques, such as CEC[94, 99], IC [100-102], FIA [103-110] and HPLC [111-115]. The concept of C^4D was also shown suitable for applications in microchip and microfluidic devices [73, 74], note however, that theoretical principles of C^4D in microchip CE are slightly different to those in conventional CE [116]. In addition, C^4D was used in miniaturized liquid chromatography and applications in capillary IC [101, 117-131] and capillary HPLC [132] were demonstrated.

4. Motivation for combination of CE with C^4D

Since its introduction, CE has proven to be a powerful analytical method, however, a serious drawback of CE, its low detection sensitivity, has been revealed already in the early days of the method development. The sensitivity of commercially available detection methods is adequate for many analytes, nevertheless, several groups of compounds are not detectable sensitively using these detection schemes or are not detectable at all. This applies to compounds that are non-UV absorbing, do not possess fluorophores and/or cannot undergo mass fragmentation in order to be detected using one of the three commonly available detection schemes of UV-Vis, LIF and MS detection, respectively. Such compounds must be derivatized (for LIF detection), detected using indirect detection modes (e.g. indirect UV-Vis and fluorescence detection), whose sensitivity is significantly reduced, or using alternative detection methods, of which amperometry, conductometry and chemiluminescence are the most popular techniques. Conductometric detection has received considerable attention as an alternative detection method in CE in particular due to its universality for determination of charged species as has been demonstrated on examples of small inorganic ions [23, 133] in late 1990s and resulted into commercial production of a contact conductivity detector [23]. However, the contact conductivity detection has never became widely adopted, probably due to omnipresent interaction of BGE solution and sample components with electrode surface and difficulties with handling the small detection cells, which had to be employed for each capillary.

C^4D has been introduced as a more robust alternative to contact conductivity detectors for two reasons. Firstly, C^4D is based on two external electrodes, which are placed on the outside of the separation capillary and are not in direct contact with liquids flowing through the capillary. This completely changes the function of the sensing electrodes since as opposed to contact conductivity detectors one set of C^4D electrodes can be used virtually without any time limitations. Secondly, the precise alignment of the sensing electrodes inside a separation capillary is avoided as the C^4D electrodes are placed externally on the capillary and the detection cell dimensions are constant for all applications and separation media as the electrodes are fixed in their exact positions in the external cell. Electronic circuitry of C^4D is very simple and can be easily built by a skilled person, additionally, it requires only very little service and maintenance. Moreover, production and running costs of a C^4D are very low as opposed to other detection methods, such as UV-Vis absorbance (spare deuterium lamps), LIF (expensive laser sources) and MS (high initial investment and high running costs) and its field of applications is relatively broad. A brief summary of suitability of C^4D and the commercially available detection methods for CE determination of inorganic, organic and biochemical ions and molecules is given in Table 1.

Table 1. Suitability of detection methods for CE determination of selected compounds.

	inorganic	organic	biochemical
C^4D	excellent	limited	good
UV-Vis	limited	excellent	limited
Indirect UV-Vis	good	limited	limited
LIF	limited	limited	limited
LIF derivatization	limited	good	excellent
MS	limited	excellent	excellent

It can be seen from Table 1 that although C^4D was originally intended for determination of inorganic compounds and best results of CE-C^4D are logically achieved in inorganic analyses, the applicability of CE-C^4D has been also extended in course of the time to determination of organic and biochemical species [58, 59, 62, 63, 70-76]. Hardly any detection technique can compete with conductivity detection in the field of inorganic analysis in terms of universality, sensitivity and simplicity and therefore this is the part of separation sciences, where CE-C^4D was generally accepted. The applications of C^4D in detection of organic analytes are limited due to the polar character of most organic compounds and C^4D sensitivity is not as good as for UV-Vis and MS detection, nevertheless, a considerable amount of scientific papers was published on CE-C^4D determination of organic ions and molecules [58, 59, 62, 63, 70-76]. Similarly, a significant contribution of C^4D has been noticed in CE determination of biochemical species [58, 59, 62, 63, 70-76]. The main advantage of C^4D over LIF detection is that no derivatization is required for detection of biochemical species and direct detection of amino acids, peptides and proteins is readily possible. The sensitivity of C^4D, of course, cannot compete with the sensitivity of LIF in derivatization mode, the simplicity of the analyses without an additional derivatization step is, however, an interesting feature of C^4D.

It is therefore evident that C^4D should not be considered as a replacement method for any of the commercially available detection mode in CE, it should be considered more as a complementary method to the detection methods available. It can be seen as a detection method of choice for CE determination of inorganic species and as a suitable detection method in CE determination of biochemical and selected organic compounds. Its main power lies in the fact that many organic and biochemical species are intact to UV-Vis and LIF detection, but conduct in liquid solutions and can therefore be determined by means of CE-C^4D. Additionally, one remarkable feature of C^4D is that although originally designed for CE, the same detection system can be applied in other analytical techniques, such as, in IC, HPLC and FIA and in micro-scaled analytical techniques, such as, in microchip electrophoresis, capillary-HPLC/IC and micro-FIA [58, 59, 62, 63, 70-76].

5. Fundamental aspects of C⁴D

The design of the developed and examined C^4D is depicted in Figure 5. The C^4D cell (lower part of the Figure) was accommodated in a metallic case, which fully encompassed the Plexiglas body, Faraday shield and current-to-voltage converter circuitry (OPA1) and contained two sockets where cables from voltage booster and to the detection circuitry were plugged in (IN and OUT in Figure 5). The IN and OUT connectors were extended using two Cu wires with soldered Cu stripes that were attached to the sensing electrodes and ensured contact between the electrodes and corresponding electronic circuits. The excitation voltage from the function generator (FG) or oscillator was boosted using a booster amplifier (PA91) to desired amplitude, connected to the IN connector and to the excitation electrode (E1). Two PTFE spacers (PTFE) and a Faraday shield (F) were placed in the middle of the cell in order to form a detection gap with exact dimensions and to minimize direct capacitive coupling between the electrodes, respectively. The AC-current flowing through the detection cell was then picked up by the second electrode (E2) and fed to current-to-voltage converter (OPA1) where it was converted to AC-voltage. The voltage was then rectified (OPA2), amplified (OPA3) and converted to digital form using an analog-to-digital (A/D) converter. OPA2 and OPA3 operational amplifiers were part of the detection circuitry, which was placed in a separate metallic case.
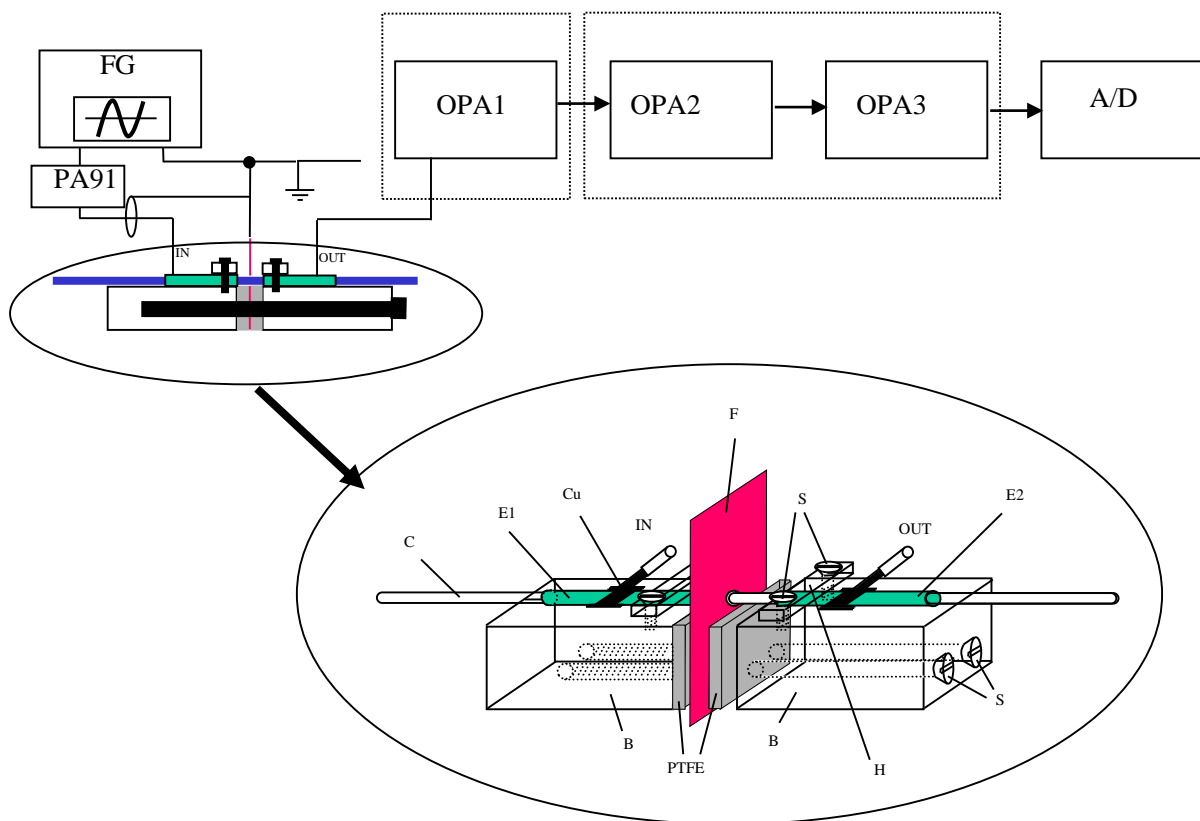

Figure 5. Schematic diagram of the C⁴D with flexible detection cell. FG – function generator/oscillator, OPA1 – current-to-voltage converter, OPA2 – rectifier, OPA3 – amplifier, PA91 – booster amplifier, A/D – analog-to-digital converter, C – separation capillary, E1 – excitation electrode, E2 – pick-up electrode, F – Faraday shield, IN – connector, OUT – connector, Cu – copper stripe, S – screws, B – Plexiglas body, PTFE – PTFE spacers, H – holders.

5.1. Modelling the electronic behaviour of C^{4}D

A schematic drawing of the standard axial C^{4}D is shown in Figure 4A. Typically two cylindrical metallic tubes act as the electrodes and are separated by a gap of several hundred μm to several mm to form the detection cell. In order to avoid direct capacitive coupling between the two electrodes a grounded Faraday shield may be employed as shown in Figure 4B. In the simplest possible equivalent circuitry for the cell, shown in Figure 4C, the two electrodes form capacitors (C) with the inside of the capillary, which are connected by a resistor (R) formed by the BGE solution. C_0 is the stray capacitance, which arises from direct coupling between the electrodes if the Faraday shield is not used. The application of an AC-voltage to the excitation electrode leads to an AC-current flowing through the cell, which is picked up at the second electrode and is transformed back to an AC-voltage at the pick-up amplifier according to the equation:

$$V_{out} = -i\, R_f \tag{1}$$

where V_{out} is the output voltage, i is the cell current and R_f is the feedback resistor value on the pick-up amplifier.

The output of the pick-up amplifier is given by the following equation, which accounts for the frequency dependence of the cell impedance (see also [134]):

$$V_{out} = -\frac{\dfrac{V_{in}}{1 + j\, 2\pi f\, R\, C_0}}{j\, 2\pi f\, (C + C_0)\left[1 + \dfrac{j\, 2\pi f\, R\, C\, C_0}{C + C_0}\right]}\, R_f \tag{2}$$

where V_{out} is the output voltage, V_{in} is the input voltage, j is the imaginary unit, f is the frequency, R_f is the feedback resistor value on the pick-up amplifier, R is the cell resistance, C is the cell capacitance and C_0 is the stray capacitance. If the stray capacitance (C_0) is absent, the equation is much simplified and corresponds to a simple, single pole high pass filter (composed of a resistor and one capacitor only).

The frequency behaviour of electronic components is usually visualized with so-called Bode plot, a log-log plot of the gain or output voltage *vs.* frequency. A Bode plot for the simple equivalent circuitry of Figure 4B without stray capacitance is depicted in Figure 6A. In Figure 6B, Bode plots for the cell model with different levels of stray capacitance are depicted. A new contribution to the signal appears at the high frequency end, which is additive to the signal of interest. The plateau region is shortened and for the higher levels of stray capacitance completely obscured. Note, that even small stray capacitances may strongly affect the signal of interest as in practice small changes of conductivity have to be monitored on top of a background signal.
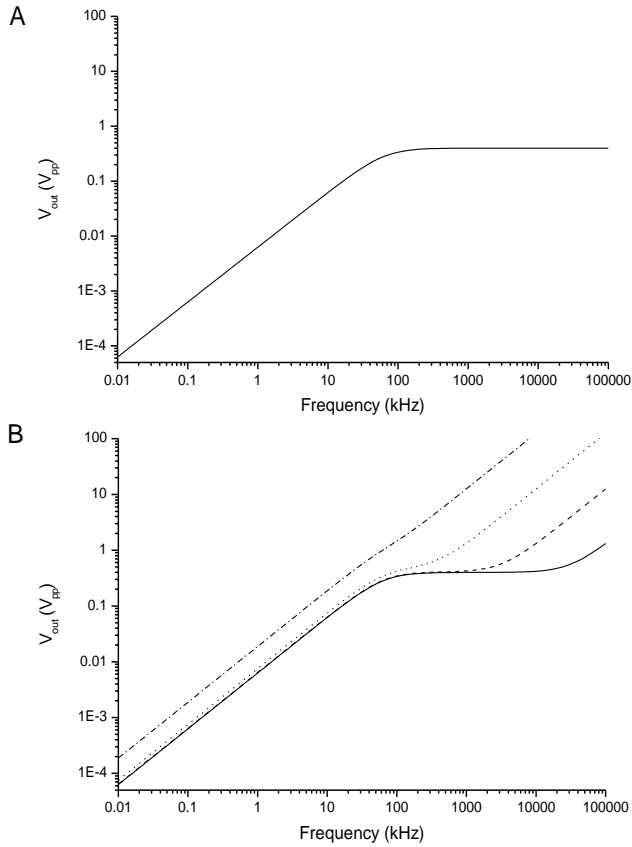
Figure 6. (A) Modelled Bode plot for the C^4D cell with shielding, R = 50 MΩ, C = 0.1 pF, R_f = 1 MΩ. (B) Modelled Bode plots for the C^4D cell without shielding, C_0 = 0.0001 pF (——), C_0 = 0.001 pF (------), C_0 = 0.01 pF (······) and C_0 = 0.1 pF (·-·-·-), other values are the same as in A [64].

5.2. Frequency behaviour of real C^4D cell

It has been found that the frequency behaviour is not only dependent on the properties of the passive components but also on the performance of the operational amplifier employed. Bode plots measured for two amplifiers, an OPA606 and an OPA655, are shown in Figure 7. The OPA606 shows a peak in gain, which is not present for the other amplifier. This means that for the OPA606 the output voltage in the region of the peak is actually higher than defined by equation 2. The output signal of the OPA655 shows an almost perfect fit with the behaviour modelled for the equivalent circuitry in Figure 6A reaching the plateau at approximately 300 kHz. The fact that no rise in signal is obtained at the high frequency end indicates that no or only negligible stray capacitance is present for the cell used. For the OPA606, the output signal shows a perfect fit in the low-frequency range (1-200 kHz) only. For higher frequencies the non-ideal behaviour of the amplifier prevails and the output signal increases steeply, peaks at 700-750 kHz and then drops down without forming a plateau.
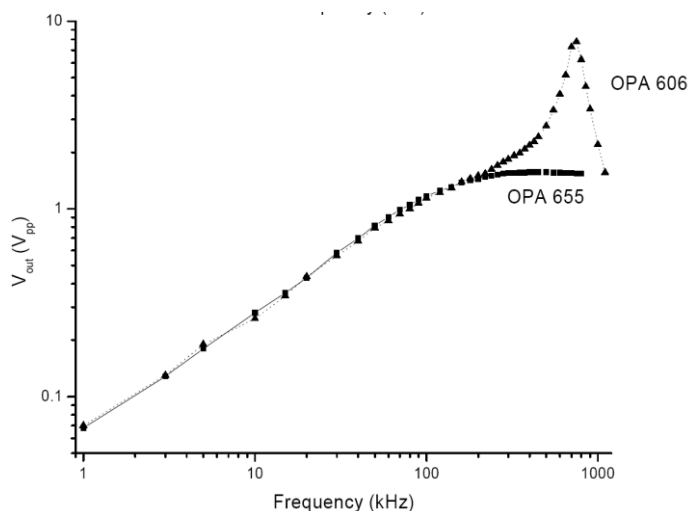
18

Figure 7. Bode plots measured for a real C⁴D cell; capillary: 50 μm ID and 375 μm OD; BGE solution: 10 mM His and 1.25 M acetic acid (pH 2.75) [64].

The Bode plots in Figure 7 clearly demonstrate that the choice of the optimal frequency is very straightforward for a unity gain stable amplifier. In other words, once the critical frequency value (the beginning of the plateau) is found, virtually any higher frequency can be used for excitation without compromising the performance of C^{4}D since the output signal remains constant throughout the whole plateau.

5.3. Effect of the cell geometry on the frequency behaviour
The effect of the electrode length was investigated with a C^{4}D cell fitted with four different lengths of electrodes (2, 4, 6 and 8 mm). Two electrodes of identical length were separated by a gap of 2 mm in all measurements. All four plots in Figure 8 show similar shapes with small differences in the lower frequency range (3 – 150 kHz) only and reach the plateau at nearly identical frequency value (260 – 280 kHz) and with nearly the same output voltage magnitude (1.29 – 1.34 V).
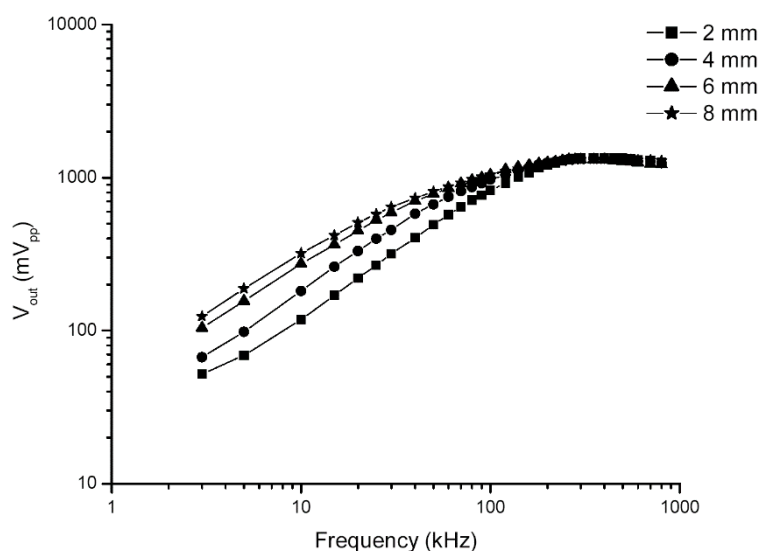

Figure 8. Bode plots measured for different electrode lengths. Gap between the electrodes: 2 mm. Capillary: 50 μm ID and 375 μm OD, BGE solution: 10 mM His and 1.25 M acetic acid (pH 2.75) [64].

19

These experimental results lead to two conclusions. Firstly, the ohmic resistance of the C^4D cell remains approximately the same independently of the electrode length. This is in agreement with the assumption that the behaviour of the actual cell can be approximated by defining the cell length (l) as the distance between the electrodes and the cell area (A) by the cross-section of the open part of the capillary. This model for the C^4D is depicted in Figure 9. Furthermore, the fact that the signal at high frequencies is identical for the different electrode lengths means that different electrodes have no or only a negligible effect on the C^4D signal intensity and appearance of the electropherogram. Secondly, the capacitance of the C^4D cell changes with different electrodes lengths demonstrating that the C^4D cell capacitance increases for longer electrodes.
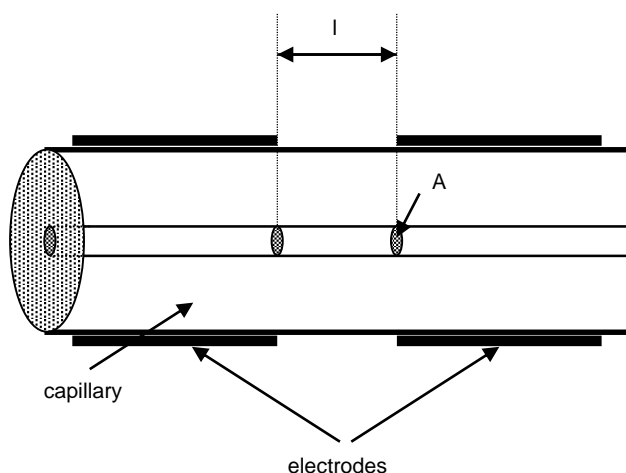

Figure 9. Sketch of the cell model that describes the basic behaviour of the C^4D [64].

The effect of the size of the detection gap was investigated with a C^4D cell consisting of two 4 mm long electrodes that were separated by a gap of 1, 2, 3 and 5 mm. The resulting plots are depicted in Figure 10 and follow the expected trend, *i.e.* the plateau level is the lower the longer the detection gap. The fact that for the larger gaps the plateau is shortened at high frequencies is a deviation from the expected behaviour. In any case, the different shapes of the frequency responses imply that optimization in that regard is dependent on the gap size. Increasing the detection gap size is expected to linearly increase the cell resistance and the experimental data derived from Figure 10 perfectly follow the values calculated from the geometrical parameters of the cell.
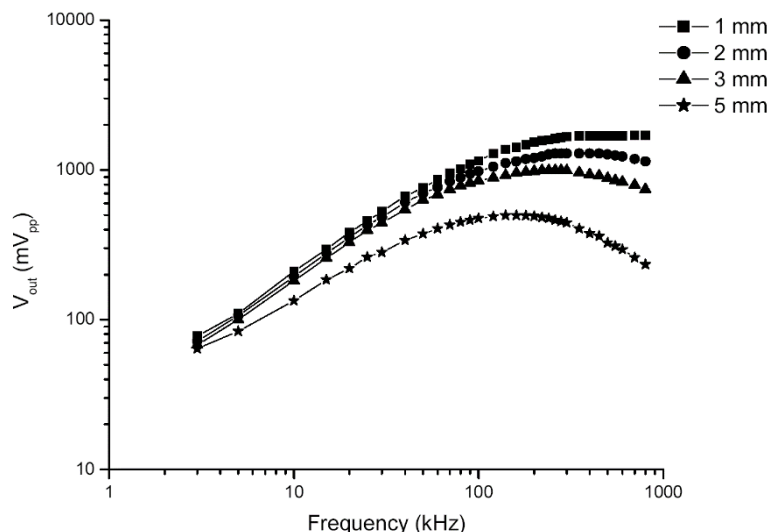
Figure 10. Bode plots measured for different gap sizes between the electrodes, electrode length: 4 mm. Capillary: 50 μm ID and 375 μm OD, BGE solution: 10 mM His and 1.25 M acetic acid (pH 2.75) [64].

5.4. Effect of the frequency on the detection of peaks

The effect of the excitation frequency on detection of electrophoretic peaks was examined with a C^4D cell consisting of two 4 mm electrodes separated by a 2 mm detection gap. Six electropherograms were recorded at excitation frequencies of 10, 30, 100, 250, 400 and 800 kHz for a standard solution of 10 mg L^{-1} K^+, Ca^{2+}, Na^+, Mg^{2+} and Li^+ and are depicted in Figure 11. It is obvious that only frequencies from the plateau region of the Bode plot ensure maximal output signal and best performance of the C^4D cell.

An artefact, known as peak overshooting, was observed in the recorded electropherograms and the corresponding overshoots are clearly visible in Figure 11 for frequencies from the rising part of the Bode plot. Furthermore, the non-optimal C^4D behaviour at low frequencies has also a direct bearing on CE-C^4D linearity as is evidenced in Table 2. The calibration curves for 20 kHz show significant non-linear behaviour that is especially pronounced for the peak areas for the three peaks (Ca^{2+}, Na^+ and Mg^{2+}), which are most seriously affected by the overshooting phenomenon.

Table 2. Correlation coefficients of the calibration curves measured at two different excitation frequencies, calibration range 1-10 mg L^{-1}, four point calibration, *n = 3*.

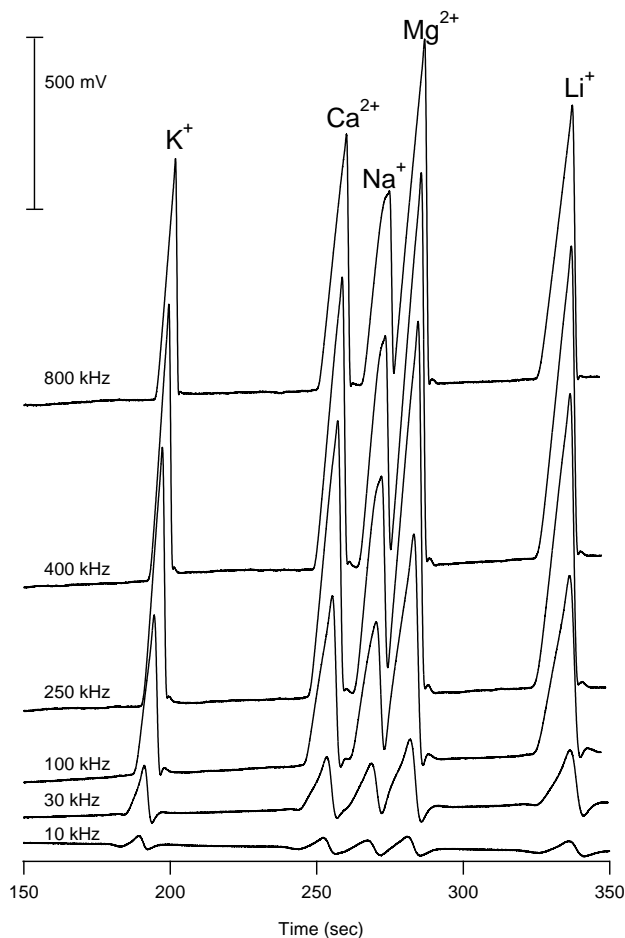|	Peak Height	Peak Area	Peak Height	Peak Area
	20 kHz		400 kHz	
K^+	0.9923	0.9992	0.9975	0.9998
Ca^{2+}	0.9978	0.9943	0.9974	0.9987
Na^+	0.9992	0.9907	0.9999	0.9998
Mg^{2+}	0.9975	0.9941	0.9985	0.9996
Li^+	0.9981	0.9984	0.9987	0.9997

Figure 11. Effect of the excitation frequency on the appearance of the electropherograms. CE conditions: capillary: 60/43 cm total/effective length, HV = 17.5 kV, BGE solution: 10 mM His and 1.25 M acetic acid (pH 2.75), hydrodynamic injection with capillary end elevated to a height of 20 cm for 10 s [64].

5.5. Effect of the excitation voltage on the signal-to-noise ratio

The effect of excitation voltage magnitude on S/N ratios is depicted in Figure 12. Clearly the use of the elevated voltages leads to a significant improvement in the S/N ratio. For frequencies near or at the plateau region, the increase of the S/N ratio is nearly proportional to the excitation voltage. The following conclusions were drawn using a more comprehensive fundamental study. Firstly, the S/N ratios are generally best for BGE solutions of low conductivity. Secondly, the S/N ratios improvement achieved by the excitation voltage increase is also dependent on the conductivity of the electrolyte solution and again best for low conductivity solutions. Thirdly, it is not possible to compensate for the use of a lower excitation voltage (and thus lower S/N ratios) by increasing the value of the feedback resistor on the pick-up amplifier.
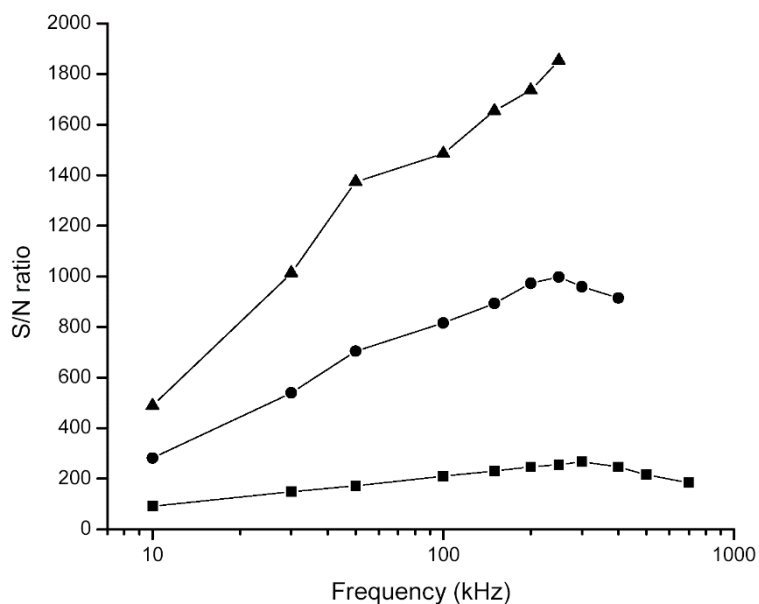
Figure 12. Effect of the excitation voltage on the S/N ratio for potassium peak: 20 V$_{pp}$ (—■—), 100 V$_{pp}$ (—●—), 200 V$_{pp}$ (—▲—). BGE solution: 10 mM CAPS/Arg (pH 9.9), HV = 17.5 kV [65].

5.6. Effect of stray capacitance on the S/N ratio and peak shapes

Stray capacitance, the direct capacitive coupling between the two excitation electrodes of a C^{4}D cell, is an undesired effect established by a current bypass, leads to an elevated background signal and adversely affects the C^{4}D performance. The effect of stray capacitance on the S/N ratios was investigated by removing the copper Faraday shield from the C^{4}D cell. Plots of the output voltage of the detector against frequency are given in Figure 13 for three different BGE solutions along with a frequency plot for a dry, air filled, capillary.
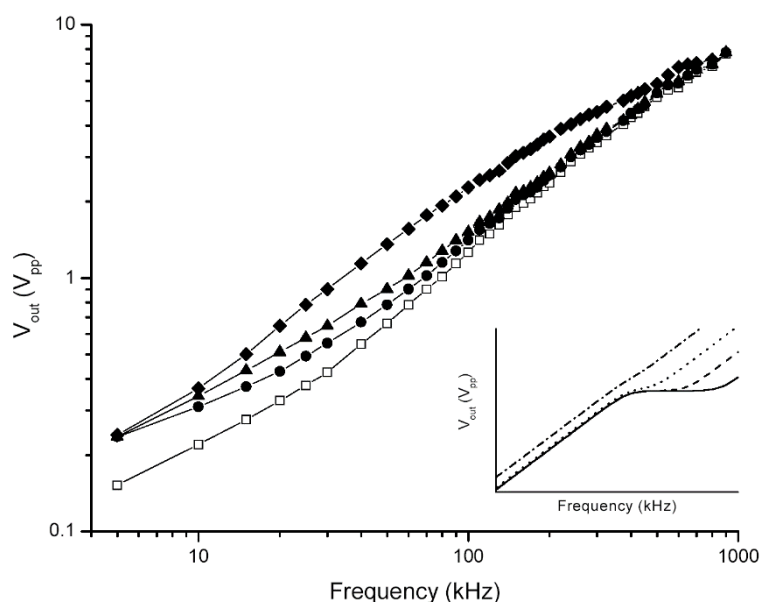

Figure 13. C^{4}D cell with stray capacitance. Effect of the excitation frequency on the output signal. Excitation voltage: 20 V$_{pp}$. Capillary filled with: air (—□—), 10 mM CAPS/Arg (—●—), 10 mM MES/His (—▲—), 10 mM His and 1.25 M acetic acid (—◆—). Insert: modelled frequency plots for different values of stray capacitance (see Figure 6B) [65].

23

All four plots showed shapes very similar to the modelled frequency behaviour, which can be expected in the presence of significant stray capacitance (see Figure 6B and the insert in Figure 13). The plateau region with stable output signal was neither achieved for the three BGE solutions nor for the dry capillary and was in stark contrast to usual behaviour of the cell with proper shielding between the excitation electrodes.

While the presence of stray capacitance had no effect on the measured sensitivity in terms of peak heights, the presence of direct capacitive coupling between the electrodes led to a deterioration of the S/N ratios (see Figure 14). This is particularly pronounced for the higher frequencies where the background signal due to the stray capacitance is highest, but also where analytical signal has its highest sensitivity.
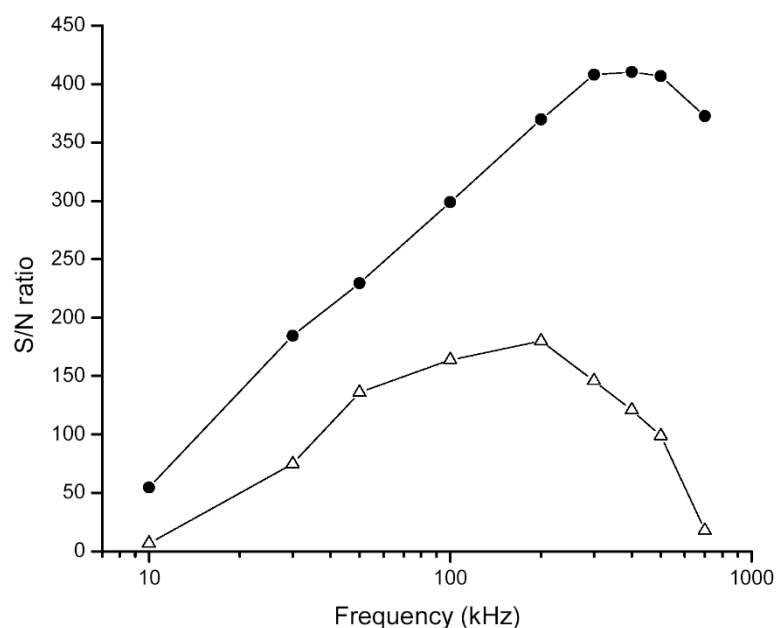

Figure 14. Comparison of S/N ratios for the cell arrangement without (——●——) and with (——△——) stray capacitance for potassium peak. Excitation voltage: 20 V_{pp}. BGE solution: 10 mM MES/His (pH 6.0), HV = 17.5 kV [65].

In addition to poor S/N ratios, the presence of stray capacitance affected the shapes of the peaks and led to overshooting phenomena. In the presence of direct coupling between the electrodes this effect was found to be most significant at high frequencies where the contribution of the stray capacitance to the background signal was highest. Figure 15 depicts CE-C⁴D traces acquired for potassium (A) and for calcium, sodium and magnesium (B) ions in presence (a) and absence (b) of the shield and clearly illustrates the effect of stray capacitance on the CE-C^4D sensitivity and on the peak shape deterioration.

24
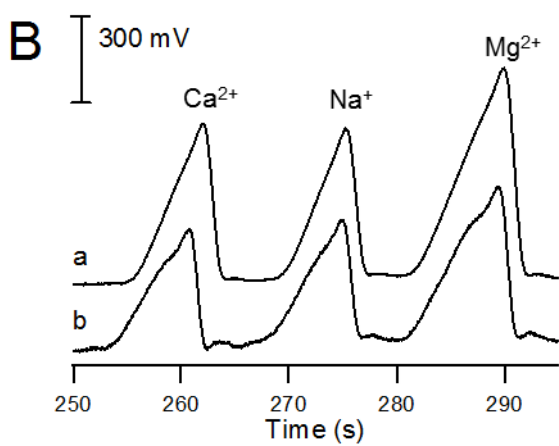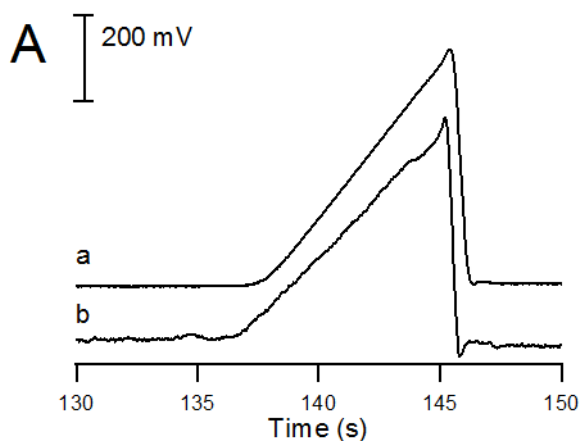
Figure 15. Effect of stray capacitance on the peak shapes. (A) Detection of a potassium peak (10 mg L^{-1}) without (a) and with (b) stray capacitance. BGE solution: 10 mM MES/His (pH 6.0). Excitation voltage 20 V$_{pp}$ and frequency 300 kHz. (B) Detection of three cations (10 mg L^{-1}) without (a) and with (b) stray capacitance. BGE solution: 10 mM His and 1.25 M acetic acid (pH 2.75). Excitation voltage 20 V$_{pp}$ and frequency 500 kHz. HV = 17.5 kV [65].

6. List of papers used in this dissertation and their characterization

The applicant has selected a set of 7 original and 6 review papers published on the topic of CE-C^{4}D for the purpose of this dissertation, which describe instrumental developments, fundamental principles and practical applications of C^{4}D and demonstrate its widespread acceptance in analytical chemistry during the last 15 years. In 12 out of the 13 papers, the applicant is the first author, which clearly evidences his substantial contribution to the presented experiments and to submission and publication process. The selected papers do not duplicate scientific papers used in previously defended Ph.D. and RNDr. theses. A brief characterization of each selected paper is included at the end of this Chapter.

1. Kubáň, P., Karlberg, B., Kubáň, P., Kubáň, V.: Application of a contactless conductometric detector for the simultaneous determination of small anions and cations by capillary electrophoresis with dual-opposite end injection. J. Chromatogr. A 964 (2002) 227-241. (IF$_{2013}$ = 4.258)

2. Kubáň, P., Kubáň, P., Kubáň, V.: Simultaneous determination of inorganic and organic anions, alkali, alkaline earth and transition metal cations by capillary electrophoresis with contactless conductivity detection. Electrophoresis 23 (2002) 3725-3734. (IF$_{2013}$ = 3.161)

3. Kubáň, P., Kubáň, P., Kubáň, V.: Speciation of chromium(III) and chromium(VI) by capillary electrophoresis with a contactless conductometric detection and dual opposite end injection. Electrophoresis 24 (2003) 1397-1403. (IF$_{2013}$ = 3.161)

4. Kubáň, P., Hauser, P.C.: Fundamental aspects of contactless conductivity detection for capillary electrophoresis. Part I: Frequency behavior and cell geometry. Electrophoresis 25 (2004) 3387-3397. (IF$_{2013}$ = 3.161)

5. Kubáň, P., Hauser, P.C.: Fundamental aspects of contactless conductivity detection for capillary electrophoresis. Part II: Signal-to-noise ratio and stray capacitance. Electrophoresis 25 (2004) 3398-3405. (IF$_{2013}$ = 3.161)

6. Kubáň, P., Nguyen, H.T.A., Macka, M., Haddad, P.R., Hauser, P.C.: New fully portable instrument for the versatile determination of cations and anions by capillary electrophoresis with contactless conductivity detection. Electroanalysis 19 (2007) 2059-2065. (IF$_{2013}$ = 2.502)

7. Kubáň, P., Evenhuis, C.J., Macka, M., Haddad, P.R., Hauser, P.C.: Comparison of different contactless conductivity detectors for the determination of small inorganic ions by capillary electrophoresis. Electroanalysis 18 (2006) 1289-1296. (IF$_{2013}$ = 2.502)

8. Kubáň, P., Hauser, P.C.: Contactless conductivity detection in capillary electrophoresis: a review. Electroanalysis 16 (2004) 2009-2021. (IF$_{2013}$ = 2.502)

9. Kubáň, P., Hauser, P.C.: A review of the recent achievements in capacitively coupled contactless conductivity detection. Anal. Chim. Acta 607 (2008) 15-29. (IF$_{2013}$ = 4.517)

10. Kubáň, P., Hauser, P.C.: Ten years of axial capacitively coupled contactless conductivity detection for CZE – a review. Electrophoresis 30 (2009) 176-188. (IF$_{2013}$ = 3.161)

11. Kubáň, P., Hauser, P.C.: Capacitively coupled contactless conductivity detection for microseparation techniques − recent developments. Electrophoresis 32 (2011) 30-42. (IF$_{2013}$ = 3.161)

12. Kubáň, P. Hauser, P.C.: Contactless conductivity detection for analytical techniques: Developments from 2010 to 2012. Electrophoresis 34 (2013) 55-69. (IF$_{2013}$ = 3.161)

13. Kubáň, P. Hauser, P.C.: Contactless conductivity detection for analytical techniques − Developments from 2012 to 2014. Electrophoresis 36 (2015) 195-211. (IF$_{2013}$ = 3.161)

Development, validation and basic optimization of a C^{4}D for CE was described in [79]. A versatile detection cell was developed that allowed for easy capillary replacement and variation of the experimental set-up, such as, electrode size and detection gap size. Basic detection parameters, i.e., excitation voltage amplitude and frequency and detection gap size were examined with respect to best detection performance. The C^{4}D was applied to determination of inorganic anions and cations, organic cations and to simultaneous determination of inorganic anions and cations.

Simultaneous CE-C^{4}D analyses using dual opposite end injection were extended to a wider range of analytes [135]. The set of common inorganic anions and cations was complemented with a set of transition metal cations, alkaline earth cations and some organic anions. Simultaneous determination of up to 22 ions was possible in the CE-C^{4}D system and was the first publication ever to determine all these groups of ions in one single analysis.

Dual opposite end injection in CE-C^{4}D was also examined for speciation analyses [136]. Chromium in oxidation state III is present as cation (Cr^{3+}) and in oxidation state VI as anion (chromate). Their physicochemical and toxicological properties are significantly different and their speciation is therefore mandatory. While Cr(III) possess no risk to human beings and environment and is considered to be an essential element in trace amounts, Cr(VI) is highly toxic due to its extreme mobility and diffusion through cell membranes. Simultaneous determination of these species together with other inorganic and organic ions was possible in industrial waste water samples.

Basic principles of axial C^{4}D in CE were investigated it two related publications [64, 65]. The two papers have arisen at the stage when C^{4}D was slowly recognized as a new alternative detection technique in CE and were intended as a fundamental basis comprehensively describing C^{4}D principles and also to explain several unclear points regarding C^{4}D, which have not been completely understood since axial C^{4}D introduction in 1998. They were also intended as a comprehensive guide for regular as well as new users of C^{4}D to ensure they can enjoy an optimized performance of the detection system.
Most importantly, it is good to see that more than a decade after publication, many of the fundamental aspects described and proven in these papers are routinely used in commercial C^{4}Ds. These can be, for example, application of unity gain stable operational amplifiers for predictable C^{4}D performance, use of elevated excitation voltages for better detection sensitivity, implementation of efficient Faraday shielding for elimination of stray capacitance, application of guiding sleeves and thus electrodes with larger IDs for easier insertion of separation capillaries into the detection cell, etc.

Standard bench-top C^{4}D was modified to a portable detector, which can be powered from lead-acid 12 V batteries [137]. This lends another dimension to this detection technique, since lab

made and commercial portable CE systems were developed more than a decade ago and coupling of portable CE and portable C^4D represents a very interesting alternative for field analysis due to its unprecedented simplicity and high sensitivity in analysis of inorganic ions. Various applications of the developed fully portable CE-C^4D were demonstrated by sensitive on-site determination of several inorganic pollutants in remote areas.

A relatively large number of axial C^4Ds has appeared since its first appearance in 1998, including several designs of home-made and commercial detectors. In the following paper [138], performance of various C^4D units was evaluated based on CE-C^4D measurements of inorganic ions. This publication covered comparison of low-voltage home-made C^4D with a miniaturized cell, home-made high-voltage C^4D, home-made battery powered portable C^4D and at that time the only accessible commercial C^4D (TraceDec, Istech, Austria). A comprehensive comparison of each respective C^4D was performed showing that optimized home-made C^4Ds are comparable in most aspects with the commercial C^4D.

A set of review articles on C^4D in CE and other analytical techniques was published in the period between 2004 and 2015 [59, 62, 63, 70-72]. They comprehensively reported on actual fundamental developments and practical applications of C^4D in CE and in other bench-top as well as down-scaled analytical techniques. They also clearly demonstrated the growing interest in the C^4D, gradual growth in usage of commercial over home-made C^4Ds and potential for applications of C^4D in various fields of analytical chemistry ranging from pharmaceutical, clinical, environmental and food chemistry to biochemistry and industrial processes.

7. Conclusions

Development and applications of C^{4}D in CE are described in this dissertation. Fundamental aspects of axial C^{4}D along with basic rules for selection of C^{4}D operational parameters can be used as a comprehensive guide for correct understanding and for proper use of C^{4}D in CE. The basic behaviour of C^{4}D can be described by the simplest possible equivalent circuitry consisting of a serial arrangement of capacitor, resistor and another capacitor. The C^{4}D cell behaves as a standard conductivity cell with dimensions corresponding to the cross-sectional area defined by the internal diameter of separation capillary and to the distance between the two C^{4}D sensing electrodes. Unity gain stable operational amplifiers should preferentially be used since they ensure stable C^{4}D performance in wide ranges of excitation frequencies. The size of the sensing electrodes does not limit the C^{4}D cell performance as long as the excitation frequency is sufficiently high, whereas C^{4}D cell size, i.e., distance between the sensing electrodes, directly affects its performance. Tight coupling between the sensing electrode and separation capillary has negligible effect on C^{4}D performance and is thus not mandatory. The most important variable characterizing C^{4}D performance, S/N ratio, is highly dependent on the overall C^{4}D design and operational parameters setting. Improved S/N ratios and thus better C^{4}D sensitivities can, for example, be achieved by using optimized operational frequencies and elevated excitation voltages and by application of BGE solutions with low conductivities. Neglecting optimization of any of these parameters can easily result in significantly reduced sensitivity; 1-2 orders of magnitude higher LODs can be obtained compared to optimized C^{4}D operational conditions. In addition, good shielding between the sensing electrodes is beneficial for higher analytical sensitivity, moreover, distorted peak shapes may be efficiently eliminated by using properly shielded C^{4}D cells. Appropriate selection of excitation frequencies also reduces the overshooting phenomena and ensures higher accuracy of the achieved quantitative data.

More than a decade after these fundamental findings, C^{4}D is considered a mature detection technique. The initial interest in developments of instrumental aspects of C^{4}D, which was mostly evidenced in the early days of axial C^{4}D existence, has evolved into routine use of C^{4}D in analytical chemistry and by applications of C^{4}D in more complex analytical procedures. Commercial production of C^{4}D has also significantly increased its popularity since it has ensured an easy access to this detection technique to a wider scientific audience and C^{4}D is thus not restricted to a limited number of electronically skilled analytical chemists only. As a consequence, the application range of C^{4}D has also been significantly broadened by the increased number of active C^{4}D users in recent years. The common features of all C^{4}D set-ups, i.e., their instrumental simplicity, low costs, virtually no need for maintenance and replacement parts, have made C^{4}D a very successful detection technique, far beyond the initially intended applications field. Consequently, C^{4}D is not only used as a detection technique of choice in CE analysis of small inorganic ions [59, 62, 63, 70-72, 75, 90], but it is often used in microchip electrophoresis [73, 74], in characterization of new chromatographic materials [139] and in modern micro-scaled separation techniques [140-142]. The application areas of CE-C^{4}D include food, environmental and pharmaceutical/clinical analysis [143, 144], CE-C^{4}D is used as a tool for development and investigation of new analytical methods [145, 146] and C^{4}D is generally used as a complementary technique to other commercially available detection techniques in CE. Direct coupling of CE-C^{4}D to liquid handling systems (e.g., FIA and SIA) is also highly interesting, since it ensures semi- to fully-automated operation of such hyphenated analytical systems [147, 148]. Moreover, these systems were shown suitable for long-term unattended operation [149] as well as for on-site operation in battery-powered regime in a fully portable format [150]. It is therefore believed that C^{4}D has been accepted as a viable detection method in CE and other microseparation techniques, will be regularly used in the future and the

fundamental investigations and practical applications of C^4D in CE, presented in this doctoral dissertation, have substantially helped to its overall success.

This success can be evidenced by more than 900 publications and by constantly growing numbers of published articles and citations on C^4D in the last 20 years (see Figure 16; WOS Core Collection: keyword "contactless cond*", 15th May 2015). The applicant has contributed to the wider acceptance of the detection technique by almost 50 original papers and a number of review papers on C^4D, especially by invited contributions on fundamental developments and applications of C^4D in CE and other analytical techniques, which are biannually published in journal Electrophoresis [63, 70-72].
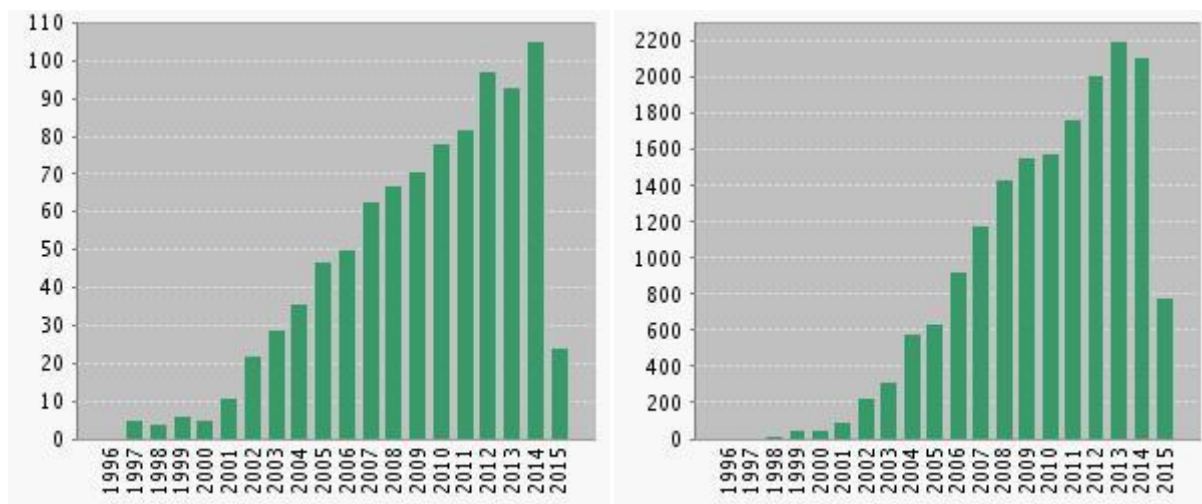

Figure 16. Number of publications and citations for the keyword "contactless cond" found on Web of Science for the time period 1996 – 2015, 15th May 2015.*

8. References

[1] Jorgenson, J. W., Lukacs, K. D., *Anal. Chem.* 1981, *53*, 1298-1302.

[2] Foret, F., Křivánková, L., Boček, P., *Capillary Zone Electrophoresis*, VCH Publishers, Inc., New York 1993.

[3] Jandik, P., Bonn, G., *Capillary Electrophoresis of Small Molecules and Ions*, VCH Publishers, Inc., New York 1993.

[4] Camilleri, P., *Capillary Electrophoresis. Theory and Practise, 2nd Edition.*, CRC Press LCC, Boca Raton 1998.

[5] Weston, A., Brown, P. R., Jandik, P., Heckenberg, A. L., Jones, W. R., *J. Chromatogr.* 1992, *608*, 395-402.

[6] Doble, P., Macka, M., Haddad, P. R., *J. Chromatogr. A* 1998, *804*, 327-336.

[7] Johns, C., Macka, M., Haddad, P. R., *Electrophoresis* 2003, *24*, 2150-2167.

[8] Padarauskas, A., *Electrophoresis* 2003, *24*, 2054-2063.

[9] Johns, C., Breadmore, M. C., Macka, M., Ryvolová, M., Haddad, P. R., *Electrophoresis* 2009, *30*, S53-S67.

[10] Poinsot, V., Rodat, A., Gavard, P., Feurer, B., Couderc, F., *Electrophoresis* 2008, *29*, 207-223.

[11] Garcia-Campana, A. M., Taverna, M., Fabre, H., *Electrophoresis* 2007, *28*, 208-232.

[12] Kašička, V., *Electrophoresis* 2010, *31*, 122-146.

[13] Kašička, V., *Electrophoresis* 2012, *33*, 48-73.

[14] Kašička, V., *Electrophoresis* 2014, *35*, 69-95.

[15] de Kort, B. J., de Jong, G. J., Somsen, G. W., *Anal. Chim. Acta* 2013, 13-33.

[16] Johnson, M. E., Landers, J. P., *Electrophoresis* 2004, *25*, 3513-3527.

[17] Dolnik, V., *Electrophoresis* 2006, *27*, 126-141.

[18] Lacroix, M., Poinsot, V., Fournier, C., Couderc, F., *Electrophoresis* 2005, *26*, 2608-2621.

[19] Mikkers, F. E. P., Everaerts, F. M., Verheggen, T. P. E. M., *J. Chromatogr.* 1979, *169*, 11-20.

[20] Huang, X. H., Luckey, J. A., Gordon, M. J., Zare, R. N., *Anal. Chem.* 1989, *61*, 766-770.

[21] Huang, X. H., Pang, T. K. J., Gordon, M. J., Zare, R. N., *Anal. Chem.* 1987, *59*, 2747-2749.

[22] Huang, X. H., Zare, R. N., *Anal. Chem.* 1991, *63*, 2193-2196.

[23] Haber, C., Jones, W. R., Soglia, J., Surve, M. A.*, et al.*, *J. Cap. Electrophoresis* 1996, *3*, 1-11.

[24] Zemann, A. J., Schnell, E., Volgger, D., Bonn, G. K., *Anal. Chem.* 1998, *70*, 563-567.

[25] da Silva, J. A. F., do Lago, C. L., *Anal. Chem.* 1998, *70*, 4339-4343.

[26] Klampfl, C. W., *Electrophoresis* 2006, *27*, 3-34.

[27] Stalmach, A., Albalat, A., Mullen, W., Mischak, H., *Electrophoresis* 2013, *34*, 1452-1464.

[28] Wang, X., Li, K. F., Adams, E., Van Schepdael, A., *Curr. Drug Metabol.* 2013, *14*, 807-813.

[29] Hirayama, A., Wakayama, M., Soga, T., *TrAC-Trends Anal. Chem.* 2014, *61*, 215-222.

[30] Ibanez, C., Simo, C., Garcia-Canas, V., Cifuentes, A., Castro-Puyana, M., *Anal. Chim. Acta* 2013, *802*, 1-13.

[31] Klepárník, K., *Electrophoresis* 2013, *34*, 70-85.

[32] Klepárník, K., *Electrophoresis* 2015, *36*, 159-178.

[33] Ramautar, R., Somsen, G. W., de Jong, G. J., *Electrophoresis* 2013, *34*, 86-98.

[34] Ramautar, R., Somsen, G. W., de Jong, G. J., *Electrophoresis* 2015, *36*, 212-224.

[35] Robledo, V. R., Smyth, W. F., *Electrophoresis* 2014, *35*, 2292-2308.

[36] Zhong, X. F., Zhang, Z. C., Jiang, S., Li, L. J., *Electrophoresis* 2014, *35*, 1214-1225.

[37] Simpson, D. C., Smith, R. D., *Electrophoresis* 2005, *26*, 1291-1305.

[38] Wittke, S., Kaiser, T., Mischak, H., *J. Chromatogr. B* 2004, *803*, 17-26.

[39] Smyth, W. F., *Electrophoresis* 2005, *26*, 1334-1357.

[40] Simo, C., Barbas, C., Cifuentes, A., *Electrophoresis* 2005, *26*, 1306-1318.

[41] Stutz, H., *Electrophoresis* 2005, *26*, 1254-1290.

[42] Willems, A. V., Deforce, D. L., Van Peteghem, C. H., Van Bocxlaer, J. F., *Electrophoresis* 2005, *26*, 1221-1253.

[43] Willems, A. V., Deforce, D. L., Van Peteghem, C. H., Van Bocxlaer, J. F., *Electrophoresis* 2005, *26*, 1412-1423.

[44] Monton, M. R. N., Terabe, S., *Anal. Sci.* 2005, *21*, 5-13.

[45] Michalke, B., *Electrophoresis* 2005, *26*, 1584-1597.

[46] Alvarez-Llamas, G., de la Campa, M. D., Sanz-Medel, A., *TrAC-Trends Anal. Chem.* 2005, *24*, 28-36.

[47] Šenk, P., Kozák, L., Foret, F., *Electrophoresis* 2004, *25*, 1447-1456.

[48] Shamsi, S. A., Miller, B. E., *Electrophoresis* 2004, *25*, 3927-3961.

[49] Kubáň, P., Hauser, P. C., *Electrophoresis* 2009, *30*, 3305-3314.

[50] Ji, X. H., Xu, Q. F., He, Z. K., *Chin. J. Anal. Chem.* 2008, *36*, 1579-1586.

[51] Lara, F. J., Garcia-Campana, A. M., Velasco, A. I., *Electrophoresis* 2010, *31*, 1998-2027.

[52] Kubáň, P., Houserová, P., Kubáň, P., Hauser, P. C., Kubáň, V., *Electrophoresis* 2007, *28*, 58-68.

[53] Suarez, C. A., Gine, M. F., *J. Anal. At. Spectrom.* 2005, *20*, 1395-1397.

[54] Viskari, P. J., Landers, J. P., *Electrophoresis* 2006, *27*, 1797-1810.

[55] Gaš, B., Demjaněnko, M., Vacík, J., *J. Chromatogr.* 1980, *192*, 253-257.

[56] Gaš, B., Vacík, J., *Chem. Listy* 1980, *74*, 652-658.

[57] Kaniansky, D., Masar, M., Marak, J., Bodor, R., *J. Chromatogr. A* 1999, *834*, 133-178.

[58] Zemann, A. J., *Electrophoresis* 2003, *24*, 2125-2137.

[59] Kubáň, P., Hauser, P. C., *Electroanalysis* 2004, *16*, 2009-2021.

[60] Guijt, R. M., Evenhuis, C. J., Macka, M., Haddad, P. R., *Electrophoresis* 2004, *25*, 4032-4057.

[61] Šolínová, V., Kašička, V., *J. Sep. Sci.* 2006, *29*, 1743-1762.

[62] Kubáň, P., Hauser, P. C., *Anal. Chim. Acta* 2008, *607*, 15-29.

[63] Kubáň, P., Hauser, P. C., *Electrophoresis* 2009, *30*, 176-188.

[64] Kubáň, P., Hauser, P. C., *Electrophoresis* 2004, *25*, 3387-3397.

[65] Kubáň, P., Hauser, P. C., *Electrophoresis* 2004, *25*, 3398-3405.

[66] Brito-Neto, J. G. A., da Silva, J. A. F., Blanes, L., do Lago, C. L., *Electroanalysis* 2005, *17*, 1198-1206.

[67] Brito-Neto, J. G. A., da Silva, J. A. F., Blanes, L., do Lago, C. L., *Electroanalysis* 2005, *17*, 1207-1214.

[68] Johnston, S. E., Fadgen, K. E., Tolley, L. T., Jorgenson, J. W., *J. Chromatogr. A* 2005, *1094*, 148-157.

[69] Opekar, F., Štulík, K., *Electroanalysis* 2006, *18*, 1282-1288.

[70] Kubáň, P., Hauser, P. C., *Electrophoresis* 2011, *32*, 30-42.

[71] Kubáň, P., Hauser, P. C., *Electrophoresis* 2013, *34*, 55-69.

[72] Kubáň, P., Hauser, P. C., *Electrophoresis* 2015, *36*, 195-211.

[73] Pumera, M., *Talanta* 2007, *74*, 358-364.

[74] Coltro, W. K. T., Lima, R. S., Segato, T. P., Carrilho, E.*, et al.*, *Anal. Methods* 2012, *4*, 25-33.

[75] Mai, T. D., Hauser, P. C., *Chem. Record* 2012, *12*, 106-113.

[76] Elbashir, A. A., Aboul-Enein, H. Y., *Biomed. Chromatogr.* 2014, *28*, 1502-1506.

[77] Zemann, A. J., *TrAC-Trends Anal. Chem.* 2001, *20*, 346-354.

[78] Tanyanyiwa, J., Leuthardt, S., Hauser, P. C., *Electrophoresis* 2002, *23*, 3659-3666.

[79] Kubáň, P., Karlberg, B., Kubáň, P., Kubáň, V., *J. Chromatogr. A* 2002, *964*, 227-241.

[80] da Silva, J. A. F., Guzman, N., do Lago, C. L., *J. Chromatogr. A* 2002, *942*, 249-258.

[81] Chvojka, T., Jelínek, I., Opekar, F., Štulík, K., *Anal. Chim. Acta* 2001, *433*, 13-21.

[82] Tůma, P., Opekar, F., Jelínek, I., *Electroanalysis* 2001, *13*, 989-992.

[83] Tůma, P., Opekar, F., Štulík, K., *Electrophoresis* 2002, *23*, 3718-3724.

[84] Tanyanyiwa, J., Galliker, B., Schwarz, M. A., Hauser, P. C., *Analyst* 2002, *127*, 214-218.

[85] Novotný, M., Opekar, F., Jelínek, I., *Chem. Listy* 2005, *99*, 132-136.

[86] Novotný, M., Opekar, F., Jelínek, I., Štulík, K., *Anal. Chim. Acta* 2004, *525*, 17-21.

[87] Novotný, M., Opekar, F. E., Štulík, K., *Electroanalysis* 2005, *17*, 1181-1186.

[88] Tan, F., Yang, B. C., Guan, Y. F., *Anal. Sci.* 2005, *21*, 583-585.

[89] Ryvolová, M., Preisler, J., Foret, F., Hauser, P. C., *et al.*, *Anal. Chem.* 2010, *82*, 129-135.

[90] Opekar, F., Štulík, K., *Electrophoresis* 2011, *32*, 795-810.

[91] Zhang, S., Wang, L., Dang, Z., Li, T., *et al.*, *Inst. Sci. Technol.* 2007, *35*, 275-294.

[92] Zhang, S. F., Wang, L. S., Dang, Z., *Chin. Chem. Lett.* 2006, *17*, 1229-1232.

[93] Nádherná, M., Opekar, F., Štulík, K., *Electroanalysis* 2007, *19*, 2413-2418.

[94] Hilder, E. F., Zemann, A. J., Macka, M., Haddad, P. R., *Electrophoresis* 2001, *22*, 1273-1281.

[95] Vuorinen, P. S., Jussila, M., Siren, H., Palonen, S., Riekkola, M. L., *J. Chromatogr. A* 2003, *990*, 45-52.

[96] Macka, M., Hutchinson, J., Zemann, A., Zhang, S. S., Haddad, P. R., *Electrophoresis* 2003, *24*, 2144-2149.

[97] TraceDec, http://www.istech.at.

[98] eDAQ, http://www.edaq.com/ER815.

[99] Kubáň, P., Kubáň, P., Kubáň, V., Hauser, P. C., Boček, P., *J. Chromatogr. A* 2008, *1190*, 377-382.

[100] Kubáň, P., Müri, M. A., Hauser, P. C., *Analyst* 2004, *129*, 82-86.

[101] Takeuchi, M., Li, Q. Y., Yang, B. C., Dasgupta, P. K., Wilde, V. E., *Talanta* 2008, *76*, 617-620.

[102] Gillespie, E., Connolly, D., Macka, M., Hauser, P., Paull, B., *Analyst* 2008, *133*, 1104-1110.

[103] Hoherčáková, Z., Opekar, F., *Anal. Chim. Acta* 2005, *551*, 132-136.

[104] Hoherčáková, Z., Opekar, F. E., Štulík, K., *Electroanalysis* 2005, *17*, 1924-1930.

[105] Míka, J., Opekar, F., Coufal, P., Štulík, K., *Anal. Chim. Acta* 2009, *650*, 189-194.

[106] Sereenonchai, K., Teerasong, S., Chan-Eam, S., Saetear, P., *et al.*, *Talanta* 2010, *81*, 1040-1044.

[107] Tian, K., Dasgupta, P. K., *Anal. Chim. Acta* 2009, *652*, 245-250.

[108] Makahleh, A., Saad, B., *Anal. Chim. Acta* 2011, *694*, 90-94.

[109] Braz, H. L., Ito, D. T., da Silva, J. A. F., do Lago, C. L., Pedrotti, J. J., *Electroanalysis* 2011, *23*, 2594-2600.

[110] Pencharee, S., Faber, P. A., Ellis, P. S., Cook, P., *et al.*, *Anal. Methods* 2012, *4*, 1278-1283.

[111] Kubáň, P., Abad-Villar, E. M., Hauser, P. C., *J. Chromatogr. A* 2006, *1107*, 159-164.

[112] Kubáň, P., Hauser, P. C., *J. Chromatogr. A* 2006, *1128*, 97-104.

[113] Hamplová, A., Coufal, P., Bosáková, Z., Opekar, F., Kubíček, V., *Chem. Listy* 2008, *102*, 194-199.

[114] Makahleh, A., Saad, B., Siang, G. H., Saleh, M. I., *et al.*, *Talanta* 2010, *81*, 20-24.

[115] Mark, J. J. P., Coufal, P., Opekar, F., Matysik, F. M., *Anal. Bioanal. Chem.* 2011, *401*, 1669-1676.

[116] Kubáň, P., Hauser, P. C., *Lab Chip* 2005, *5*, 407-415.

[117] Zakaria, P., Hutchinson, J. P., Avdalovic, N., Liu, Y., Haddad, P. R., *Anal. Chem.* 2005, *77*, 417-423.

[118] Gillespie, E., Macka, M., Connolly, D., Paull, B., *Analyst* 2006, *131*, 886-888.

[119] Yang, B. C., Takeuchi, M., Dasgupta, P. K., Umemura, T.*, et al.*, *Anal. Chem.* 2007, *79*, 769-772.

[120] Kubáň, P., Dasgupta, P. K., Pohl, C. A., *Anal. Chem.* 2007, *79*, 5462-5467.

[121] Kubáň, P., Pelcová, P., Kubáň, V., Klakurková, L., Dasgupta, P. K., *J. Sep. Sci.* 2008, *31*, 2745-2753.

[122] O Riordain, C., Gillespie, E., Connolly, D., Nesterenko, P. N., Paull, B., *J. Chromatogr. A* 2007, *1142*, 185-193.

[123] Connolly, D., Paull, B., *J. Sep. Sci.* 2009, *32*, 2653-2658.

[124] Sedyohutomo, A., Lim, L. W., Takeuchi, T., *J. Chromatogr. A* 2008, *1203*, 239-242.

[125] Yang, B. C., Takeuchi, M., Dasgupta, P. K., *Anal. Chem.* 2008, *80*, 40-47.

[126] Yang, B. C., Zhang, F. F., Liang, X. M., Dasgupta, P. K., *J. Chromatogr. A* 2009, *1216*, 2412-2416.

[127] Gillespie, E., Connolly, D., Macka, M., Nesterenko, P. N., Paull, B., *Analyst* 2007, *132*, 1238-1245.

[128] Shen, D. Z., Li, D. D., Yang, X. W., Zhu, Y.*, et al.*, *Talanta* 2011, *84*, 42-48.

[129] Zhang, Z. L., Li, D. D., Liu, X. Y., Subhani, Q.*, et al.*, *Analyst* 2012, *137*, 2876-2883.

[130] Rong, L., Liu, Z. G., Ma, M. L., Liu, J. Q.*, et al.*, *Anal. Sci.* 2012, *28*, 367-371.

[131] Kiplagat, I. K., Kubáň, P., Pelcová, P., Kubáň, V., *J. Chromatogr. A* 2010, *1217*, 5116-5123.

[132] Kubáň, P., Hauser, P. C., *J. Chromatogr. A* 2007, *1176*, 185-191.

[133] Jones, W. R., Soglia, J., McGlynn, M., Haber, C.*, et al.*, *Am. Lab.* 1996, *28*, 25-28.

[134] Baltussen, E., Guijt, R. M., van der Steen, G., Laugere, F.*, et al.*, *Electrophoresis* 2002, *23*, 2888-2893.

[135] Kubáň, P., Kubáň, P., Kubáň, V., *Electrophoresis* 2002, *23*, 3725-3734.

[136] Kubáň, P., Kubáň, P., Kubáň, V., *Electrophoresis* 2003, *24*, 1397-1403.

[137] Kubáň, P., Nguyen, H. T. A., Macka, M., Haddad, P. R., Hauser, P. C., *Electroanalysis* 2007, *19*, 2059-2065.

[138] Kubáň, P., Evenhuis, C. J., Macka, M., Haddad, P. R., Hauser, P. C., *Electroanalysis* 2006, *18*, 1289-1296.

[139] Connolly, D., Floris, P., Nesterenko, P. N., Paull, B., *TrAC-Trends Anal. Chem.* 2010, *29*, 870-884.

[140] Yang, B. C., Zhang, M., Kanyanee, T., Stamos, B. N., Dasgupta, P. K., *Anal. Chem.* 2014, *86*, 11554-11561.

[141] Zhang, M., Stamos, B. N., Amornthammarong, N., Dasgupta, P. K., *Anal. Chem.* 2014, *86*, 11538-11546.

[142] Zhang, M., Stamos, B. N., Dasgupta, P. K., *Anal. Chem.* 2014, *86*, 11547-11553.

[143] Elbashir, A. A., Aboul-Enein, H. Y., *Biomed. Chromatogr.* 2010, *24*, 1038-1044.

[144] Elbashir, A. A., Aboul-Enein, H. Y., *Biomed. Chromatogr.* 2012, *26*, 990-1000.

[145] Kubáň, P., Boček, P., *J. Chromatogr. A* 2012, *1267*, 96-101.

[146] Kubáň, P., Foret, F., *Anal. Chim. Acta* 2013, *805*, 1-18.

[147] Kubáň, P., Kubáň, P., Hauser, P. C., Kubáň, V., *Electrophoresis* 2004, *25*, 35-42.

[148] Wuersig, A., Kubáň, P., Khaloo, S. S., Hauser, P. C., *Analyst* 2006, *131*, 944-949.

[149] Gaudry, A. J., Guijt, R. M., Macka, M., Hutchinson, J. P.*, et al.*, *Anal. Chim. Acta* 2013, *781*, 80-87.

[150] Mai, T. D., Pham, T. T. T., Pham, H. V., Saiz, J.*, et al.*, *Anal. Chem.* 2013, *85*, 2333-2339.

9. List of all publications

1. Kubáň, P., Kubáň, P., Kubáň V.: Simultaneous capillary electrophoretic separation of small anions and cations after complexation with EDTA. J. Chromatogr. A 836 (1999) 75-80.

2. Kubáň, P., Kubáň, P., Kubáň, V.: Capillary electrophoretic determination of inorganic anions in drainage and surface water samples. J. Chromatogr. A 848 (1999) 555-561.

3. Kubáň, P. Flowers, H.: Ion chromatographic determination of the decomposition products of tecnazene solution irradiated by UV light. Inorganic and organic anions. J. Agric. Food Chem. 49 (2001) 2848-2852.

4. Kubáň, P., Flowers, H.: On-line pre-concentration for the ion chromatographic determination of chlorophenolic and phenolic compounds in environmental samples using electrochemical detection. Anal. Chim. Acta 437 (2001) 115-122.

5. Kubáň, P., Flowers, H.: Determination of nitroaromatic photodecomposition products of tecnazene using liquid chromatography with amperometric detection. Collect. Czech. Chem. Commun. 67 (2002) 1181-1192.

6. Kubáň, P., Karlberg, B., Kubáň, P., Kubáň, V.: Application of a contactless conductometric detector for the simultaneous determination of small anions and cations by capillary electrophoresis with dual-opposite end injection. J. Chromatogr. A 964 (2002) 227-241.

7. Kubáň, P., Kubáň, P., Kubáň, V.: Simultaneous determination of inorganic and organic anions, alkali, alkaline earth and transition metal cations by capillary electrophoresis with contactless conductivity detection. Electrophoresis 23 (2002) 3725-3734.

8. Kubáň, P., Kubáň, P., Kubáň, V.: Speciation of chromium(III) and chromium(VI) by capillary electrophoresis with a contactless conductometric detection and dual opposite end injection. Electrophoresis 24 (2003) 1397-1403.

9. Kubáň, P., Kubáň, P., Kubáň, V.: Flow injection-capillary electrophoresis system with contactless conductivity detection and hydrostatic pressure generated flow. Application to the quantitative analysis of inorganic anions in water samples. Electrophoresis 24 (2003) 1935-1943.

10. Kubáň, P., Kubáň, P., Kubáň, V.: Rapid speciation of Se(IV) and Se(VI) by flow injection – capillary electrophoresis system with contactless conductivity detection. Anal. Bioanal. Chem. 378 (2004) 378-382.

11. Kubáň, P., Kubáň, P., Hauser, P.C., Kubáň, V.: A flow injection-capillary electrophoresis system with high-voltage contactless conductivity detection for automated dual opposite end injection. Electrophoresis 25 (2004) 35-42.

12. Kubáň, P., Müri, M.A., Hauser, P.C.: Application of a contactless conductivity detector to the determination of inorganic ions in ion chromatography. Analyst 129 (2004) 82-86.

13. Kubáň, P., Reinhardt, M., Müller, B., Hauser, P.C.: On-site simultaneous determination of anions and cations in drainage water using a flow injection-capillary electrophoresis system with contactless conductivity detection. J. Environ. Monit. 6 (2004) 169-174.

14. Kubáň, P., Hauser, P.C.: Fundamental aspects of contactless conductivity detection for capillary electrophoresis. Part I: Frequency behavior and cell geometry. Electrophoresis 25 (2004) 3387-3397.

15. Kubáň, P., Hauser, P.C.: Fundamental aspects of contactless conductivity detection for capillary electrophoresis. Part II: Signal-to-noise ratio and stray capacitance. Electrophoresis 25 (2004) 3398-3405.

16. Kubáň, P., Hauser, P.C.: Contactless conductivity detection in capillary electrophoresis: a review. Electroanalysis 16 (2004) 2009-2021.

17. Wan, Q.J., Kubáň, P., Tanyanyiwa, J., Rainelli, A., Hauser, P.C.: Determination of major inorganic ions in blood serum and urine by capillary electrophoresis with contactless conductivity detection. Anal. Chim. Acta, 525 (2004) 11-16.

18. Kubáň, P., Guchardi, R., Hauser, P.C.: Trace metal analysis with separation methods. TrAC-Trends Anal. Chem., 24 (2005) 192-197.

19. Kubáň, P., Hauser, P.C.: Effects of the cell geometry and operating parameters on the performance of an external contactless conductivity detector for microchip electrophoresis. Lab on a Chip, 5 (2005) 407-415.

20. Kubáň, P., Hauser, P.C.: Application of an external contactless conductivity detector for the analysis of beverages by microchip capillary electrophoresis. Electrophoresis 26 (2005) 3169-3178.

21. Abad-Villar, E.M., Kubáň, P., Hauser, P.C.: Determination of biochemical species on electrophoresis chips with an external contactless conductivity detector. Electrophoresis 26 (2005) 3609-3614.

22. Gong, X.Y., Kubáň, P., Tanyanyiwa, J., Hauser, P.C.: Separation of enantiomers in capillary electrophoresis with contactless conductivity detection. J. Chromatogr. A 1082 (2005) 230-234.

23. Law, W.S., Kubáň, P., Zhao, J.H., Li, S.F.Y., Hauser, P.C.: Determination of vitamin C and preservatives in beverages by conventional capillary electrophoresis and microchip electrophoresis with capacitively coupled contactless conductivity detection. Electrophoresis 26 (2005) 4648-4655.

24. Abad-Villar, E.M., Kubáň, P., Hauser, P.C.: Evaluation of the detection of biomolecules in capillary electrophoresis by contactless conductivity measurement. J. Sep. Sci. 29 (2006) 1031-1037.

25. Kubáň, P., Abad-Villar, E.M., Hauser, P.C.: Evaluation of contactless conductivity detection for the determination of UV-absorbing and non-UV-absorbing species in reversed phase high performance liquid chromatography. J. Chromatogr. A 1107 (2006) 159-164.

26. Law, W.S., Kubáň, P., Yuan, L.L., Zhao, J.H., Li, S.F.Y., Hauser, P.C.: Determination of Tobramycin in human serum by capillary electrophoresis with contactless conductivity detection. Electrophoresis 27 (2006) 1932-1938.

27. Kubáň, P., Evenhuis, C.J., Macka, M., Haddad, P.R., Hauser, P.C.: Comparison of different contactless conductivity detectors for the determination of small inorganic ions by capillary electrophoresis. Electroanalysis 18 (2006) 1289-1296.

28. Wuersig, A., Kubáň, P., Khaloo, S.S., Hauser, P.C.: Rapid electrophoretic separations in short capillaries using contactless conductivity detection and a sequential injection analysis manifold for hydrodynamic sample loading. Analyst 131 (2006) 944-949.

29. Kubáň, P., Hauser, P.C.: Application of gradient programs for the determination of underivatized amino acids and small peptides in reversed-phase high-performance liquid chromatography with contactless conductivity detection. J. Chromatogr. A 1128 (2006) 97-104.

30. Zhang, L., Khaloo, S.S., Kubáň, P., Hauser, P.C.: Analysis of electroplating baths by capillary electrophoresis with high voltage contactless conductivity detection. Meas. Sci. Technol. 17 (2006) 3317-3322.

31. Kubáň, P., Houserová, P., Kubáň, P., Hauser, P.C., Kubáň, V.: Mercury speciation by capillary electrophoresis. A review. Electrophoresis 28 (2007) 58-68.

32. Kubáň, P., Houserová, P., Kubáň, P., Hauser, P.C., Kubáň, V.: Sensitive capillary electrophoretic determination of mercury species with amperometric detection at a copper electrode after cation exchange preconcentration. J. Sep. Sci. 30 (2007) 1070-1076.

33. Nguyen, H.T.A., Kubáň, P., Pham, V.H., Hauser, P.C.: Study of the determination of inorganic arsenic species by capillary electrophoresis with capacitively coupled contactless conductivity detection. Electrophoresis 28 (2007) 3500-3506.

34. Kubáň, P., Nguyen, H.T.A., Macka, M., Haddad, P.R., Hauser, P.C.: New fully portable instrument for the versatile determination of cations and anions by capillary electrophoresis with contactless conductivity detection. Electroanalysis 19 (2007) 2059-2065.

35. Schuchert-Shi, A., Kubáň, P., Hauser, P.C.: Monitoring of enzymatic reactions using conventional and on-chip capillary electrophoresis with contactless conductivity detection. Electrophoresis 28 (2007) 4690-4696.

36. Kubáň, P., Hauser, P.C.: High performance liquid chromatography with contactless conductivity detection for the determination of peptides and proteins using a monolithic capillary column. J. Chromatogr. A 1176 (2007) 185-191.

37. Kubáň, P., Hauser, P.C.: A review of the recent achievements in capacitively coupled contactless conductivity detection. Anal. Chim. Acta 607 (2008) 15-29.

38. Kubáň, P., Hauser, P.C.: Flow analysis – capillary electrophoresis. In: *Advances in Flow Injection Analysis and Related Techniques, 54*, Kolev, S. and McKelvie, I. (Editors), 2008, pp. 287-307, Elsevier, The Netherlands.

39. Kubáň, P., Kubáň, P., Kubáň, V., Boček, P., Hauser, P.C.: Capillary electrochromatography of inorganic cations in open tubular columns with a controllable capacity multilayered stationary phase architecture. J. Chromatogr. A 1190 (2008) 377-382.

40. Kubáň, P., Hauser, P.C.: Evaluation of microchip capillary electrophoresis with external contactless conductivity detection for the determination of major inorganic ions and lithium in serum and urine samples. Lab on a Chip 8 (2008) 1829-1836.

41. Gong, X.Y., Kubáň, P., Scholer, A., Hauser, P.C.: Determination of γ-hydroxybutyric acid (GHB) in clinical samples using capillary electrophoresis with contactless conductivity detection. J. Chromatogr. A 1213 (2008) 100-104.

42. Kubáň, P., Hauser, P.C.: Ten years of axial capacitively coupled contactless conductivity detection for CZE – a review. Electrophoresis 30 (2009) 176-188.

43. Kubáň, P., Hauser, P.C.: Fundamentals of electrochemical detection techniques for capillary and microchip electrophoresis. Electrophoresis 30 (2009) 3305-3314.

44. Kubáň, P., Šlampová, A., Boček, P.: Electric field enhanced transport across phase boundaries and membranes and its potential use in sample pretreatment for bioanalysis. Electrophoresis 31 (2010) 768-785.

45. Kubáň, P., Hauser, P.C.: Capacitively coupled contactless conductivity detection for microseparation techniques – recent developments. Electrophoresis 32 (2011) 30-42.

46. Kiplagat, I.K., Thi, K.O.D., Kubáň, P., Kubáň, P., Boček, P.: Use of disposable open tubular ion exchange pre-columns for in-line clean-up of serum and plasma samples prior to capillary electrophoretic analysis of inorganic cations. J. Chromatogr. A 1218 (2011) 856-859.

47. Thi, K.O.D., Kubáň, P., Kubáň, P., Kiplagat, I.K., Boček, P.: Analysis of inorganic cations in biological samples by the combination of micro-electrodialysis and capillary electrophoresis with capacitively coupled contactless conductivity detection. Electrophoresis 32 (2011) 464-471.

48. Kubáň, P., Strieglerová, L., Gebauer, P., Boček, P.: Electromembrane extraction of heavy metal cations followed by capillary electrophoresis with capacitively coupled contactless conductivity detection. Electrophoresis 32 (2011) 1025-1032.

49. Strieglerová, L., Kubáň, P., Boček, P.: Rapid and simple pretreatment of human body fluids using electromembrane extraction across supported liquid membrane for capillary electrophoretic determination of lithium. Electrophoresis 32 (2011) 1182-1189.

50. Strieglerová, L., Kubáň, P., Boček, P.: Electromembrane extraction of amino acids from body fluids followed by capillary electrophoresis with capacitively coupled contactless conductivity detection. J. Chromatogr. A 1218 (2011) 6248-6255.

51. Kiplagat, I.K., Thi, K.O.D., Kubáň, P., Boček, P.: Trace analysis of perchlorate by a combination of electromembrane extraction and capillary electrophoresis with capacitively coupled contactless conductivity detection. Electrophoresis 32 (2011) 3008-3015.

52. Kubáň, P., Boček, P.: On-line coupling of a clean-up device with supported liquid membrane to capillary electrophoresis for direct injection and analysis of serum and plasma samples. J. Chromatogr. A 1234 (2012) 2-8.

53. Šlampová, A., Kubáň, P., Boček, P.: Electromembrane extraction using stabilized constant d.c. electric current – a simple tool for improvement of extraction performance. J. Chromatogr. A 1234 (2012) 32-37.

54. Kubáň, P., Kiplagat, I.K., Boček, P.: Electrokinetic injection across supported liquid membranes – new sample pretreatment technique for on-line coupling to capillary electrophoresis. Direct analysis of perchlorate in biological samples. Electrophoresis 33 (2012) 2695-2702.

55. Kubáň, P., Boček, P.: Capillary electrophoresis with capacitively coupled contactless conductivity detection – a universal tool for the determination of supported liquid membrane selectivity in electromembrane extraction of complex samples. J. Chromatogr. A 1267 (2012) 96-101.

56. Kubáň, P. Hauser, P.C.: Contactless conductivity detection for analytical techniques: Developments from 2010 to 2012. Electrophoresis 34 (2013) 55-69.

57. Pantůčková, P., Kubáň, P., Boček, P.: A simple sample pretreatment device with supported liquid membrane for direct injection of untreated body fluids and in-line coupling to capillary electrophoresis. Electrophoresis 34 (2013) 289-296.

58. Kubáň, P., Boček, P.: Direct analysis of formate in human plasma, serum and whole blood by in-line coupling of microdialysis to capillary electrophoresis for rapid diagnosis of methanol poisoning. Anal. Chim. Acta 768 (2013) 82-89.

59. Kubáň, P., Boček, P.: Direct coupling of supported liquid membranes to capillary electrophoresis for analysis of complex samples: A tutorial. Anal. Chim. Acta 787 (2013) 10-23.

60. Pantůčková, P., Kubáň, P., Boček, P.: Supported liquid membrane extraction coupled in-line to commercial capillary electrophoresis for rapid determination of formate in undiluted blood samples. J. Chromatogr. A 1299 (2013) 33-39.

61. Kubáň, P., Boček, P.: Micro-electromembrane extractions across free liquid membranes. Instrumentation and basic principles. J. Chromatogr. A 1346 (2014) 25-33.

62. Kubáň, P., Boček, P.: Micro-electromembrane extractions across free liquid membranes. Extractions of basic drugs from undiluted biological samples. J. Chromatogr. A 1337 (2014) 32-39.

63. Šlampová, A., Kubáň, P., Boček, P.: Effects of selected operational parameters on efficacy and selectivity of electromembrane extraction. Chlorophenols as model analytes. Electrophoresis 35 (2014) 2429-2437.

64. Kubáň, P., Boček, P.: Preconcentration in micro-electromembrane extractions across free liquid membranes. Anal. Chim. Acta 848 (2014) 43-50.

65. Šlampová, A., Kubáň, P., Boček, P.: Fine-tuning of electromembrane extraction selectivity using 18-crown-6 ethers as supported liquid membrane modifiers. Electrophoresis 35 (2014) 3317-3320.

66. Kubáň, P. Hauser, P.C.: Contactless conductivity detection for analytical techniques – Developments from 2012 to 2014. Electrophoresis 36 (2015) 195-211.

67. Oszwaldowski, S., Kubáň, P.: Capillary electrophoresis study on phase of mixed micelles and its role in transport phenomena of particles. Anal. Chim. Acta 864 (2015) 85-93.

68. Pantůčková, P., Kubáň, P., Boček, P.: Sensitivity enhancement in direct coupling of supported liquid membrane extractions to capillary electrophoresis by means of transient isotachophoresis and large electrokinetic injections. J. Chromatogr. A 1389 (2015) 1-7.

69. Kubáň, P., Boček, P.: The effects of electrolysis on operational solutions in electromembrane extraction. The role of acceptor solution. J. Chromatogr. A 1398 (2015) 11-19.

70. Oszwaldowski, S., Kubáň, P.: Capillary electrophoresis study on segment/segment system for segments based on phase of mixed micelles and its role in transport of particles between the two segments. J. Chromatogr. A xxxx (2015) submitted.