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Integration of a contactless conductivity detector into a commercial capillary cassette
Detection of inorganic cations and catecholamines

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Abstract

A contactless conductivity detector integrated into the capillary cassette of Agilent ^{3D}CE equipment is described. The detector is user-friendly, compact and easily modified. The UV detector of the ^{3D}CE equipment is available parallel with the contactless conductivity detector increasing the detection power. Two electrolyte solutions, 2-(*N*-morpholino)ethanesulfonic acid–histidine solution (20 m*M*, pH 6.0) and ammonium acetate (10 m*M*, pH 4.0), were used as the separation media for inorganic cations and organic catecholamines, respectively. The detection limit for all metal cations except barium was under 0.5 mg/l, and that for four catecholamines was ca. 10 mg/l. This last value was the same order of magnitude as achieved with parallel UV detection.

Keywords: Contactless conductivity detection; Conductivity detection; Detection, electrophoresis; Instrumentation; Inorganic cations; Catecholamines

1. Introduction

Capillary electrophoresis (CE) is a highly efficient separation technique for charged compounds owing to the low sample consumption and good resolution. Online detection is more challenging in CE than in conventional chromatographic methods, however, because sample volumes are smaller. UV detection is the standard detection method in commercial CE instruments and is in widest use. A serious drawback of the UV detector is that many analytes do not absorb UV light [1]. Moreover, the sensitivity for the UV absorbing analytes tends to be inadequate because of a narrow optical path length. Laser-induced fluorescence may be more sensitive than UV, but it is limited to fluorescent analytes. Both direct and indirect detection methods are widely used with fluorescence detection, but the need for chemical derivatization and the cost of the equipment do not rule in its favour. Derivatization also sets limitations on the use of fluorescence detection.

Electrochemical detection methods have been investigated for many years as a way of overcoming the limitations of optical detectors. In general, electrochemical detectors are simple [2,3]. Normally, the hardware consists of two or three electrodes and basic electronics. The electrical response is also readily available without special transducers (e.g. photomultipliers). The conductivity detector can be

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considered to be a universal detector suitable for all manner of compounds.

Over the years, many different arrangements have been employed to integrate the electrodes in contact with the solution inside the capillary and thereby improve the detection sensitivity. Huang et al. [4] were among the first to investigate an on-capillary conductivity detector. In their first version, two Pt wires were inserted into the capillary through laser-drilled holes. Later, an improved end-capillary cell structure, mounted directly at the outlet end of the capillary, was introduced [5,6]. Dasgupta and co-workers [7,8] developed a suppressed conductometric CE system, where the conductivity of the background electrolyte is decreased before the detection cell. However, when the conductivity cell is mounted on the capillary, some dead volume remains in the junction. In addition to the dead volume, peak broadening occurs due to the relatively large volume of the conductivity cell. Furthermore, the adsorption of impurities and analytes on the electrodes causes baseline drifting and reduction in sensitivity. Bodor et al. [9] have investigated the solution mediated contact electrodes, which reduce the need for flushing and washing of the electrodes.

Recently Zemann and co-workers [10,11] and Fracassi and co-workers [12,13] have introduced a contactless conductivity detection (CCD) system that was based on two tubular cylinders acting as capacitive electrodes, mounted on the outer surface of the capillary. In the CCD device, a radiofrequency (rf) signal is transferred to one of the electrodes, while the other electrode is connected to the amplifier-rectifier unit of the CCD system. The conductivity of the solution between the electrodes determines the strength of the rf signal measured at the electrode. The signal is rectified, amplified and filtered before being sent to the data acquisition unit. The response of the detector depends on the ratio of the mobilities of the analyte and the co-ion of the background electrolyte (BGE). Because the electrodes are not directly in contact with the background electrolyte solution, interferences (e.g. contamination) from the solution are prevented. In addition, electrode alignment and fabrication of the CCD system are simpler than with conventional conductivity detectors.

Electrode fabrication arrangements differ widely. Cut-off metal syringe needles have been used to simplify changing the capillary [10,14]. The electrical isolation effect of the air between capillary and metal cylinder, however, interferes with the coupling of the rf signal to the solution. In one case, the isolation effect has been prevented by covering the capillary surface with silver paint [12]. Tight fitting has also been obtained by a wire-wrapping technique, which at the same time simplifies capillary replacement [13]. The use of metal cylinders has been shown to allow movable electrodes along the capillary making the distance of the detection point easier to change [15]. Multi-detection [16,17] and integration of the CCD system to portable devices have also been reported [18]. Unfortunately, the sensitivity obtained with CCD has so far been lower than the sensitivity of contact conductivity detection, though many have been working to rectify this problem. One promising approach to increase sensitivity is to use high peak-to-peak voltage of the rf signal [19].

CCD is commonly used in the detection of small inorganic cations and anions and low conductivity organic compounds [20]. Recently, the usefulness of CCD has been improved to allow analyses in nonaqueous background electrolytes [21], the separation of aliphatic alcohols in micellar electrokinetic chromatography [22] and the separation of cyclodextrins and herbicides and their metabolites [23,24].

Catecholamines are of interest as a group of phenolic aromatic cationic compounds, which act as neurotransmitters in the central and peripheral nervous systems. They are important in the diagnosis of Parkinson's disease and investigations of the stress system. Conventionally, catecholamines have been determined by high-performance liquid chromatography with electron-capture detection, but recently, Sirén and Karjalainen [25] and Wallenborg et al. [26] have demonstrated the usefulness of CE for the analysis of catecholamines and their metabolites. Detection methods such as UV [25,27], amperometry [26] and mass spectrometry [28,29] have been used for the detection of catecholamines.

We describe the integration of the electronics of a CCD system into the ^{3D}CE device fabricated by Agilent. The electronics, like that of Fracassi da Silva and do Logo [12], was located inside the capillary cassette to ease handling of the detector as well as to allow parallel UV detection. The CCD was

tested with seven inorganic cations and its applicability to the detection of catecholamines was studied.

2. Experimental

2.1. Contactless conductivity detector

A diagram of the electronic printed circuit board (PCB) of the detector inside the capillary cassette is shown in Fig. 1A. The printed circuit board was designed to fit tightly into the cassette to prevent movement of the detector during runs. Two electrodes were fabricated by a wire-wrapping technique to reduce the air isolation problem. A thin copper wire ($d=100$ μm) was wound around a thicker wire ($d=200$ μm) to obtain a 7-mm-long coil, which was covered with silver paint to connect adjacent wires. The electrodes were mounted directly to the printed circuit board with epoxy glue and the distance 0.4 mm between the electrodes was measured with a microscope (Fig. 1B). The silver paint prevented the glue from penetrating into the cavities of the coil.

A diagram of the CCD electronics is presented in Fig. 2. Extra diodes protecting the operational amplifier were added to the circuit to prevent the over voltages. The output of the detector circuit was connected to an A/D converter through an active filter, which was designed to cut off all frequencies above 16 Hz and so decrease the background noise of the rectified and amplified signal. A 12-bit A/D converter (ADC 10/12 Pico, Pico Technology, Cambridgeshire, UK) was connected via the printer port to the computer. Operational amplifiers A1–A3 (OPA2604AU, Burr-Brown, USA) and A4 (LF356H, National Semiconductor, USA) and other basic components were purchased from local electronics dealers. The peak-to-peak voltage of the rf generator, model FG-32 (RSR Electronics, USA), was 20 V and this was amplified with OPA606KP (Burr-Brown) to 25 V. All collected data were analysed with an in-house Epeaks data analysis script written for Matlab (Mathworks, Natick, USA).

2.2. Chemicals

$MgCl_2 \cdot 2H_2O$, $MnCl_2$, $BaCl_2$ and histidine (His) were obtained from Merck (Darmstadt, Germany), KCl from Riedel-de Haen (Seelze, Germany), $CaCl_2$ and 2-(*N*-morpholino)ethanesulfonic (MES) acid from Sigma–Aldrich (Steinheim, Germany), NaCl from J.T. Baker (Deventer, The Netherlands) and CH_3COOLi from Acros Organics (NJ, USA).

The catecholamines—dopamine (3-hydroxytyramine hydrochloride), DL-normetanephrine (3-

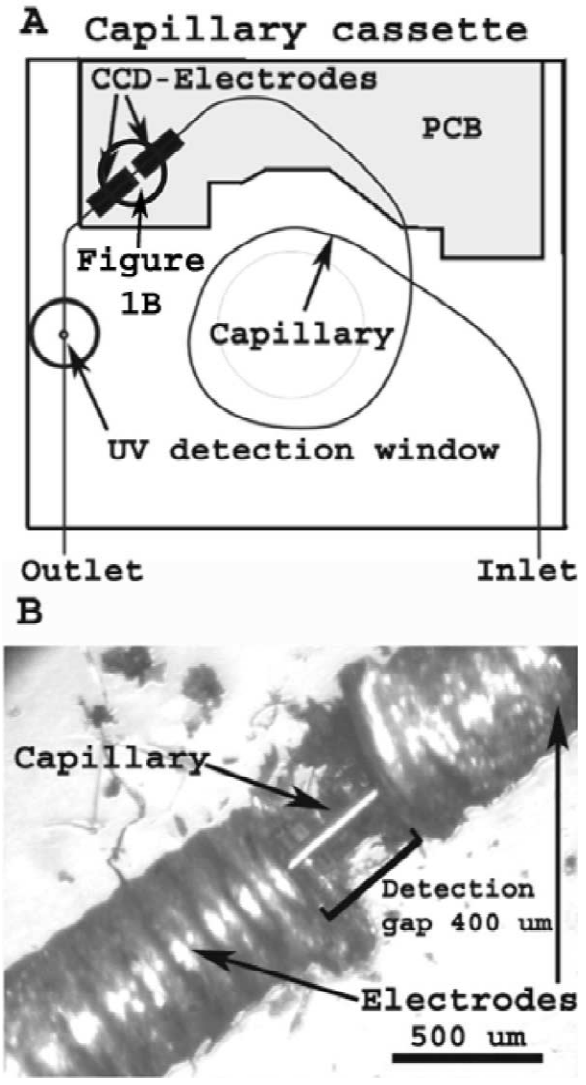

Fig. 1. (A) Diagram of the contactless conductivity detector set-up in the capillary cassette of ^{3D}CE. Tailor-made printed circuit board (PCB) is shown as the grey area. (B) Photograph of the CCD electrodes taken with microscope (circled area in A).

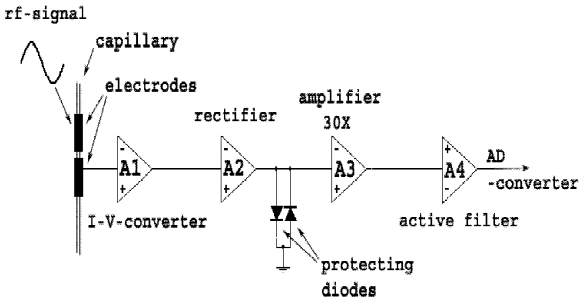

Fig. 2. The electronics diagram for the contactless conductivity detector.

methoxybenzenemethanol hydrochloride), DL-metanephrine (DL-*m*-*O*-methylepinephrine hydrochloride) and 4-hydroxy-3-methoxybenzylamine hydrochloride (HMBA)—were obtained from Sigma–Aldrich. Ammonium acetate was obtained from Fisher (Loughborough, UK) and glacial acetic acid from J.T. Baker. All reagents were of analytical grade. The purified water used in the experiments was distilled and ion-exchanged with a Milli-Q system (Millipore, Molsheim, France).

2.3. Samples and solutions

The 1000 mg/l stock solutions of inorganic cations (K^+, Na^+, Ba^+, Ca^{2+}, Mg^{2+}, Mn^{2+}) were prepared separately in deionised water from chloride salts of the cations. Li^+ was prepared from its acetate salt. The 20 m*M* buffer for separation of the cations was prepared from MES and the pH was adjusted to 6.0 with 0.1 *M* His solution. The 1000 mg/l stock solutions of the catecholamines were prepared in 2.5% (v/v) aqueous solution of glacial acetic acid. For the separation of catecholamines, the pH of the ammonium acetate background electrolyte was adjusted to 4.0 with 2.5% acetic acid solution. All solutions were filtered before use and stored at +8 °C. The final diluted sample solutions (e.g. 5 mg/l) of cations were prepared in deionised water, and catecholamine samples (e.g. 10 mg/l) were prepared in 2.5% acetic acid solution, directly from their stock solutions.

2.4. Capillary electrophoresis

The tests and analyses of the CCD system were carried out with ^{3D}CE equipment (Agilent, Waldbronn, Germany). Both on-line conductivity and UV absorption were measured. The temperature of the capillary cassette was adjusted to 25 °C. The capillaries (50 μm I.D.×150 μm O.D.) were purchased from Composite Metal Services (Hallow, UK). The injection of inorganic cations was carried out electrokinetically with +10 kV for 2 s and the applied separation voltage was +30 kV. The total length of the separation capillary was 63.0 cm and the lengths to the conductivity detector and UV detector were 50.0 and 54.5 cm, respectively. Catecholamines were injected hydrodynamically with a pressure of 50 mbar for 5 s. The voltage applied during separation was 20 kV. The total length of the capillary was 68 cm and the lengths to the conductivity detector and UV detector were 55.0 and 59.5 cm, respectively. Before separations, new capillaries were conditioned for 15 min with 0.1 *M* NaOH, for 15 min with deionised water and for 15–30 min with BGE solution. Between analyses, the capillaries were flushed for 3–4 min with the BGE solution. The UV detector, which was coupled in series with the CCD system, was set to monitor at a wavelength of 214 nm. The wavelength for the UV detection was optimised elsewhere [27].

3. Results and discussion

The installation of capillaries into the cassette and the installation of the cassette into the CE equipment were not more difficult with than without the CCD system in the cassette. The out-going wires, which were led through the ventilation tube, did not affect the temperature control of the capillary cassette. The surface-mounted components were soldered on the top side of the board, because the thermal noise and drift of the electronic components were then minimized by the more efficient cooling of the thermostatic system of the cassette. No drifting of the baseline was observed during the analyses.

The electrodes were set relatively close (0.4 mm) to each other to increase the sensitivity and improve resolution [13]. High resolution was especially important for the separation of HMBA and dopamine, whose migration times were similar. Electrodes were fabricated from wire of 100 μm O.D. to obtain as

cylindrical a shape as possible. The 0.4-mm distance between the electrodes proved to be short enough and the analyte peaks were sufficiently well separated. Before experiments, the frequency of the rf signal was optimised. The highest response was obtained at 300 kHz, but the frequency was adjusted to 291 kHz because the background noise level was then lower. To increase the sensitivity, a pre-amplifier was used to raise the peak-to-peak voltage of the rf signal to 25 V.

3.1. Direct detection of cations

The performance of the CCD system was tested in the separation of cations Li^+, Na^+, K^+, Ba^{2+}, Ca^{2+}, Mg^{2+} and Mn^{2+}. BGE solution of low conductivity relative to the analytes was needed to achieve acceptable sensitivity. On this basis, we chose 20 m*M* MES background electrolyte and HIS as pH modifier. Samples were prepared in deionised water to increase the stacking effect. Ba^{2+} was added in twofold concentration compared with the other inorganic cations because of the lower sensitivity of CCD for this ion. Electrokinetic injection was chosen, because it provided better peak shapes and narrower peak widths than hydrodynamic injection. Fig. 3 shows an example of separation of the cations and Table 1 presents the linearity and limit of detection (LOD) values. LODs were determined for cations using a signal-to-noise ratio (S/N) of 3. The LOD values were of the same order of magnitude as reported elsewhere [12]. Eight repetitions were made at each concentration. In the range examined (1–5 mg/l), the linearity based on the peak areas was fairly good for all inorganic cations except Na^+. The poorer linearity of Na^+ was probably due to peak overlapping. Sodium concentrations are normally very high in environmental samples, however, and 2–5 mg/l can be considered adequate for sodium determinations. The linearity range for other cations was not investigated at concentrations over 5 mg/l because of the peak overlapping at higher concentrations.

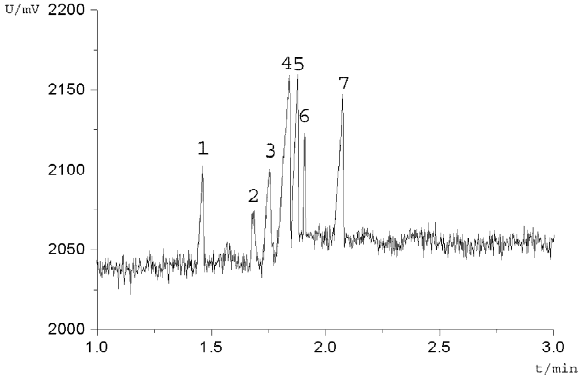

Fig. 3. Example electropherogram of the separation of inorganic cations with CCD. Samples: 1=K^+, 2=Ba^{2+}, 3=Ca^{2+}, 4=Na^+, 5=Mg^{2+}, 6=Mn^{2+} and 7=Li^+; Concentrations of cations were 1 mg/l each except for Ba^{2+}, which was 2 mg/l. Buffer 20 m*M* MES–His, pH 6, voltage 30 kV, injection 10 kV for 2 s, total length 63 cm, length to the CCD system 50 cm.

Table 1
Linearity ranges and limits of detection (LODs) for separated cations obtained with CCD

Ion	Linearity[a] range (mg/l)	r^2	LOD (mg/l), $S/N=3$
1 K^+	1–5	0.969	0.24
2 Ba^{2+}	1–5	0.978	2.14
3 Ca^{2+}	1–5	0.969	0.15
4 Na^+	2–5	0.994	0.12
5 Mg^{2+}	1–5	0.920	0.18
6 Mn^{2+}	1–5	0.931	0.34
7 Li^+	1–5	0.975	0.22

[a] Linearity is determined based on peak areas of different concentrations.

3.2. Indirect detection of catecholamines

Usually, poorly conductive analytes are detected by indirect detection with the use of highly conductive buffer. The 20 m*M* ammonium acetate buffer was selected for its relatively high conductivity. The suitability of lithium and sodium acetates was also investigated, in an attempt to reduce the relatively high background noise with ammonium acetate. Fig. 4 shows electropherograms without injection and, as can be seen, sodium acetate BGE showed the lowest noise level and only one system peak (Fig. 4A–C). In the end, ammonium acetate was chosen, because CCD provided better sensitivity for catecholamines when ammonium acetate was used. In addition, the effects of ionic strengths of 10 and 20 m*M* and pH values from 4.0 to 6.0 were studied. The background noise level was lower and the baseline stability was improved at lower ionic strength (Fig. 4C,D) with no

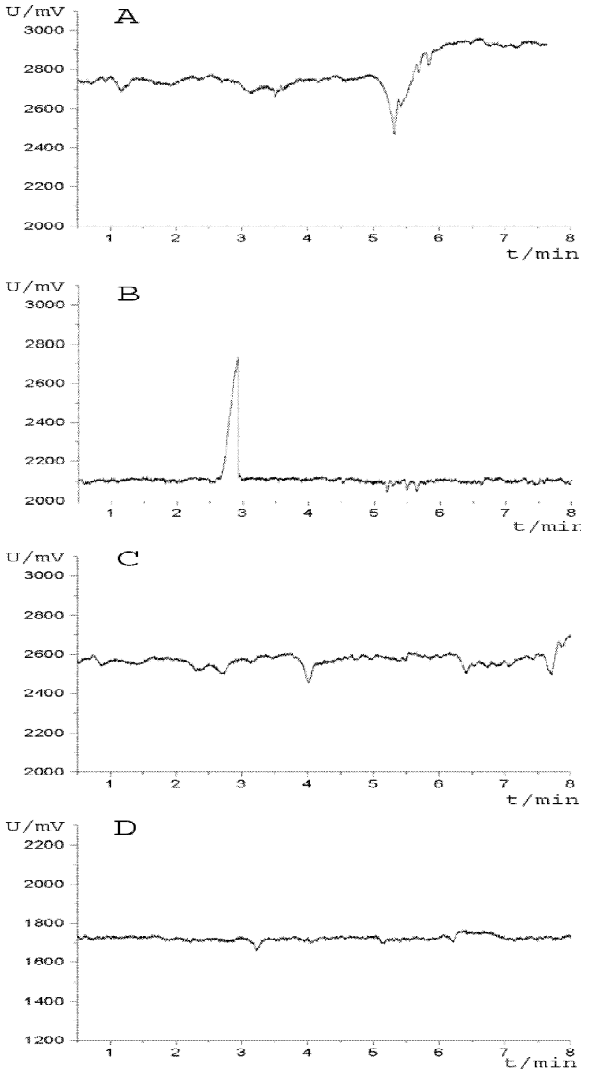

Fig. 4. Baselines and system peaks obtained with CCD using different background electrolytes: (A) 20 m*M* potassium acetate, (B) 20 m*M* sodium acetate, (C) 20 m*M* ammonium acetate and (D) 10 m*M* ammonium acetate.

loss of signal intensity. Furthermore, the characteristic system peak of ammonium acetate did not interfere with the catecholamine peaks in the lower ionic strength buffer because of its shorter migration time. At higher pH, the migration times decreased, leading to overlapping of HMBA and dopamine. On the basis of these results, 10 m*M* ammonium acetate buffer and pH 4 were chosen. The separation of the 20 mg/l catecholamines is illustrated in Fig. 5.

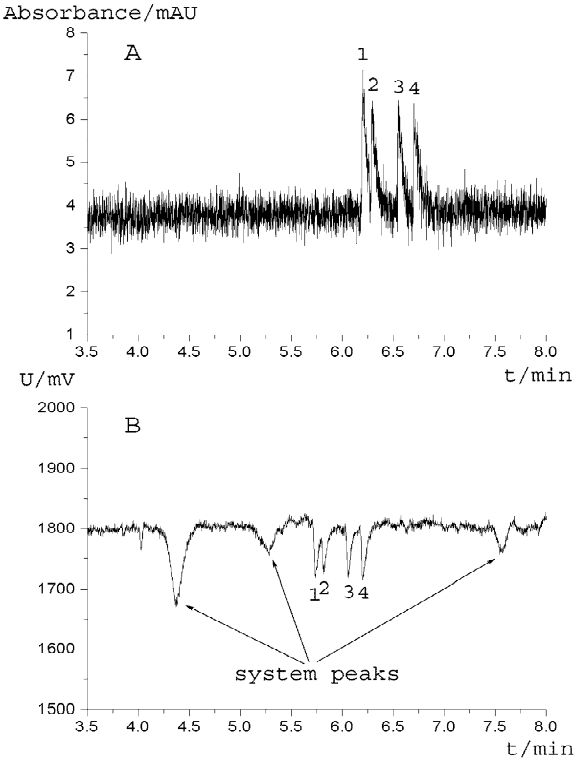

Fig. 5. Electropherograms for separation of catecholamines with (A) UV detector and (B) CCD. Peak identification: 1=HMBA, 2=dopamine, 3=DL-normetanephrine and 4=DL-metanephrine. The concentration of each catecholamine was 20 mg/l. Buffer 10 m*M* ammonium acetate, pH 4, voltage 20 kV, injection 50 mbar for 5 s, total length 68 cm, length to the CCD system 55 cm, length to the UV detector 59.5 cm.

The linear concentration ranges for CCD and UV detection were determined by analysing samples containing different concentrations of the analytes. Peak areas were determined in eight repetitions at each concentration. The limit of detection was taken as S/N ratio 3 (Table 2). The linearity as well as the LODs were of the same order of magnitude for CCD and UV. The values of the correlation coefficient (r^2) were slightly higher for CCD than for UV, while the limits of detection for both CCD and UV were approximately 10 mg/l. Slopes of the calibration plots were steeper for CCD than for UV (Table 2).

The RSD values of the analyte peak areas for CCD and UV were calculated as a measure of the repeatability investigations of results with the two detectors. Table 2 shows the RSD values for peak

Table 2
Linearity, limits of detection (LODs), slope and area RSD values for catecholamines from both CCD and UV detection

Compound	LOD[a] (mg/l)		Linearity range (mg/l)	Correlation coefficient, r^2		Slope of the calibration plots		RSD (%) for area values of 30 mg/l	
	CCD	UV		CCD	UV	CCD	UV	CCD	UV
1 HMBA	9.0	9.1	5–50	0.916	0.904	0.119	0.004	3.4	8.4
2 Dopamine	12.4	11.2	5–50	0.924	0.935	0.150	0.005	8.4	13.0
3 DL-Normetanephrine	10.4	11.2	5–50	0.935	0.917	0.142	0.004	9.9	4.5
4 DL-Metanephrine	10.1	12.0	5–50	0.967	0.942	0.177	0.004	17.2	9.9

[a] Linearity is determined based on peak areas of different concentrations for CCD and UV.

areas of 30 mg/l samples. The RSD values were of the same order of magnitude for CCD and UV, except near the limit of detection, where the relative standard deviations were about twice as large for conductivity as for UV detection. The conductivity signal is sensitive to external interferences and changes in the buffer composition and these probably increased the RSD values at lower concentrations.

The effect of the detection window of CCD (0.4 mm) was estimated by comparing the peak widths with those obtained with UV, which has a well-defined aperture length (0.7 mm). The peak width and plate numbers are presented in Table 3. The three largest concentrations were compared because of the better peak shapes. The plate numbers and the peak widths were similar, even though the distance between the electrodes was 0.3 mm shorter than the aperture length of the UV detection. The effect of the detection window width was probably too small to observe, or perhaps the actual effective detection window of CCD was wider; it has not been proved that the distance between the electrodes is exactly the effective detection gap of CCD.

4. Conclusions

The compact electronics of the CCD system was successfully integrated into a commercial capillary cassette. The CCD system was user-friendly and enabled parallel detection with the UV-detector of the ^{3D}CE equipment. The electronic parts were thermostated inside the cassette with an air ventilation system that reduced the effects of thermal noise of the components and baseline drifting. The seven inorganic cations were separated with direct conductivity detection, with a sensitivity of 0.25–2 mg/l depending on the cation. The catecholamines were separated simultaneously with indirect conductivity detection and direct UV detection. The detection

Table 3
Comparison of peak widths and plate numbers for catecholamines determined with CCD and UV

Compound	50 mg/l		40 mg/l		30 mg/l	
	CCD	UV	CCD	UV	CCD	UV
Peak width						
HMBA	0.069	0.080	0.095	0.104	0.080	0.084
Dopamine	0.139	0.169	0.165	0.184	0.105	0.123
DL-Normetanephrine	0.091	0.099	0.113	0.125	0.084	0.084
DL-Metanephrine	0.121	0.133	0.139	0.149	0.137	0.095
Plate number						
HMBA	87 847	75 107	64 773	62 649	79 675	84 772
Dopamine	22 763	17 458	23 210	20 741	48 021	42 582
DL-Normetanephrine	56 566	54 740	52 611	49 473	81 463	93 637
DL-Metanephrine	32 933	31 968	35 665	36 603	35 491	78 348

sensitivity of CCD was approximately the same as achieved with UV detection. However, CCD gave more information about changes in buffer composition during the analyses. CCD is a highly promising detection method owing to its universality.

Acknowledgements

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