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Review

Electrochemical detection in the capillary electrophoresis analysis of inorganic compounds

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Abstract

Recent advances in the design and application of electrochemical detection systems in capillary electrophoresis of inorganic compounds are reviewed. The review covers amperometric, potentiometric and conductimetric detection techniques. Determination and speciation of transition metal compounds by amperometric detection systems are shown to be a very promising field of work from both sensitivity and specificity points of view. The feasibility of detection of inorganic anions and cations by ion selective microelectrodes has been demonstrated, but the detection limits and ruggedness of these detection systems are still not satisfying. End-column nonsuppressed and suppressed conductivity and contactless conductivity systems are described and the potential and limits of these different approaches are discussed.

Keywords: Reviews; Electrochemical detection; Detection electrophoresis; Conductivity detection; Water analysis; Inorganic anions; Inorganic cations; Transition metals

Contents

1. Introduction

One of the advantages of capillary electrophoresis, as compared to conventional chromatographic techniques, is that there is almost no limit to the miniaturisation of electrophoretic separation systems, allowing the injection of sample volumes down to the 10 fl range [1]. However, the low volume characteristics and small diameter of the CE capillary place volume constraints on the method of detection used. The detection device must be so sensitive as to respond to even a few ions. For a CE analysis performed on an analyte solution of 10^{-10} *M* with a

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total run time of 200 s in a capillary of 50 cm×10 μm I.D., the detector must respond to 20 zmol (ca. 10 000 ions)/s [2]. Absolute sensitivity and feasibility of miniaturisation have been well accomplished by electrochemical detection (ED), which was coupled to CE for the first time by Wallingford and Ewing in 1987 [3]. The development of CE–ED systems can be followed by reading the biennial issue 'Fundamental Reviews' published by Analytical Chemistry [4–8]. Significant advances in CE–ED, with particular emphasis on amperometric systems, have been recently reviewed [9–11].

Most efforts in developing methods using electrochemical detection have been carried out for the analysis of biologically relevant molecules (catecholamines, amino acids, carbohydrates and sulphur compounds) in order to study biochemical and pharmacological processes in single cells nearly in real time [10]. The diffusion of this detection technique in other analytical fields (e.g. industrial process and environmental monitoring) has been hampered by the lack of commercially available instruments. The application of CE–ED in the analysis of inorganic compounds is in fact rather limited, even if many inorganic species are electrochemically active and can be detected without any derivatisation step.

The aim of the present review surveying the last five-year literature is to show the possibilities of this detection technique in the field of inorganic analysis, and to discuss the actual limits in real sample analysis. Papers are gathered according to the electrochemical technique, i.e. amperometry, potentiometry with ion selective electrodes, and conductivity, and tables are used to show potentially useful methods.

2. Amperometry

The use of an ultramicroelectrode (ca. 10 μm) as an electrochemical detector in CE offers some advantages over photometric techniques. The ultramicroelectrode response is not limited by a small detection volume and ultramicroelectrodes show high sensitivity, good selectivity and low cost. The main problem in combining CE and ED is the need to isolate the electrochemical detector from the potential used in the separation and the difficulty encountered in aligning the working electrode with the end of the capillary. In the first off-column ED system, developed by Wallingford and Ewing [3], porous glass or Nafion tubing was used to connect the separation capillary with a short piece of capillary used for detection. In this way the separation current passing through the capillary was separated from the detection cell, and background noise, proportional to the applied voltage, was reduced. The major drawback was the difficulty of making the porous joint.

Kaniansky et al. [12] proposed a galvanic decoupling of a postcolumn amperometric detector for CE. The high insulating resistance achieved in this way reduced leak currents from the electrophoretic equipment through the detector to the low pA level. The detector was tested by ITP carried out in a hydrodynamically closed separation compartment. ITP resolution of nitrite and ascorbate on a glassy carbon electrode was demonstrated.

An alternative way to decouple detection electrodes from the CE power unit was to place the electrode outside the capillary (end column detection), using a very small capillary (5 μm) [13]. Lu et al. [14] described the use of an end-column ultramicroelectrode with a 10–25-μm capillaries without a porous joint system. The detection of inorganic anions at a carbon-fiber electrode, and of transition metal cations at a Hg-film electrode was used to illustrate the potential of this electrode system. Detection limits of $2 \cdot 10^{-8}$ *M* for Pb^{2+} and $1 \cdot 10^{-5}$ *M* for NO_2^- were achieved by this system. A study of the detector response as a function of the position of the electrode showed that accurate location of the electrode was important for sensitive and reproducible detection. These studies also showed that differences between the density of the electrolyte exiting the capillary and the electrolyte in the detection cell could cause anomalous electrode response depending on the location of the electrode relative to the end of the capillary.

Using the same CE detection cell, Pt, Au, carbon-fiber, and Hg-film deposited on a gold substrate were evaluated as working electrodes for amperometric detection of metal ions under constant and pulsed

voltage conditions [15]. The detector response was not reproducible for solid electrodes operating under constant voltage, but was considerably improved for Hg-film electrodes. Detection of 14 metal ions separated in a 25-μm capillary gave detection limits in the range of concentration from 10 amol to 50 fmol without removal of the background signal of oxygen. Long-term reproducibility of the Hg electrodes was limited by the reproducibility of the procedures used to renew the Hg film (5%). An evaluation of pulsed stripping at gold and carbon-fiber electrodes showed that the solid electrode surfaces were reproducibly (2% variation) conditioned by the pulse train, even when operated continuously in relatively high concentrations (10^{-4} *M*). The potential of an ultramicroelectrode PAD system for cathodic and anodic detection of metal ions separated by CE was studied by Wen and Cassidy [16]. The metal ions were detected at Au and Pt 25-μm disk electrodes via both cathodic and anodic stripping. Comparison with constant-voltage detection showed that the pulsed detection system gave superior stability, and detector stability was consistent within 5% over a 14-h period. The cathodic analysis showed detection limits in the 2–20 μ*M* range, while LODs of anodic analysis were more than 10-fold smaller than those for cathodic detection. Linearity was demonstrated for more than two orders of magnitude. A comparison of PAD for spiked and real snow samples with AAS showed good agreement. The reliability of sample stacking was also examined briefly to enhance detection for metal ions.

The possibility of having wider working potential ranges by using nonaqueous media in the amperometric detection of inorganic anions was explored by Salimi-Moosavi and Cassidy [17]. Amperometric detection at a 25-μm Pt disk electrode gave detection limits in the range 10^{-8}–10^{-9} *M* for some anions, but calibration curves were linear only over small ranges of concentrations. Nonlinearity could not be corrected even by using pulsed amperometric detection to clean the electrode surface between two consecutive analyses. Peak shapes were good, and theoretical plate counts were in the range of 140 000–450 000.

Amperometric detection in the CE analysis of inorganic compounds found only two applications on real samples (Table 1). Speciation of mercury species in contaminated sediments from the Great Lakes was shown, with detection limits ranging from 0.2 μg l^{-1} for Hg^{2+} to 3 μg l^{-1} for CH_3Hg^+ [18,19] (Fig. 1). Selective detection of these electrochemically active species was attained by controlling the reduction potential applied on the micro-electrode. For Hg^{2+}, an optimal potential of −0.2 V on gold micro-disk electrode was used to prevent interference by less electroactive toxic metals and other substances found in complex environmental samples. Using a laboratory-built off-column detection cell and an amperometric detector, determination of sulphite and nitrite at sub-μ*M* level in bulk rain samples was demonstrated [20].

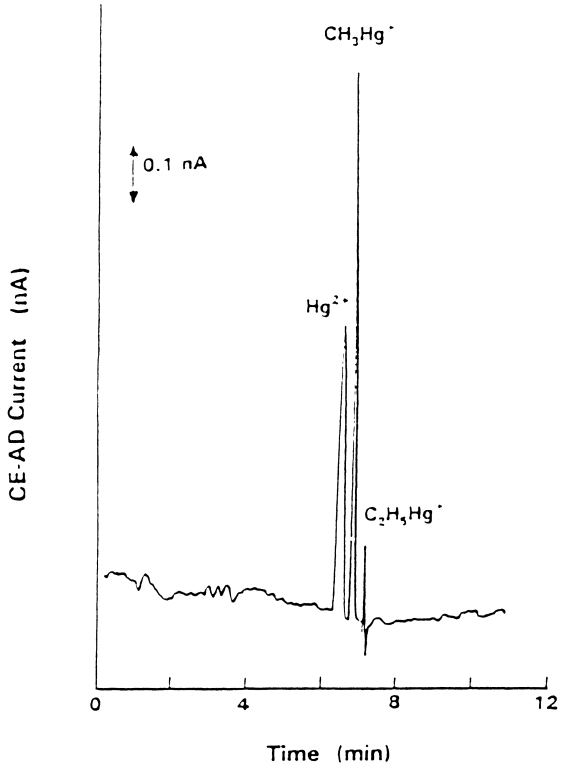

Fig. 1. Separation of 83 μg l^{-1} Hg^{2+}, 8 mg l^{-1} methylmercury and 6 mg l^{-1} ethylmercury by CE–AD, using a selective WE potential of −0.2 V. Electrophoretic conditions as in Table 1. From Ref. [19].

Table 1
Capillary electrophoresis methods with amperometric detection

Compound	Matrix	Sample injection	Electrolyte buffer, separation voltage	Working electrode and electrochemical detection potential	Ref.
Thallium, lead, cadmium, copper	Deionized water	Electrokinetic injection (10 kV for 2 s)	0.05 *M* DMBA, 0.0065 *M* HIBA (pH 4.9); 30 kV	Mercury film; −1100 mV	[14]
Nitrite	Deionized water	Electrokinetic injection (8 kV For 1 s)	0.005 *M* sodium phosphate, 0.005 *M* CTAC (pH 6.2); 8 kV	Cylindrical carbon fiber; 700 mV	[14]
14 Metal ions	Deionized water	Electrokinetic injection (20 kV for 1 s)	0.03 *M* creatinine, 0.008 *M* HIBA (pH 4.8); 30 kV	Mercury film; −1100 mV	[15]
9 Metal ions	Deionized water, snow	Electrokinetic injection (5 kV for 10 s); stacking (12% electrophoretic buffer)	0.03–0.05 *M* creatinine, 0.008–0.010 *M* HIBA (pH 4.8); 20–30 kV	Gold and platinum disk; cathodic detection: −700 mV for 96 ms and 0 mV for 96 ms with data collected over last 48 ms; anodic detection: −800/1100 mV for 72/96 ms and 100 mV for 96/144 ms with data collected over first 48/144 ms	[16]
Nitrite, iodide, thiocyanate, azide	Deionized water	Electrokinetic injection (1 kV for 15 s)	0.5 *M* tetraethylammonium perchlorate, 0.1 *M* *n*-$BuNH_2$, 20% (v/v) acetonitrile in DMF; 30 kV	Platinum disk; 1700 mV for 44 ms and 0 mV for 16 ms	[17]
Inorganic and organic mercury compounds	Sediment	Electrokinetic injection (25 kV for 5 s)	0.1 *M* creatinine, HOAc (pH 4.8); 25 kV	Gold micro-disk; −200/−500 mV	[19]
Nitrite, sulphite	Rain water	Hydrodynamic injection (110 mbar for 5 min)	5 m*M* phosphate buffer (pH 6.7); 10 kV	Gold; 900 mV	[20]

3. Ion selective microelectrodes

In order to overcome the major drawback of amperometric detectors, which are applicable only to electroactive compounds, ion selective microelectrodes (ISMEs) have been introduced as detection systems of inorganic ions (Table 2). For electrochemical inert systems and very small detection volumes, ISMEs have certain advantages. In a single run, it is possible to detect widely different ions. In addition, the detection limits for specially designed ISMEs may be lower than those for conductivity detectors.

In the first application of ISME as a CE detector [21], the microelectrode tip was positioned several micrometers behind the capillary end (post-column) to avoid drifting and noisy potentials due to the electrophoretic field inside the capillary. Owing to irreproducible turbulence, this configuration led to distorted peak shapes, so that conditions for maximum resolution were not fulfilled. On the contrary, using an ISME as CE detector in an on-column position, drift and noise problems are encountered, mainly because the ISME is not decoupled from the electrophoretic field and because temporary instabilities in its position give rise to potential changes, which are superimposed on the Nerstian response. To stabilise the position of the ISME with a precision of at least ± 10 nm would be very costly. Nann and Simon [22] described a procedure for drastically reducing drift and noise by etching the capillary detector end with hydrofluoric acid to a conical aperture (Fig. 2). The field strength at the tip of the ISME is considerably reduced compared with that of the remaining capillary, and so the use of a decoupling system is avoided. This system was used for the determination of potassium, sodium, rubidium, calcium, and dopamine in blood plasma [23]. The logarithmic response obtained with potentiometric sensors allowed detection over a wide dynamic range. The method was shown to be reliable in real samples especially when an internal standard with simultaneous analyte addition was used to compensate matrix effects and sampling biases. Generally the lifetime of the ISMEs described was about 2–3 days under CE conditions. To achieve accurate results, recalibration every 12 h was recommended. The same detector configuration was applied to the determination of anions, using ISMEs based on anion-exchanger liquid membranes [2]. Their selectivity coefficients depend on the free enthalpy of hydration of the analyte anions, i.e. the more lipophilic the anion, the higher is the sensitivity of the ISME. With this kind of electrode (consisting in 10% TDMAC in 2-nitrophenyl octyl ether) the use of surfactants (especially lipophilic additives like CTAB) was avoided because the anion-selective liquid membrane phase was spontaneously washed out of the microelectrode. So polymer (Polybrene)-coated capillaries were employed in order to cover the glass surface with a positively charged layer. Several inorganic anions (bromide, iodide, nitrate, perchlorate and thiocyanate) plus an organic one (salicylate) were separated within 4 min. For perchlorate, which is the ion the electrode is most responsive to, a plate number of 300 000 and a LOD$<10^{-7}$ M (10 μg l^{-1}) were achieved. According to the procedure described for the cation determination [23], a calibration plot allowed quantitative determination of perchlorate in spiked tap water.

A very similar system was used by Hauser et al. [24] for the separation and detection of inorganic anions with LODs ranging from 10 $\mu g\, l^{-1}$ for perchlorate to 3.5 $mg\, l^{-1}$ for chloride. The same group studied the effect of surface charge reversal of silica capillaries in the determination of inorganic anions with an ISME as a detector [25]. The authors concluded that, in contrast to the results reported in the case of indirect photometric detection of anions, coating was not found to be of benefit when potentiometric detectors were used.

All the aforementioned microelectrodes are based on glass micropipettes. Their construction requires a special pipette drawing instrument and is fairly complicated. Moreover the lifetime of these electrodes does not usually exceed a few hours, so that they must be prepared immediately before use. An alternative to glass microelectrodes is the use of coated-wire electrodes where the polymer matrix is in direct contact with a metallic conductor. They are much easier to prepare and are mechanically robust. As they do not possess an inner reference electrolyte they sometimes lack long term stability and reproducibility of the potential. Kappes et al. [26] compared coated-wire electrode and micropipette-based electrodes in the CE determination of monovalent

Table 2
Capillary electrophoresis methods with ion selective microelectrode detection

Compound	Matrix	Electrokinetic injection	Electrolyte buffer, separation voltage	Microelectrode	Membrane phase	Ref.
Potassium	10 m*M* magnesium acetate	5 kV for 5 s	20 m*M* $Mg(OAc)_2$; 30 kV	Liquid membrane	Potassium TCB (10 mg) in NOE (500 mg)	[22]
Potassium, lithium, sodium, rubidium, calcium	Electrolyte buffer; blood serum	5 kV for 5 s	9 m*M* $Mg(OAc)_2$, 1 m*M* $MgCl_2$, ca 17 m*M* HOAc (pH 4.8); 30 kV	Liquid membrane	2,2′-[1,2-Phenylenebis(oxy-2,1-ethanediyloxy)]bis(*N*,*N*-diphenylacetamide) (6.2 mg, 100 mol%), PTCB (3.4 mg, 68.5 mol%) in NOE (620 mg)	[23]
Bromide, iodide, nitrate, perchlorate, thiocyanate	Deionized water; tap water	2–10 kV for 3–10 s	20 m*M* Tris–formate (pH 7.0); 25 kV 20 m*M* sodium sulphate, sulphuric acid (pH 2.5); 30 kV	Liquid membrane	10% TDMAC in NOE	[2]
Chloride, nitrate, nitrite, bromide, iodide, perchlorate	Deionized water	5 kV for 7 s; hydrostatic injection 10 cm for 30 s	10 m*M* HOAc–NaOAc (pH 5.0); 30 kV 10 m*M* potassium sulphate; 30 kV	Liquid membrane	3% TDMAC in NOE; 5.0% TmTPP< 1.0% TDMA–TCB, 4.0% 1-decanol, 90.0% NOE	[24]
Chloride, nitrate, nitrate, bromide, iodide, perchlorate, thiocyanate	Deionized water	5 kV for 7 s	10 m*M* potassium sulphate; 30 kV	Solid-state coated-wire	5.0% MnTPP, 1.0% TDMA–TCB, 4.0% 1-decanol, 60.0% NOE, 30.0% PVC in THF	[26]
Lithium, sodium, potassium, rubidium, cesium, ammonium, calcium, strontium, barium	Deionized water	5 kV for 7 s	10 m*M* $Mg(OAc)_2$, 18 m*M* HOAc (pH 4.7); 30 kV	Solid-state coated-wire	1.2% *N*,*N*,*N*′,*N*′-Tetracyclohexyl-oxybis(*o*-phenyleneoxy)-diacetamide (V163), 0.8% potassium TCB, 65.3% NOE, 32.7% PVC in THF	[26]

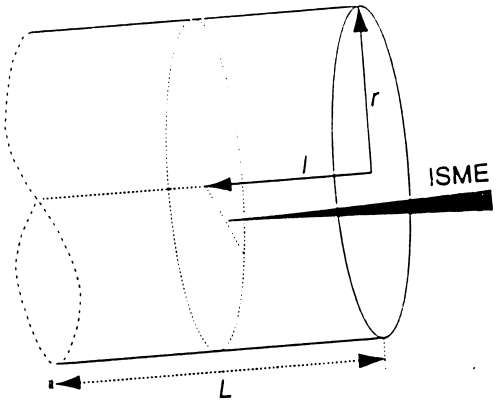

Fig. 2. On column positioning of an ISME in a cylindrical capillary aperture. L=Total lenght of the capillary; l=distance from capillary end to ISME tip; r=inner radius of the capillary. From Ref. [22].

inorganic anions and of alkali and earth-alkaline cations. Relative responses of different species varied with the two electrodes but sensitivity was generally comparable. A slight loss of resolution due to post-column band broadening was observed in the case of the coated-wire electrode. On the other hand this electrode gave satisfying results up to the eighth day, while the micropipette electrode could not be used for more than one day at best.

4. Conductivity

Detection of inorganic ions is usually performed by indirect or direct UV [27], but this detection mode suffers from lack of sensitivity (LOD $>10^{-4}$ M) and it can be hardly applied to minor inorganic constituents without the use of a preconcentration technique. A suitable alternative to specifically detect small ions which do not have a chromophore and are not electrochemically active, is to measure the differences in conductivity of the solute zones and the separation electrolyte (Table 3). By using separation electrolytes with a low conductivity, a sensitive detection is possible. Three possible designs for a CE conductivity detector are on-column, off-column, and end-column configurations. In the first on-column detection system platinum sensing electrodes are sealed on the opposite sides of the column through two laser beam-punched holes [28]. Because CE typically has a voltage drop of 300 V/cm along the length of the capillary, the sensing electrodes need to be aligned carefully and an isolation transformer must be used in measuring the conductance.

On column conductivity detection, previously developed for ITP measurements, was used by Kaniansky et al. [29–31] for anion and cation analysis. By using a hydrodynamically closed separation compartment in a FEP capillary, inorganic anions were determined at 50–100 ppb levels in drinking, river and rain water [29]. No interfering constituents were detected in the tested samples. Preconcentration obtained by on-line coupled ITP–CE with conductivity detection allowed to determine anions at low ppb concentrations when the concentrations of sulphate and chloride in the samples were higher than 1000 ppm [30]. LODs from 200 to 600 ppt were determined for nitrite, fluoride and phosphate.

Off-column conductivity detectors may be constructed by grounding the capillary prior to the sensing electrode. They do not suffer from electrical interference caused by the applied high voltage during the CE separation. Huang et al. [13,32] developed end-column designs for conductimetric and amperometric detection in CE, in which the sensing electrode is placed at the outlet of the fused-silica capillary. Conductivity changes in the effluent were measured between this sensing electrode contacting the surrounding bulk solution near the capillary outlet. Such detector configuration demonstrated sensitivities approaching those of previous on column conductivity detectors with only a small sacrifice in resolution, due to an extra band broadening of approximately 25%. Practical considerations about fouling of the electrode and maintenance suggest that a conductivity cell assembly for CE should be accessible and interchangeable from the sensor side (e.g. electrode fouling) as well as the capillary side (e.g. plugged or broken capillary). Based on these assumptions, the first commercial end-column conductivity detector especially designed for CE was developed [33,34]. The detection end of the capillary is permanently encapsulated in a stainless steel coupling connector, which also serves as a precision spacer and centres the detection end of the fused-silica capillary (Fig. 3). Two channels positioned perpendicular to each other on the capillary connec-

Table 3
Capillary electrophoresis methods with conductimetric detection

Compound	Matrix	Sample injection	Electrolyte buffer, separation voltage	Conductivity detector	Ref.
Inorganic anions	Deionized water, drinking, rain and river water	200–400 nl sample injection loop	7 m*M* succinate, bis-tris propane, 0.2% (v/v) MHEC, polyvinylpyrrolidone (pH 3.5); 20–30 μA	On-column	[29]
Inorganic anions	Deionized water, river water	ITP	10 m*M* aspartic acid, β-alanine, 0.2% (v/v) MHEC (pH 3.4); 75 μA	On-column	[30]
Inorganic and organic anions	Deionized water, soft drink, urine	Hydrodynamic (25 mbar for 12 s)	50–100 m*M* CHES, 20–40 m*M* LiOH, 0.03% Triton X-100, 3 m*M* HMOH (pH 9.2); 25 kV	Nonsuppressed end-column	[34]
Alkali and earths alkaline metals, organic amines	Deionized water, saliva	Hydrodynamic (14–25 mbar for 12 s)	30 m*M* HIS/MES, 2–3 m*M* 18-crown-6 (pH 6.1); 20–25 kV	Nonsuppressed end-column	[34]
Cations, transition metals	Deionized water	Electrokinetic (5 kV for 6 s)	0.1 *M* HOAc, 1 m*M* oxalic acid (pH 2.84); 30 kV	Nonsuppressed end-column	[34]
Bromide, chloride, iodide, nitrite, sulphate, nitrate	Multivitamin supplement	Hydrodynamic (25 mbar for 12 s)	75 m*M* boric acid, 30 m*M* LiOH, 0.75 m*M* HMOH (pH 8.95); 25 kV	Nonsuppressed end-column	[34]
Chloride, nitrate, nitrite, sulphate	2 ppm ammonia and 50 ppb hydrazine water solution	ITP	50 m*M* CHES, 20 m*M* LiOH, 0.03% (w/w) Triton X-100 (pH 9.2); 25 kV	Nonsuppressed end-column	[35]
Chloride, nitrite, nitrate, sulphate, phosphate, bicarbonate	Airway surface fluid	100 nl	100 m*M* CHES, 40 m*M* LiOH, 8% (v/v) 2-propanol, 80 μ*M* spermine (pH 9.3); −278 V/cm	Nonsuppressed end-column	[38]
Sodium, potassium, calcium, magnesium	Airway surface fluid	100 nl	100 m*M* MES, 100 m*M* His, 20 m*M* HIBA (pH 5.6); 222 V/cm	Nonsuppressed end-column	[38]
Inorganic and organic anions	Deionized water, rain water	Hydrodynamic (500 mbar for 0.4 min.)	100 m*M* CHES, 40 m*M* LiOH, 0.02% Triton X-100 (pH 9.2); 25 kV	Nonsuppressed end-column	[41]
Arsenic and selenium species	Waste water	Hydrodynamic (25 mbar for 0.2 min.)	50 m*M* CHES, 20 m*M* LiOH, 0.03% Triton x-100 (pH 9.4); 25 kV	Nonsuppressed end-column	[42]
Inorganic anions	Electrodeposition coating sample	Hydrodynamic (25 mbar for 0.2 min)	50 m*M* CHES, 30 m*M* Arg. (pH 9.0); 30 kV	Nonsuppressed end-column	[43]
Inorganic and organic anions	Deionized water, tap water, grape juice	Hydrodynamic (1 psi for 2 s)	2 m*M* borax buffer; 24 kV	Suppressed end-column	[45]
Inorganic and organic anions	Deionized water, beer, soy sauce, Chinese green tea, Swedish coffee, saliva	Hydrodynamic (5 psi for 2 s)	2 m*M* sodium tetraborate (pH 10–10.5); 20 kV	Suppressed end-column	[46]
Inorganic and organic anions	Deionized water	Hydrostatic (40 mm for 5 s)	1–5 m*M* sodium tetraborate (pH 10–10.5); 24 kV 4.0 m*M* sodium glycinate (pH 10.5); 20 kV 4.0 m*M* sodium taurinate (pH 10.5); 20 kV 2.0 m*M* sodium tetraborate, 0.02 m*M* barium borate; 24 kV	Suppressed end-column	[47]
Inorganic and organic anions	Deionized water	Hydrodynamic (5 p.s.i. for 2 s)	2 m*M* sodium tetraborate (pH 10); 20 kV	Suppressed end-column	[49]
Soluble ionogenic atmospheric gases	Air	Electrokinetic, hydrodynamic hydrostatic (10 cm for 20–50 s)	2 m*M* sodium tetraborate; 15 kV	Suppressed end-column	[51]
Inorganic anions	Deionized water	Hydrostatic (10 cm for 10 s)	20 m*M* MES, 20 m*M* His, 0.0001% hexadimethrine bromide (pH 6); 20 kV	Contactless on-column	[54]
Inorganic cations	Deionized water	Hydrostatic (10 cm for 10–30 s)	10 m*M* lactic acid, 8 m*M* 4-methylbenzylamine, 15% methanol (pH 4.9); 20 kV 20 m*M* MES, 20 m*M* His (pH 6); 20 kV	Contactless on-column	[54]

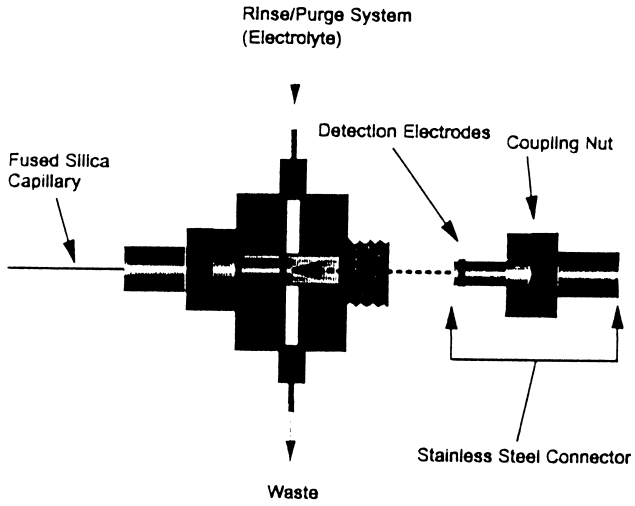

Fig. 3. End column nonsuppressed conductivity cell. From Ref. [34].

tor permit unimpeded flow of bulk liquid to and from the detector cell, when both components are connected together. The capillary end is accurately cut and precisely arranged with its outlet protruding from the centre cross to yield a working distance (gap) in contact with the detector connector, which is optimised for sensitivity and band broadening. Direct conductivity detection was used for the analysis of small inorganic and organic ions in various actual matrices (urine, saliva, soft drinks, multivitamin tablets) using low mobility electrolytes (CHES for anions and His-MES for cations), and the method was shown to be at least ten times more sensitive than indirect UV for the early migrating ions. Indirect mode allowed sensitive detection of larger ions with low ionic mobility using dilute organic acids (acetic and oxalic) as electrolytes. The linear range of the conductivity detector was determined to be greater than three orders of magnitude. Detection limits for selected anions were extended into the ppb/ppt range by a transient ITP stacking procedure [35]. The characteristics of this end-column nonsuppressed conductivity detector were investigated using solutes and background electrolytes that possess a range of mobilities [36]. Indirect detection was shown to be most sensitive for low- to medium-mobility solutes. A generalised response expression was developed for conductivity detection based on the similarities in conductivity and electrophoretic mobility. This commercial detector has found application in different analytical fields (Table 3). Good reproducibility was observed even in a loaded matrix such as coffee [37]. Airway surface fluid from rats was collected using a small sampling capillary inserted into the trachea and inorganic anion and cation content was directly determined by CE with the commercial conductivity detector [38]. In comparison to the previously published method using indirect UV detection [39], conductivity detection allowed more flexibility in terms of operating parameters, resolution of a great number of species, and lower limits of detection. The drawbacks of the conductivity methods are the need to make coupled capillaries (the sampling capillary and the separation capillary are permanently joined to the detector cell assembly) and the time taken to re-equilibrate the system if the conductivity sensor becomes fouled. Magnesium determination in drinking waters by CE equipped with this conductivity detector was validated by comparison with results obtained by the ion selective electrode [40]. The same commercial system was used for the determination of anions in rainwater [41]. Linear calibration plots were generated from 0.050 to 20 mg l^{-1}, which is the range generally found in wet depositions. The method was demonstrated to be accurate and reliable by comparison with standard IC analysis and by analysing certified reference standards. Conductivity detection

was compared with direct UV detection in the speciation analysis of arsenic and selenium compounds [42]. The use of the commercial conductivity detection system showed a slight sensitivity improvement with respect to the photometric mode. This method was applied to a contaminated water sample of a tailing of tin ore processing. The relatively low ionic strength of this water matrix and the absence of charged organic contaminants like humic acids are advantageous for conductivity detection, simplifying the signal evaluation. Limitations of the unspecific detection mode appeared in other experiments covering plant extracts, where, in spite of the high sensitivity of conductivity monitoring, extended interference by charged organic compounds might occur. The same end column conductivity detector was used for the quantitative determination of small anionic compounds in electrodeposition coating samples after CE separation [43]. At last, CE separation system with this conductivity detector was used for determining inorganic impurities and acetate counter ions in pharmaceutical drug substances [44].

Despite their refinement, conductivity detectors for CE are nonselective bulk property detectors. The signal arises from the difference in equivalent conductance or mobility of the charge carrier electrolyte ion and the analyte ion. The LOD of such a system is rarely better than 10^{-5} *M*. However unlike single column IC, where such detection is commonly used and where there is no special restriction on the choice of the mobility of the eluent ion, a large difference in mobility of the carrier electrolyte ion leads to excessive peak tailing/fronting in CE. This conflict between optimum sensitivity and separation efficiency represents the ultimate limitation of nonsuppressed conductivity detection in CE.

To determine the levels of solute ions in the presence of vast amounts of bulk buffer ions, it is necessary to convert the buffer ions into a weakly conducting form. This allows one to make a conductivity detector act as a specific solute property detector. Two suppressed conductimetric CE separation systems (SuCCESS) have been described [45–48]. These systems provided significantly better LODs than nonsuppressed systems by lowering the conductivity of the carrier electrolyte through an ion-exchange process. In one system, suppressors were made from short ion-exchange membrane capillaries and were placed between the separation capillary and the detector cell (Fig. 4). The suppressor was placed in the destination vial, which contained regenerant and the ground electrode [45]. In the other system a tubular cation-exchange membrane suppressor, housed in a static reservoir of dilute acid regenerant solution, was installed at the end of the fused-silica separation capillary [46]. The conductivity detector that follows the suppressor was constructed by inserting two 100-μm diameter Pt wires through the wall of a 190-μm I.D. PVC capillary in parallel and as close to each other as possible. A positive high voltage is applied to the capillary inlet and the regenerant solution is grounded. Thus, the detector is isolated from the electric field, and the EOF generated in the capillary carries the solution through the suppressor and the detector. The system provides LODs at the tens of micrograms per litre level for a variety of anions without any preconcentration [45,46]. Efficiencies were little worse than those obtained with a photometric detection system. The relative immunity of SuCCESS to sample constituents, that adversely affect a chromatographic column, was demonstrated by analysing a variety of sample types, including beverages, physiological fluids, medications and household items [45,46]. A comparison was made between several electrolytes for SuCCESS determination of inorganic anions in terms of efficiency of separation and peak shape [47]. Two constraints, i.e. the ability to be suppressed to a weakly conducting species and a suitable electrophoretic mobility similar to that of the analytes, are the major considerations in picking an electrolyte for SuCCESS. For the analysis of inorganic anions, borate and glycinate appear the best suited.

Because of the difficulty in achieving the same spacing between the wires each time, it is problematic to reproducibly construct suppressed conductivity detectors with similar cell constants. A novel and simple design of a conductivity detection cell, based on bifilar wire electrodes that is rugged and easy to construct, was described by Kar et al. [49]. A PC-based bipolar pulse conductance detection system that significantly exceeds the performance of commercial conductance detectors was also described. Results obtained with the new cell and electronic design in a SuCCESS system were presented. The

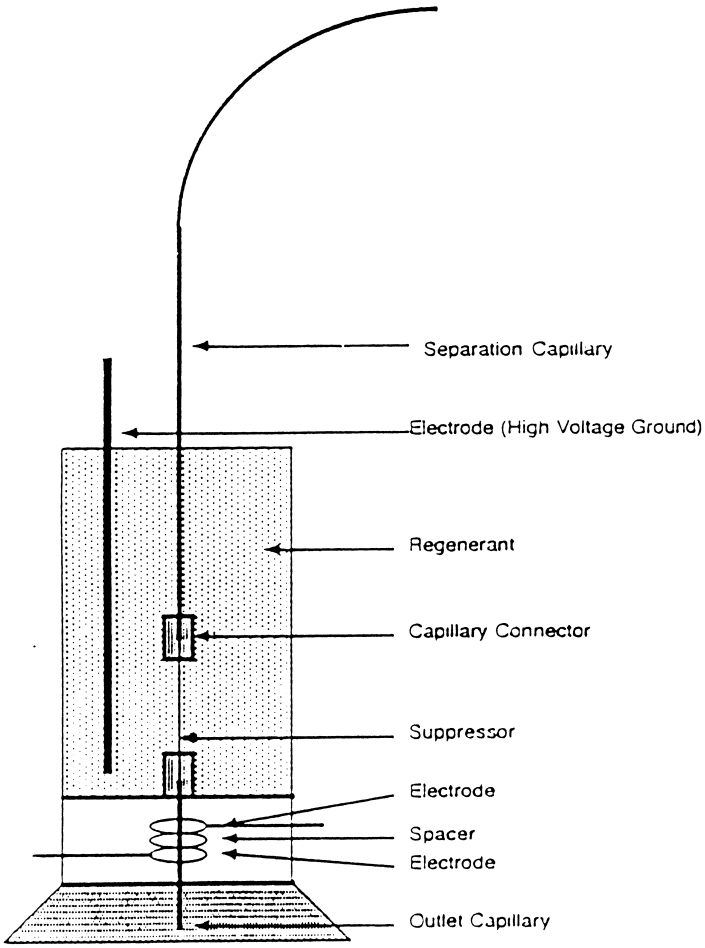

Fig. 4. Scheme of capillary-suppressor–conductivity cell assembly. From Ref. [47].

resistance of this cell to fluid flow was smaller, resulting in perceptibly decreased migration times and providing superior separation efficiency both in terms of plate counts and plates for unit time.

SuCCESS systems are still not commercialised and they have found limited application. Two approaches to interfacing a suppressed IC system with a SuCCESS system were described [50]. The first interface uses a six-port loop valve which allows selective introduction of any segment of an IC eluate band into the CE system. This arrangement is also suitable as a sample delivery system, especially with CE systems using relatively short capillaries and high electric fields. In the second interface, a small fraction of the IC effluent passes through a length of Nafion tube immersed in a solution of saturated sodium tetraborate. Thus, sufficient borax is introduced by Donnan penetration to increase the ionic strength of the flowing stream to an extent appropriate for carrying out CE separations. This valveless design allows the continuous injection of all IC eluate bands into the CE system without additional electrolyte preparation for CE.

A very innovative application of SuCCESS technique in atmospheric sciences is the direct measurement of soluble ionogenic atmospheric gases [51]. The sampling was performed by a small wire loop deployed at the tip of the capillary. When the loop was dipped into a solution and withdrawn, a liquid film, which could be used as an absorber for gases

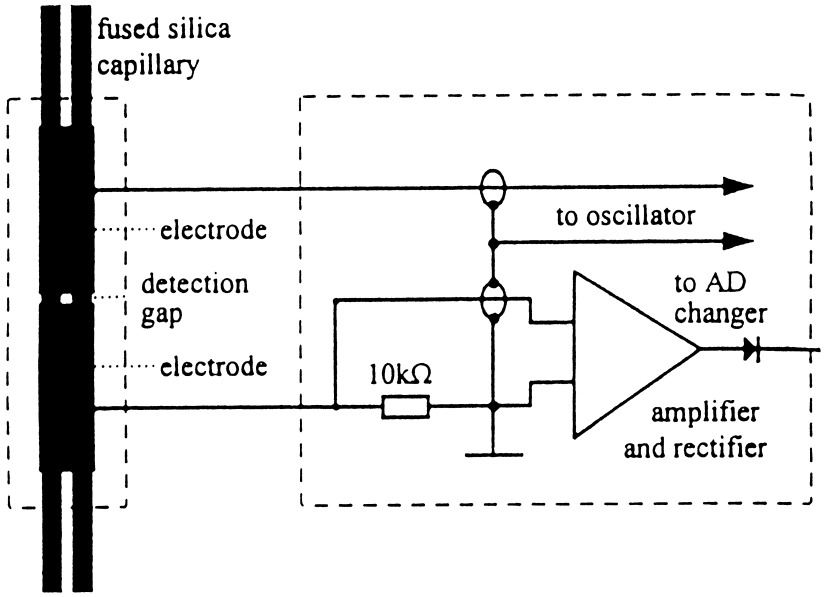

Fig. 5. Scheme of the capacitively coupled conductivity detector. From Ref. [54].

collected in a sample chamber, was formed on it. The film was in fluid communication with the capillary and acted as a microreservoir. Under hydrostatic injection conditions 1 ppb SO_2 could be detected by a suppressed conductimetric detection. The determination of soluble gases and organic vapours was also demonstrated [52].

A certain drawback of instruments working with conductivity detection measured by direct contact of electrodes and sample are the high instrument costs, as the capillary has to be modified to a specific extent and a rather complicated geometry and construction are required. As a consequence, for CE purpose, a detector without galvanic connection of solutes and electrodes with, at the same time, equal sensitivity as compared to a directly connected conductivity detector is a certain demand. Contactless methods require inductively or capacitively devices using high frequencies [53,54]. The contactless capacitively coupled conductivity detector, developed by Zemann et al. [54], consists of two syringe cannulas that are mounted 2-mm apart so that the CE capillary can be pushed through both of them (Fig. 5). The cannulas act as capacitors that, together with a home-made frequency generator and amplifying electronics, measure the electrolyte conductivity inside the gap between them.. This design allows a fast and easy to use adjustment of the detector on virtually any position along the capillary. The conductivity is measured via the capacitive properties of the detection unit, which is in fact a resistor–capacitor unit when an electric frequency (20–40 Hz) is applied. By this design, conductivity is measured axially along the capillary (over the length of the gap between electrodes) and not across the capillary diameter. Therefore capillaries of different diameter give similar signals. The detector linearity is excellent (from less than 1 ppm to more than 1000 ppm for sodium and chloride). The LODs of sodium and chloride are 200 ppb. By improving the electronic components, further reductions in LOD is likely. As the device works contactlessly, any cleaning or flushing of the detection cell is not necessary and it can be easily used without modification of the geometry of the capillary or of the polyimide coating.

5. Conclusions

ED techniques have reached such a stage of development that they have a significant impact on

CE usage in selected areas of analysis, such as biochemical studies [9,10]. On the contrary the application of ED systems to inorganic analysis is still limited. Determination and speciation of transition metal compounds by amperometric detection systems are shown to be a very promising field of work from both sensitivity and specificity points of view. The simultaneous determination of inorganic and organic mercury species in contaminated sediments has been reported indeed [19]. The feasibility of detection of inorganic anions and cations by ion selective microelectrodes has been demonstrated, but the detection limits and ruggedness of these detection systems are still not satisfying.

Much work has been devoted to the development of conductivity detection systems, but till now, despite a certain number of patents, only an instrument has become commercially available [33]. This detector, which is ten-fold more sensitive than an indirect UV detector, has found a good number of applications in various analytical fields, but it is more prone to be fouled by sample matrix constituents. Suppressed conductivity detection systems have achieved very promising detection limits, but the suppression process limits drastically the choice of available running buffers. Contactless conductivity detection has still a great margin of improvement, and future work is needed to explore the possibilities and the limits of this technique.

Nevertheless, in order to widen the availability and the diffusion of analytical methods based on CE separation with ED, it is urgent that CE–ED systems are marketed by analytical instrument suppliers.

6. Abbreviations

AAS	Atomic absorption spectrometry
AD	Amperometric detection
CE	Capillary electrophoresis
CHES	2-(*N*-Cyclohexylamino)ethanesulphonic acid
CTAB	Cetyltrimethylammonium bromide
CTAC	Cetyltrimethylammonium chloride
DMBA	*N*,*N*-Dimethylbenzyl-amine
	Dimethylformamide
ED	Electrochemical detection
EDTA	Ethylenediaminetetraacetic acid
EOF	Electroendoosmotic flow
HIBA	α-Hydroxyisobutyric acid
His	Histidine
HMOH	Hexamethonium hydroxide
I.D.	Internal diameter
IC	Ion chromatography
ISME	Ion selective microelectrode
ITP	Isotachophoresis
LOD	Limit of detection
MES	2-(*N*-Morpholino)ethanesulphonic acid
MHEC	Methylhydroxyethylcellulose
MnTPP	5,10,15,20-Tetraphenyl-21H-23H-porphin manganese (III) chloride
NOE	2-Nitrophenyl octyl ether
OAc	acetate
PAD	Pulsed amperometric detection
PC	Personal computer
PVC	Poly(vinylchloride)
SDS	Sodium dodecylsulphate
SuCCESS	Suppressed conductivity–capillary electrophoresis separation system
TCB	Tetrakis (4-chlorophenyl) borate
TDMA	Tridodecylmethyl ammonium
TDMAC	Tridodecylmethyl ammonium chloride
THF	Tetrahydrofuran
UV	Ultraviolet
WE	Working electrode

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