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Determination of amino acids by capillary electrophoresis with differential resonant contactless conductivity detector

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ABSTRACT

The impedance of a capacitively coupled contactless conductivity detector (C^4D) in capillary electrophoresis (CE) was measured by an impedance analysis method. The influence of solution conductivity and capillary dimension on impedance parameters was investigated. Under the experimental conditions used, 86–99.9% of the total impedance of a C^4D is composed by its imaginary part from the capillary wall capacitor. With increasing inner diameter of capillary and solution conductivity in detection zone, the wall capacitance increases, which results in the increase in the response signal of C^4D. But the wall capacitance is only 0.5–12% of the predicted value according to a cylinder capacitor model. As the change in solution resistance is detected in a resonant C^4D (RC^4D), the sensitivity of contactless conductivity detection in CE is improved. The application of an end-to-end differential RC^4D (DRC^4D) system in CE was demonstrated in the determination of 10 amino acids. The running buffer consisted of 2 M acetic acid and 0.1% hydroxyethylcellulose (pH 2.1). The limit of detection for amino acids is in the range of 0.1–0.4 μM. Under our experimental conditions, the sensitivity of DRC^4D enhances by a factor of 15–29 as compared with C^4D.

1. Introduction

Since the works of Zemann et al. [1] and da Silva and do Lago [2] in 1998, a capacitively coupled contactless conductive detector (C^4D) has received considerable attention as an alternative detection method in capillary electrophoresis (CE) and microchip electrophoresis [3–9]. Besides, the applications of C^4D in high-performance liquid chromatography [10,11], ion chromatography [12,13] and flow injection [14], non-invasive characterization of monolithic stationary-phase coatings [15,16], and measurement of electroosmotic flow (EOF) [17] and conductivity of aqueous droplets in segmented flow [18] were reported. Other measurement models such as four electrode arrangement [19,20], integration electrodes in a miniaturized detection system [21–23] were demonstrated. The influence of the factors such as operating frequency [24–26], detection cell geometry [27], wall thickness of capillary [28], and stray capacitance [29,30] on the response of C^4D was discussed.

To enhance the sensitivity of C^4D, a higher actuator voltage of 500 V [31], thinner insulating layer of 30 nm [32], end-to-end differential model [33] was reported. In our previous papers [34–36], a resonant C^4D (RC^4D) was proposed to improve the sensitivity of C^4D in microchip electrophoresis and ion chromatography. In this work, the impedance parameters of C^4D under CE conditions were measured. The influence of solution conductivity and capillary dimension on the impedance of C^4D was investigated. The determination of amino acids by CE with end-to-end differential RC^4D (DRC^4D) model was performed. The limit of detection for 10 amino acids is in the range of 0.1–0.4 μM. Compared with C^4D, the sensitivity of DRC^4D enhances by a factor of 15–29 under our experimental conditions.

2. Experimental section

2.1. Chemicals and materials

Polyimide-coated fused silica capillaries (YN-025365, YN-050365, YN-075365, YN-100365) were the product of Yongnian Ruifeng Chromatographic Component Co. Ltd (Hebei, China). All chemicals were of analytical grade purity and used as received. Deionised Milli-Q water (Millipore, Bedford, MA, USA) was used throughout. Ten amino acids (Alanine, arginine, glutamic acid, glycine, leucine, lysine, phenylalanine, praline, threonine and valine) were purchased from Fluka. Hydroxyethylcellulose (HEC) was purchased from Sigma. Stock solutions of individual amino acids (1 mM) were prepared by water and acetonitrile 1:1 (v/v) and stored in the refrigerator at 4 °C. The mixture of 10 amino acids,

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which was made from the stock solutions by diluting with water and acetonitrile 1:1 (v/v), was used as the model sample in CE separation. All solutions were degassed by ultrasonication for 10 min and filtered through 0.22 μm nylon syringe filters (Shanghai Liangzheng Technology Co., China) prior to use.

2.2. Apparatus and setup

Chromatographic experiments were performed with a purpose-built CE system with DRC^{4}D (Fig. 1). The separation voltage was provided by a high voltage power supply unit (DW-P303, Tianjing Wendong High Voltage Power Supply Co. Ltd., China). A polyimide-coated fused silica capillary (with inner diameter of 50 μm, with outer diameter of 365 μm, total length of 65 cm) was used for amino acid separation. Two C^{4}Ds were fixed to the capillary at the distance of 10 cm from the near ends. The input and signal electrodes were prepared by silvered wire (with diameter of 0.1 mm) twisted tight on capillary. The electrode length was 2 mm and the gap between the input and signal electrodes was 0.6 mm. The C^{4}D was mounted in a ground Faraday shielding box to minimize the leakage capacitance. The capillary between two shielding boxes was enveloped by a water bath at the controlled temperature of 25 °C. A purpose-built function generator was designed to provide an actuator voltage of 20 V (peak-to-peak) with frequency breadth less than 1 Hz and voltage stability better than 1 mV. The actuator voltage was applied to the series combination of 10 piezoelectric quartz crystal (PQC) resonators (200 kHz, Beijing Chenjin Quartz Crystal Manufactory, China) and C^{4}D on the capillary. The circuitry on the pick-up side comprises a current-to-voltage converter, followed by rectification, low-pass filtering. Prior to chromatographic detection, the capillary was filled the running buffer and the operating frequency of the actuator was scanned. The frequency corresponding to the maximum output signal was chosen as the operating frequency for RC^{4}D. In the end-to-end differential measurement model, the preamplified signals from the upstream and downstream detectors are subtracted from each other. The resulting differential signal is postamplified and recorded by a chromatographic working station.

Each new capillary was activated by sequentially flowing (pressure-driven by a medicine syringe) 0.5 M solution of sodium hydroxide, deionised water, and running buffer for 20 min, respectively. The capillary was then equilibrated in the running buffer under a voltage of 25 kV for 20 min prior to sample injection. Sample was injected hydrostatically by elevating the sample vials to a height of 10 cm for 10 s. As two C^{4}Ds were equipped at both ends of the capillary, sampled injection was performed by turning the whole equipment box around its rotary axis. Separation was taken place at +25 kV. Between each consecutive run, the capillary was flushed with deionised water followed by running buffer for 10 min each for reproducible results.

The impedance of C^{4}D was measured in an impedance analyzer (Model 4294A, Agilent) at the constant laboratory temperature of 25 ± 1 °C. To increase the signal-to-noise ratio in impedance measurement, a parallel arrangement of 10 C^{4}Ds with the same electrode geometry was employed (Fig. 2). The impedance of a single C^{4}D is 10 times of that of the C^{4}D array, which was set in a grounded shielding box to minimize the stray capacitance. The contactless electrodes were fabricated from syringe cannulas with length of 10 mm. The gap between the electrodes was 2 mm. The test solution was injected into capillary array by a pressure-driven medicine syringe. The impedance was scanned three times (with 401 points in a scan) for each solution, the averaged values were reported. Capillary array with different inner diameter (25, 50, 75, 100 μm) was exchanged after a group measurements.

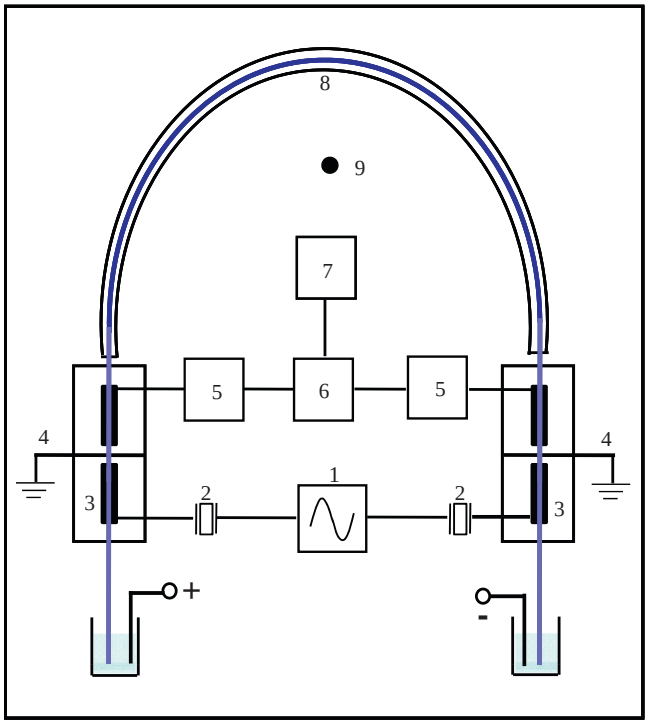

Fig. 1. Schematic drawing of end-to-end differential resonant C^{4}D for capillary electrophoresis (not to scale). (1) function generator; (2) piezoelectric quartz crystal resonator array; (3) contactless electrode; (4) ground shielding box; (5) current detection circuitry; (6) differential detection circuitry; (7) chromatographic working station; (8) separation capillary; (9) rotary axis for hydrostatic sample injection.

3. Results and discussion

3.1. Measurement of the impedance of C^{4}D in capillary electrophoresis

Analysis of the impedance of C^{4}D shows how the detector can be optimized. Fig. 3A presents an simplified equivalent circuit

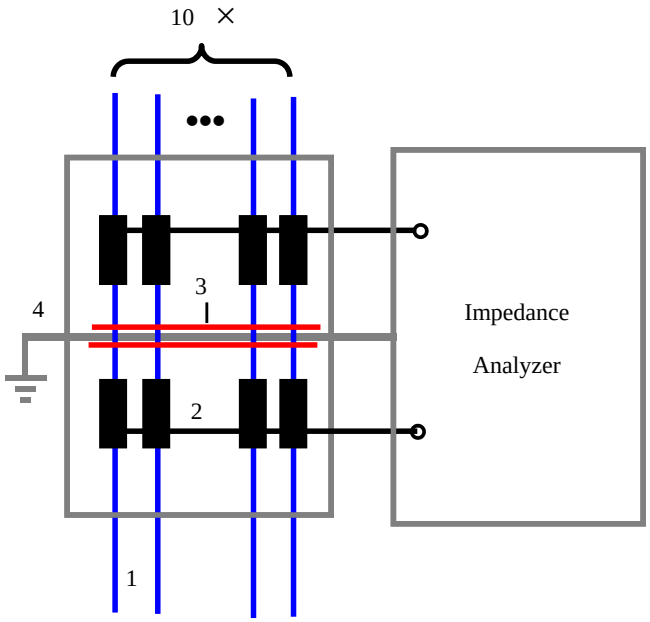

Fig. 2. Schematic drawing of C^{4}D array for impedance measurement (not to scale). (1) capillary array; (2) contactless electrode array; (3) Teflon insulation film; (4) ground shielding box.

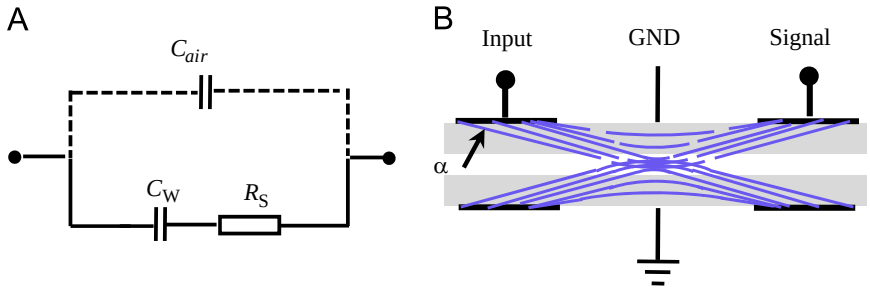

Fig. 3. Simplified models for equivalent circuit (A) and electrical line distribution in C^{4}D (B, not to scale). Where C_{air}, C_w and R_s are the leakage capacitor, wall capacitor and solution resistor, respectively.

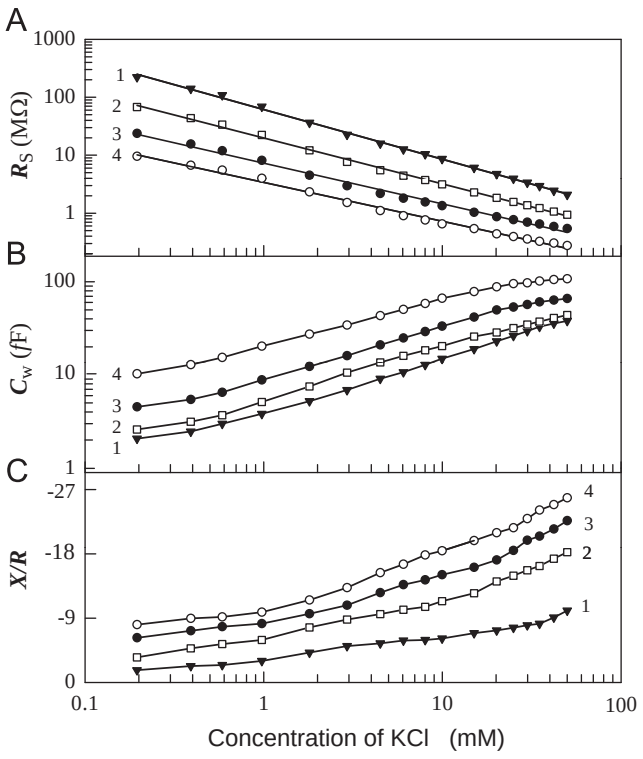

Fig. 4. Dependence of the solution resistance (A), wall capacitance (B) and impedance ratio (C) in C^{4}D on concentration of KCl solution in different capillaries. The experimental conditions: L=10 mm, f=200 kHz, d=2 mm, r_2=365 μm, capillary inner diameter: (1) r_1=25 μm, (2) r_1=50 μm; (3) r_1=75 μm; (4) r_1=100 μm.

model for C^{4}D [37,38], where C_{air}, C_W and R_S are the leakage capacitor, wall capacitor and solution resistor, respectively. Under well designed shielding conditions, the impedance of C_{air} is much larger than that of the series branch of C_W and R_S (data were not shown). Hence, the influence of C_{air} on the total impedance of C^{4}D is negligible. Thus, the values of C_W and R_S were estimated from the imaginary part (X) and real part (R) of the total impedance of C^{4}D, respectively. As shown in Fig. 4A, the value of R_S decreases with near-linearly increasing electrolyte (KCl) concentration in the double- logarithmic coordinates figure. On the other hand, with increasing capillary inner diameter (r_1), the conducting area of the solution in capillary increases, the value of R_S decreases.

Usually, the value of C_W is estimated according to a cylinder capacitor model by the following equation [37,39]

$$C_w = \frac{2\pi L \varepsilon_0 \varepsilon_r}{\ln(r_2/r_1)} \quad (1)$$

where L is the electrode length, r_2 the inter and outer diameter of the capillary, $\varepsilon_0 = 8.854 \times 10^{-12}$ F/m, ε_r the relative permittivity of the wall, respectively.

According to Eq. (1), the value of C_W depends mainly on the values of L, ε_r and r_2/r_1. For a C^{4}D with given dimensions, the value of C_W seems to be constant. As shown in Fig. 4B, however, the value of C_W increases with increasing KCl concentration. On the other hand, the value of C_W is much less than the prediction from Eq. (1), especially in solution of low conductivity. For example, the fused silica capillaries used in this work were coated by polyimide layer with thickness of 20 μm. According to Eq. (1), the wall capacitance of the polyimide layer (ε_r=3.5, r_1=325 μm, r_2=365 μm, L=10 mm) and quartz wall (ε_r=4.4, r_1=50 μm, r_2=325 μm, L=10 mm) is 16.8 and 1.31 pF, respectively. The total series wall capacitance between the two contactless electrodes is evaluated to be 0.61 pF. But the measuring value of C_W in C^{4}D with capillary filled 1 and 50 mM KCl is 5.1 and 44 fF, which are 0.8 and 7% of the prediction (610 fF), respectively. With increasing r_1, the ratio of r_2/r_1 decreases and the value of C_W increases. The change in C_W may be ascribed to the change in electrical line distribution in C^{4}D (Fig. 3B). With increasing solution conductivity, the angle between the contactless electrode and electrical line (α) increases, the conducting distance for the electrical lines from the electrode through the capillary wall to solution reduces and the value of C_W increases.

As shown in Fig. 4C, the absolute value of X/R in the C^{4}Ds is in the range from 1.7 to 26. Hence, the magnitude of the impedance ($|Z| = \sqrt{R^2+X^2}$) in C^{4}D depends mainly on that of X (86–99.9%) under the experimental condition used in this work, especially in capillary with larger inner diameter and filled solution with higher conductivity.

3.2. Determination of amino acids by CE with end-to-end differential C^{4}D

Determination of amino acids by CE with C^{4}D was reported in references [40–43]. In this work, the approaches of end-to-end differential model and RC^{4}D were combined to enhance the sensitivity of contactless conductivity detection for the determination of amino acids. As shown in Fig. 1, two C^{4}Ds were equipped near both ends of the separation capillary. It should be mentioned that the response of a normal C^{4}D was recorded if only the signal in the downstream C^{4}D (with PQC replaced by wire) was measured. In the end-to-end differential model, the signal difference between the upstream RC^{4}D (or C^{4}D) and downstream RC^{4}D (or C^{4}D) was measured. Hence, the drift in baseline and noise level can be suppressed effectively [33].

In this work, the running buffer consisted of 2.0 M HAc+0.1% HEC (pH 2.1) was used for the separation of amino acids. The addition of 0.1% HEC to the running buffer prevents the adsorption of amino acids on the capillary wall and decreases the EOF [40]. A mixture of 10 amino acids was chosen as the model sample. As shown in Fig. 5, the signal in DRC^{4}D is much higher than that in end-to-end differential C^{4}D (DC^{4}D). The analytical data are evaluated from the baseline subtracted chromatograms

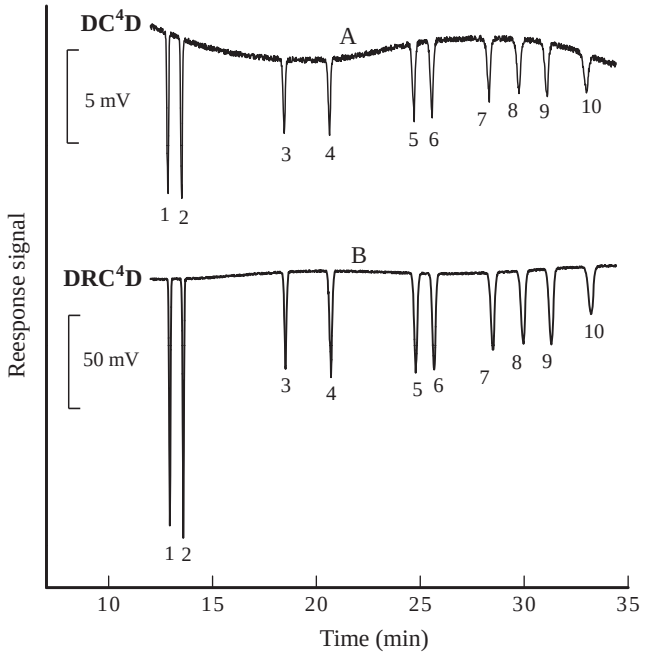

Fig. 5. Capillary electrophoresis separation of a model mixture of 10 amino acids (5 μM) with DC^{4}D (A) and DRC^{4}D (B). Peak identification: (1) lysine; (2) arginine; (3) glycine; (4) alanine; (5) valine; (6) leucine; (7) threonine; (8) glutamic acid; (9) phenylalanine; (10) praline.

Table 1
Performance parameters for determination of amino acids by CE with C^{4}D, DC^{4}D and DRC^{4}D detectors.

Amino acid	Limit of detection (μM)			Enhancement factor[a]		RSD of peak area (%)		
	C^{4}D	DC^{4}D	DRC^{4}D	DC^{4}D	DRC^{4}D	C^{4}D	DC^{4}D	DRC^{4}D
Alanine	4.3	1.1	0.25	3.9	17	3.1	2.8	2.3
Arginine	1.8	0.52	0.12	3.5	15	2.9	2.5	1.8
Glutamic acid	5.2	1.4	0.34	3.7	15	2.8	2.7	2.3
Glycine	3.8	0.78	0.14	4.9	27	3.3	2.2	2.4
Leucine	3.5	0.70	0.12	5.0	29	3.0	2.6	2.5
Lysine	2.4	0.55	0.10	4.4	24	2.6	2.4	1.9
Phenylalanine	4.8	1.3	0.31	3.7	15	3.2	3.2	2.1
Praline	7.5	1.8	0.40	4.2	19	3.5	3.3	2.7
Threonine	4.5	1.2	0.28	3.8	16	3.4	2.1	2.1
Valine	4.0	0.94	0.16	4.3	25	3.1	2.7	2.2

[a] Enhancement factor is the ratio of limit of detection of C^{4}D to that of DC^{4}D or DRC^{4}D.

and are summarized in Table 1. As compared with a normal C^{4}D, the sensitivity for determination of amino acids is enhanced by a factor of 3–5 in DC^{4}D and 15–29 in DRC^{4}D, respectively. The limit of detection for the 10 amino acids is in the range of 0.1–0.4 μM in CE with DRC^{4}D. The relative standard deviation (RSD) in peak area is in the range of 1.8–2.7%. The higher sensitivity with DRC^{4}D is explained below.

As shown in Fig. 4, there is $R_S \ll |X| \approx (2\pi f C_W)^{-1}$ in C^{4}D under our CE conditions. Hence, the response of C^{4}D is ascribed to the change in C_W, which increases with increasing conductivity in the detection zone. Thus, the response of DC^{4}D, ΔV_1, is expressed as

$$\Delta V_1 \approx 2\pi f k(C_{W1} - C_{W0}) \quad (2)$$

where k is a constant related to the actuator voltage and magnification of the detection circuit, C_{W1} and C_{W0} are the wall capacitance in sample zone and running buffer, respectively.

In an RC^{4}D, the capacitive impedance of C_W is neutralized by an equivalent inductive impedance from PQC resonator under resonance, the total impedance is close to solution resistance. Accordingly, the response of DRC^{4}D, ΔV_2, is given by

$$\Delta V_2 \approx k\left(\frac{1}{R_{S1}} - \frac{1}{R_{S0}}\right) \quad (3)$$

where R_{S1} and R_{S0} are the solution resistance in the sample zone and running buffer, respectively.

Under the CE conditions used in this work, there is $|\Delta V_2| \gg |\Delta V_1|$ because $R_S^{-1} \gg 2\pi f C_W$. As shown in Fig. 4, the value of R_S is more sensitive to the change in conductivity in detection zone than that of C_W. Hence, the sensitivity of DRC^{4}D is higher than that of DC^{4}D with the same cell dimension and separation conditions. DRC^{4}D is an effective approach to enhance the sensitivity of contactless conductivity detectors.

4. Conclusions

The impedance of C^{4}D in CE depends mainly on that from the capillary wall capacitance. With increasing solution conductivity, the wall capacitance increases, which results in the decrease in the impedance and increase in response signal of C^{4}D. Under our experimental conditions, however, the wall capacitance is only 0.5–12% of the prediction from a cylinder capacitor model. The determination of amino acids by CE with DRC^{4}D was demonstrated. The limit of detection for amino acids is in the range of 0.1–0.4 μM. Under our experimental conditions, the sensitivity of DRC^{4}D enhances by a factor of ca.15–29 as compared with C^{4}D.

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