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End-Column Amperometric Detection in Capillary Electrophoresis: Influence of Separation-Related Parameters on the Observed Half-Wave Potential for Dopamine and Catechol

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Abstract

Capillary electrophoresis (CE) was coupled to a micro-electrode-based end-column amperometric detector. The influences of separation voltage, CE buffer concentration, and capillary-to-electrode distance on the observed hydrodynamic voltammetry of dopamine and catechol were studied using a separation capillary with an i.d. of 25 μm. It was found that an increased CE voltage, increased buffer concentration, or decreased capillary-to-electrode distance resulted in a positive shift of the observed half-wave potentials for both dopamine and catechol. At a constant separation current of 1.6 μA, the observed half-wave potential was found to increase with applied separation voltage. Furthermore, when experiments were carried out with a platinum quasi-reference electrode instead of a Ag/AgCl reference electrode, similar shifts in half-wave potential were observed. These results indicate that the observed shifts are an effect of the separation voltage rather than the separation current or a change in the reference potential. The characteristics of end-column detection with and without a fracture decoupler were compared. It was found that the effects of separation voltage, CE buffer concentration, and capillary-to-electrode distance were minimized by the use of a decoupling device. The observed half-wave potentials for dopamine and catechol were more positive when a CE capillary without a decoupler was employed compared to when a decoupler was used. Additionally, using the fracture decoupler, the observed half-wave potentials for both dopamine and catechol were approximately the same as when no CE voltage was applied (i.e., when the hydrodynamic voltammograms were recorded under flow injection conditions).

Capillary electrophoresis (CE) is a powerful and highly efficient separation technique. Most commercial CE instruments are equipped with a UV absorbance detector. However, due to a limited path length of the light, this detection technique may lack sufficient sensitivity for certain applications.[1] To increase the detection sensitivity in CE, laser-induced fluorescence[2,3] and amperometric[1,4-8] detection have been successfully employed. While LIF detection generally requires a derivatization of the analytes and comprises expensive and complex instrumentation, electrochemical detectors are relatively simple and inexpensive. However, the use of CE with electrochemical detection (CEEC) has been limited. In our opinion, this is mainly due to the perceived difficulty in assembling a CEEC system. The difficulty arises from the need to either use very small inner diameter (i.d.) capillaries and carefully align a microelectrode, or to construct an electrical decoupler between the CE separation capillary and the detection capillary.

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The use of CEEC was first reported by Wallingford and Ewing in 1987.[9] In that first report, a porous glass joint was employed to electrically decouple the amperometric detector from the electrophoresis system. Several approaches to isolate the detector have since been published. The main approach involves the introduction of a fracture near the end of the capillary, typically 1–2 cm from the point of detection. The fracture is immersed in a buffer reservoir housing the CE ground electrode. The electroomotic flow (EOF) produced in the separation capillary is generally sufficient to push the analytes through the short detection capillary to the detector. Fracture decouplers presented in the literature have been either bare[10] or coated. Coatings have been fabricated from porous glass,[6] Nafion,[11-13] cellulose acetate,[7,14] porous graphite,[15] and a semipermeable membrane.[16] In addition to the fracture decouplers, devices based on a palladium solid-state field connector[17-19] have been employed. Recently, Park et al. presented on-column[4,20] and end-column[21] cast Nafion decouplers.

Another approach to minimize the influence of the CE voltage/current on the detector is to employ narrow i.d. capillaries (≤25 μm i.d.).[5,22-25] The use of narrow capillaries ensures that most of the voltage drop occurs over the separation capillary, especially when used in conjugation with high-resistance buffers.[5,23,25] In this approach (generally termed end-column detection), the working electrode is placed just outside the capillary end. Matysik recently showed that end-column detection can be used in capillaries with inner diameters as large as 50 μm[26] in aqueous solution and as large as 75 μm[27] in nonaqueous CE. In these two cases, it was found necessary to increase the detection potential by up to +500 mV in order to compensate for the influence of the CE system. Matysik observed larger shifts of the apparent half-wave potential with 50-μm capillaries compared to that with 25-μm capillaries.[26]

A drawback with on-column decouplers is decreased separation efficiencies, which is mainly due to laminar flow generated in the detection capillary[21] and the dead volume within the decoupler.[4,17] In addition, the introduction of a decoupling device adds a significant difficulty in the fabrication of the CEEC system. This is especially true since the success rate of producing such devices is not 100%.

To better understand end-column amperometric detection in CE, the effects of various separation-related parameters on the detector response were studied. In particular, the influence on the observed half-wave potential for two test analytes was determined. The influence of the separation system on the detector noise will not be discussed since this issue has been extensively studied previously.[4,21,28]

EXPERIMENTAL SECTION

CE Apparatus

The instrumentation included a high-voltage power supply (Glassman High Voltage, Whitehouse Station, NJ), a laboratory-built device for pressure injection, a Plexiglas safety box with dual interlocks, and a Faraday cage housing the electrochemical detector. All injections were made at 20 psi for 2 or 3 s. The separations were performed using 50-cm × 25-μm-i.d. fused silica capillaries (Polymicro Technologies, Phoenix, AZ). The separation capillary was mounted on an *xyz*-micropositioner (Newport Corp., Irvine, CA). Each day, the capillaries were flushed with 0.1 M NaOH, water, and run buffer for 10 min each. The fracture decouplers were prepared according to previously published procedures,[14,29] with the exception that the polyimide coating on the capillary was not burned off prior to making the fracture. The separation capillaries were 47 cm × 25 μm i.d., and the detection capillaries were 1.5–2 cm × 25 μm i.d.

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Amperometric Detector

An LC-4B or an LC-4CE potentiostat (BAS, West Lafayette, IN) was used to control the detection potential and to measure the current. The working electrode consisted of a 7-cm Pt wire with a diameter of 25 μm (Goodfellow Ltd., Cambridge, UK). The wire was glued (Ultra Super Glue, Conros Corp., Taylor, MI) into a 6-cm × 150-μm-i.d. piece of fused silica capillary (Polymicro). One end of the platinum wire was attached to a copper wire (used as an external connection) by the use of liquid silver paint (Ted Pella Inc., Redding, CA). The other end was cut to a disk using a scalpel. The working electrode was mounted on an *xy*-micropositioner (Newport Corp.). For end-column detection the disk electrode was placed outside the end of the separation capillary, while for decoupled detection the disk electrode was placed outside the detection capillary. The positioning of the electrode in relation to the end of the capillary was made under a microscope with 40× magnification with a calibrated reticule.

The auxiliary electrode consisted of a 5-cm × 1-mm-diameter Pt wire, while a Ag/AgCl electrode obtained from BAS was employed as the reference electrode. While not in use, the reference electrode was stored in 3 M NaCl. The reference electrode was checked versus other Ag/AgCl reference electrodes throughout the study using a voltmeter. The maximum observed difference in potential was 10 mV. All potentials are reported versus the Ag/AgCl reference electrode.

Each day, the tip of the working electrode was polished using a polishing cloth (BAS) and alumina powder (0.3 μm) (Fisher Scientific, Pittsburgh, PA) and then sonicated in ethanol/water (1:1) for approximately 1 min. After the electrodes had been mounted in the detector cell, the new working electrode was electrochemically pretreated using a 10-kHz square wave of ±1.5–2 V for 2 min in 0.5 M H_2SO_4. The waveform was applied using a function generator (Global Specialties, New Haven, CT), and the waveform and magnitude were checked with an oscilloscope (BK Precision, Chicago, IL). When repeated HDVs were recorded, the same pretreatment was performed in situ in run buffer between each HDV, using either the function generator or the LC-4CE's built-in square-wave generator. When the LC-4CE was used, the frequency was 100 Hz, which is the upper frequency limit of this instrument. No difference in detector performance (e.g., current levels) was observed between the different activation frequencies (i.e., 10 kHz and 100 Hz). The main advantage of using the LC-4CE was that no connections between the working electrode and the function generator had to be made between the repeated HDVs. This was a major advantage since the capillary-to-electrode distance was found to be a critical parameter for the reproducibility of the observed half-wave potentials (see below).

Chemicals

Dopamine, catechol (Sigma, St. Louis, MO), Tris (tris(hydroxymethyl)aminomethane) (Aldrich), and phosphoric acid (Fischer) were used as received. All other reagents were of analytical grade. The buffers were prepared from 1 M phosphoric acid, 1 M Tris, and nanopure water (Sybron-Barnstead, Boston, MA). The pH was carefully checked using a pH meter and adjusted to 6.5 with the 1 M Tris solution. All buffer solutions were filtered through a 0.22-μm Nylon membrane filter (Fisher). Immediately before use in the CE system, the buffers were filtered through a Milliex-GP 0.22-μm syringe filter (Millipore, Bedford, MA). The buffers were stored in a refrigerator. Each day, the buffers were brought to room temperature and degassed by sonication for 20 min.

Data Handling

All electropherograms were recorded using a Chromjet integrator (SpectraPysics, San Jose, CA) connected to a Chromnet/Labnet system (SpectraPhysics). The data were transferred to a Gateway 2000 computer (Gateway 2000 Inc., North Sioux City, SD) and converted to "readable

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files" using a conversion program obtained from SpectraPhysics. Peak height measurements were done with Igor (Wave Metrics, Lake Oswego, OR), a program for general graphing and analysis.

Hydrodynamic Voltammetry

Hydrodynamic voltammograms (HDVs) at various conditions (specified in the Results and Discussion section) were recorded for 30–50 μM dopamine and catechol. The two test substances were injected simultaneously. Two or more HDVs were recorded for each condition. Before an HDV was recorded, the electrode surface was regenerated by the in situ pretreatment described above. All data that were to be compared (e.g., HDVs recorded at different CE voltages, at different capillary-to-electrode distances, or at different buffer concentrations) were recorded sequentially in order to eliminate variations due to experimental conditions (e.g., capillary-to-electrode distance).

RESULTS AND DISCUSSION

Influence of Capillary-to-Electrode Distance

To minimize the diffusional and convective broadening of the analyte zone, the distance between the end of the capillary and the detection electrode should be as short as possible.[22,30] This is especially important as zone broadening results in a smaller peak height and, therefore, poorer detection limits. In general, the farther the detection electrode is positioned away from the end of the capillary, the lower the sensitivity. This was seen for end-column detection of dopamine with the electrode positioned 20 and 40 μm from the end of the capillary (Figure 1). However, at a detection potential of +0.75 V, the response for catechol increased as the capillary-to-electrode distance was increased (Figure 1a), while at a detection potential of +1.15 V, the expected decrease in signal was observed (Figure 1b). To investigate this observation, the effect of the capillary-to-electrode distance on peak height for dopamine and catechol was examined in more detail. Figure 2 shows the plot of normalized peak height at increasing capillary-to-electrode distances, using a CEEC system with and without a fracture decoupler. When end-column detection without a decoupler was employed, the peak height for dopamine continuously decreased as the distance between the end of the capillary and the electrode was increased at detection potentials of both +0.75 and +1.10 V. The peak height for catechol, on the other hand, significantly increased as the electrode was moved from 20 to 40 μm at a detection potential of +0.75 V. After this initial increase, the response for catechol declined with distance. When the detection potential was increased to +1.10 V, the peak height for catechol decreased in a way similar to that of dopamine when the electrode was moved farther away from the end of the capillary. Using the system with a fracture decoupler (Figure 2b), the signal for both analytes decreased when the distance between the end of the capillary and the electrode was increased at both +0.75 and +1.10 V as the detection potential. Also interesting to note in Figure 2 is the more rapid decrease in peak height when the fracture decoupler was used compared to that when no decoupler was employed. This is most likely because the flow velocity in the short detection capillary was slower than the EOF. Hence, the liquid "jet" emerging from the capillary end dissipated more rapidly when a fracture decoupler was employed, and thus the analyte zones experienced more diffusional broadening.

Matysik[26] showed a shift in half-wave potential for ferrocene-monocarboxylic acid when CE voltages of 5–20 kV were applied. The shift in half-wave potential was attributed to a shift in the working electrode potential as a consequence of the applied CE voltage. In addition, Gerhardt et al.[30] recently reported a shift of ca. 250 mV in the voltammograms when using end-column square-wave voltammetric detection in CE (40-cm × 25-μm-i.d. capillary and a CE voltage of 30 kV). The shift was observed in comparison to the potential when no CE voltage was applied.[30] To check if a shift in apparent half-wave potential was causing the

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previous result, hydrodynamic voltammograms were recorded for dopamine and catechol at two different capillary-to-electrode distances. When using end-column detection without a decoupler, the apparent half-wave potential was shifted 100 mV in the positive direction when the electrode was moved closer to the end of the capillary (Figure 3a). A detection potential of +0.75 V was on the diffusion-limited plateau of the HDV for dopamine, even at the shorter capillary-to-electrode distance. For catechol, a detection potential of +0.75 V was on the slope of the voltammetric wave at both distances. The larger capillary-to-electrode distance, therefore, resulted in a higher current response because the detection potential was shifted in relation to the HDV. A detection potential of +1.10 V was on the diffusion-limited plateau of the HDV for both analytes at both distances. For comparison, HDVs for dopamine and catechol were recorded at the same capillary-to-electrode distances using a capillary with a fracture decoupler (Figure 3b). As can be seen, the effect of capillary-to-electrode distance on the HDVs was negligible when such a decoupling device was used. Additionally, it should be noted that the half-wave potentials were significantly higher when no decoupler was used compared to the half-wave potentials when a fracture decoupler was employed.

This shift in half-wave potential explains the increase in peak height for catechol as the electrode was moved away from the capillary at a detection potential of +0.75 V (Figure 2a). To compensate for the shift, a higher detection potential has to be applied when the distance between the capillary and electrode is decreased. Additionally, since the required detection potential is strongly dependent on the capillary-to-electrode distance, it is very important to have a reproducible way of horizontally positioning the electrode relative to the capillary outlet. Alternatively, the detection potential can be increased enough to ensure that a steady-state response is obtained at all distances. Although the importance of positioning of the electrode has been discussed previously.[5,22,23,28,31] the influence of capillary-to-electrode distance on the apparent half-wave potential has not been considered. Here, it is clearly shown that this effect has to be taken into account when using CEEC with end-column amperometric detection.

Effect of Reference Electrode

A possible cause of the apparent shift in half-wave potential was poor control of the reference potential. This could be due to a current through the reference electrode or formation of a ground loop.[32] To check if a drifting reference potential could have caused the observed shifts in half-wave potentials, HDVs were recorded for dopamine and catechol at two different capillary-to-electrode distances (20 and 60 μm) using a platinum wire as a quasi-reference electrode (QRE). The observed shifts in half-wave potential were similar to those when the Ag/AgCl reference electrode was used. If a drifting reference electrode was the cause of the shift in observed half-wave potential, the shifts in half-wave potential should have been much more severe when the QRE was used. As this was not the case, it was concluded that the stability of the reference electrode was not the explanation for the observed shifts in half-wave potential.

Influence of CE Voltage and Current

A higher applied separation voltage results in a higher field strength, which in turn leads to faster separations. Provided that the Joule heat is effectively dissipated, a higher field strength also results in higher separation efficiencies. It was, therefore, of interest to study the influence of the applied voltage on the amperometric detector. Figure 4 shows the HDVs obtained for dopamine and catechol for separation voltages of 15 and 30 kV using end-column detection without a decoupler at a capillary-to-electrode distance of 20 μm. As can be seen, a higher separation voltage resulted in a 130 mV more positive half-wave potential for both dopamine and catechol. When the fracture decoupler was used, no significant shifts in the half-wave potentials were observed. Additionally, the half-wave potential for both dopamine and catechol using a separation voltage of up to 30 kV and a fracture decoupler was found to be comparable to that when no high voltage was applied (i.e., using a pressure-driven flow) (Figure 5).

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The increase in half-wave potential for end-column detection at higher CE voltages is in agreement with the findings of Matysik,[26] who showed a linear relationship between the applied CE voltage and the observed shift in working electrode potential for both 50- and 25-μm-i.d. capillaries. An increase in CE voltage, however, induces a simultaneous increase in separation current. Experiments were thus carried out to distinguish between the effects of the separation voltage and the separation current. In the first experiment, the buffer concentration and the CE voltage were altered simultaneously. The buffer concentration was selected to give a constant electrophoretic current as the separation voltage was changed. In the second experiment, only the buffer concentration was changed. In this case, the electrophoretic current was varied at a constant separation voltage. The result of the constant current experiment using end-column detection without a decoupler is shown in Figure 6. As can be seen, there was a significant shift in the observed half-wave potentials for the two conditions. This indicates that it is the separation voltage, rather than the electrophoretic current, that causes the observed shift of the half-wave potential. However, as can be seen in Figure 7a, a constant separation voltage of 30 kV resulted in similar shifts of the half-wave potentials when no decoupler was used (170 mV for dopamine and 140 mV for catechol). These results are not unexpected since, provided that the capillary length and inner diameter are kept constant, a higher buffer concentration results in lower resistance through the capillary, and thus a smaller voltage drop over the capillary. A larger voltage will therefore be present at the end of the capillary when a higher buffer concentration is employed. This electric field is likely to influence the potential of the detection electrode[28] and thus cause the shift in observed half-wave potential. As shown in Figure 7b, the effect of the buffer concentration on the position of the apparent half-wave potential was minimized if a capillary with a fracture decoupler was used. The shift in half-wave potential using a fracture decoupler was 40 mV for dopamine, compared to a shift of 170 mV without the decoupler, and 50 mV for catechol, compared to a shift of 140 mV without the decoupler.

CONCLUSIONS

It has been shown that the apparent half-wave potentials for dopamine and catechol using end-column detection without a decoupler are strongly dependent on separation-related parameters such as CE voltage, CE buffer concentration, and capillary-to-electrode distance. These results were all obtained using 25-μm-i.d. separation capillaries. This is interesting to note since end-column detection generally is considered suitable for capillaries with an i.d. $\leq$25 μm. The shifts in apparent half-wave potential should be larger with larger i.d. capillary, as previously indicated by Matysik.[26]

For simple amperometric detection, the observed shifts in half-wave potential are not regarded to be a major problem, since a larger detection potential can be applied to compensate for the effects from the separation system. However, to realize reproducible results, it is very important to precisely control separation parameters such as capillary-to-electrode distance, separation voltage, and buffer concentration. Hydrodynamic voltammetry should be performed to determine the appropriate detection potential. Additionally, if the separation conditions are changed, new voltammograms should be recorded.

In terms of voltammetric characterization of analytes, the influence of the separation parameters on the observed half-wave potentials is more serious. The dependence of the voltammetry on the separation conditions means that the half-wave potentials in this case not are thermodynamically relevant. Proper decoupling of the electrophoretic separation system and the electrochemical detector can eliminate this problem. For a decoupled system, the half-wave potentials observed by CEEC are thermodynamically relevant and can thus be directly compared to values obtained by other experiments.

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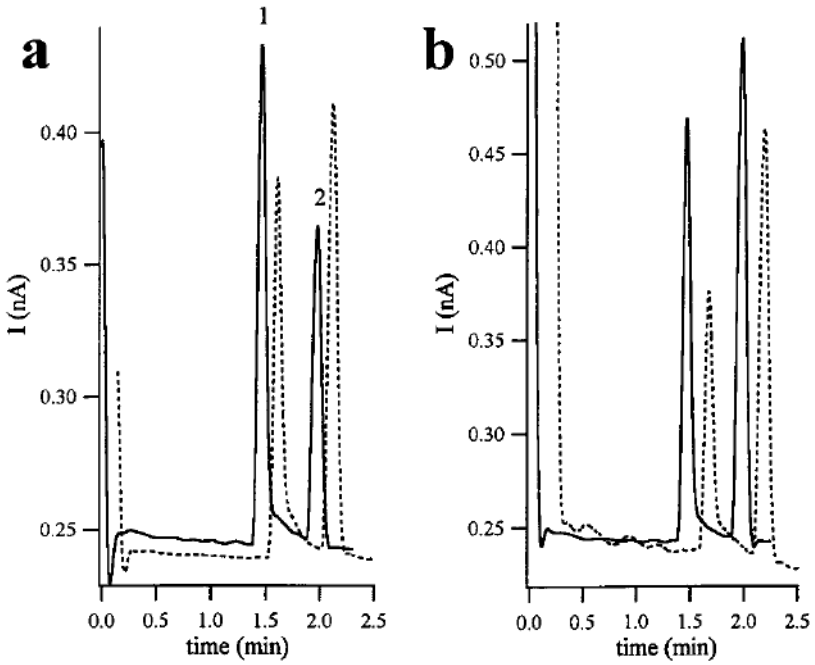

Figure 1.

Electropherogram for the detection of dopamine and catechol using end-column detection without a decoupler at a capillary-to-electrode distance of 20 (solid line) and 40 μm (dashed line) using a detection potential of +0.75 (a) and +1.15 V (b). The electropherograms obtained at 40 μm have been slightly shifted in time. Peak identification, dopamine (1) and catechol (2). Conditions; analyte concentration, 25 μM; buffer, 25 mM phosphate/Tris at pH 6.5; CE voltage, 30 kV; CE current, 1.5 μA.

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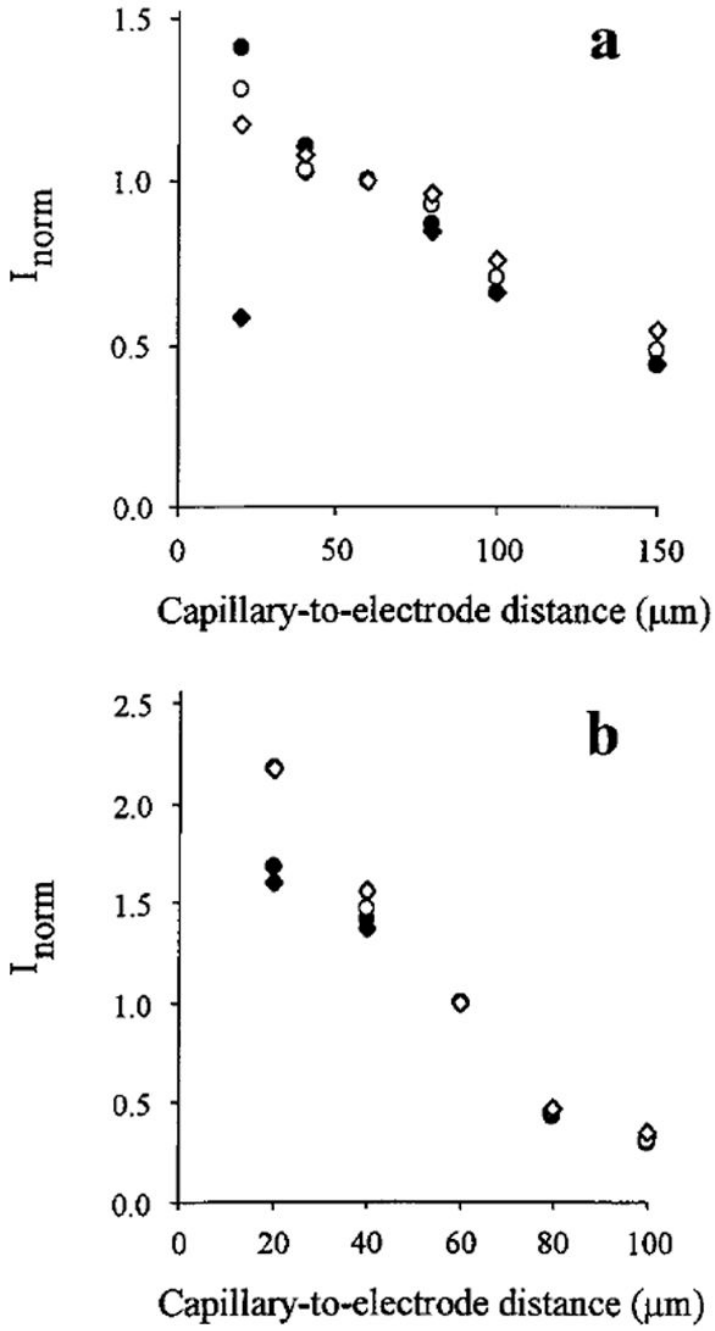

Figure 2.

Plot of normalized peak current versus capillary-to-electrode distance for CEEC without (a) and with (b) a fracture decoupler. Peak currents have been normalized versus the peak current obtained at a capillary-to-electrode distance of 60 μm. Two injections were made at each distance, at each potential. The detection potentials were +0.75 and +1.10 V. Symbols: dopamine at +0.75 V (●), catechol at +0.75 V (◆), dopamine at +1.10 V (○), and catechol at +1.10 V (◇). Conditions; analyte concentration, 30 μM; buffer, 25 mM phosphate/Tris at pH 6.5; CE voltage, 30 kV; CE current, 1.2 (a) and 1.3 μA (b).

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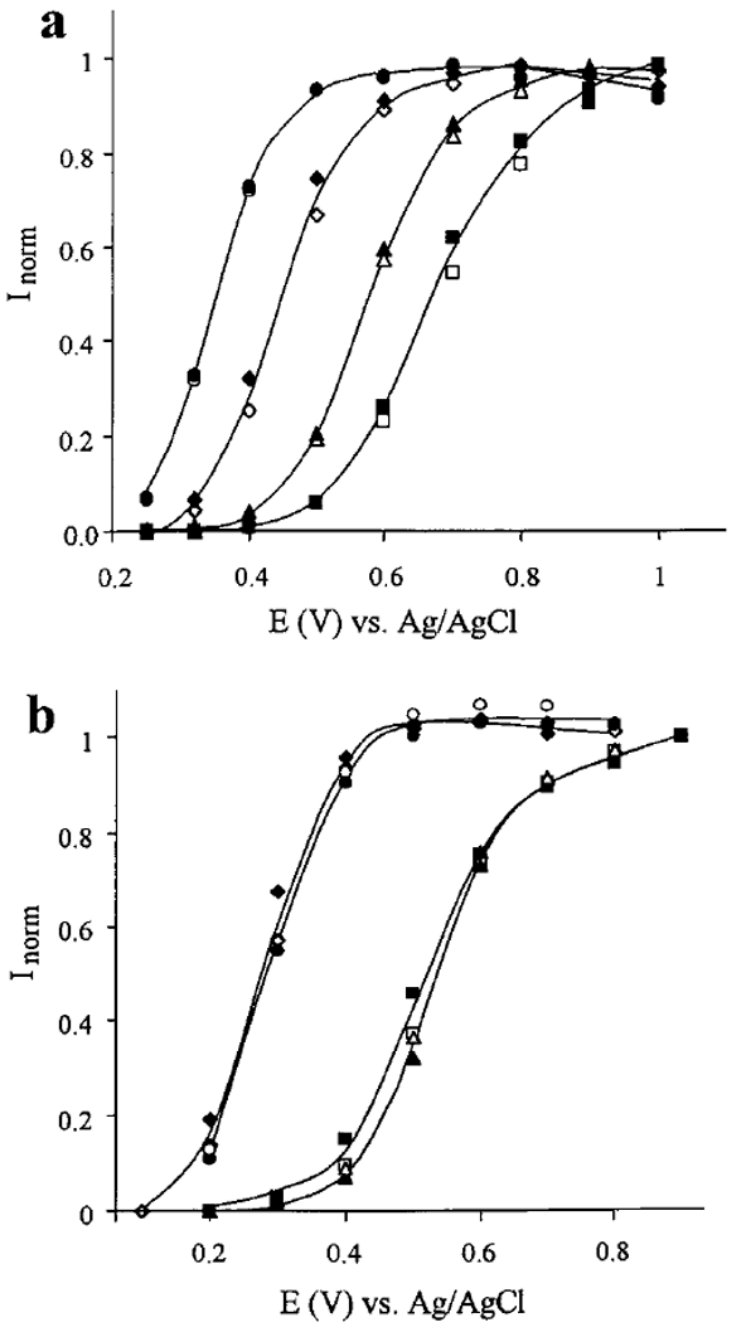

Figure 3.

HDVs recorded for dopamine and catechol using end-column detection at capillary-to-electrode distances of 20 and 60 μm without (a) and with (b) a fracture decoupler. Symbols: dopamine (◆,◇) and catechol (■,□) at 20 μm, dopamine (●,○) and catechol (▲,△) at 60 μm. Two HDVs were recorded at each distance; the open and closed symbols represent the different trials. Analyte concentrations: 30 (a) and 40 μM (b). Conditions: buffer, 25 mM phosphate/Tris at pH 6.5; CE voltage, 30 kV; CE currents, 1.3–1.6 μA.

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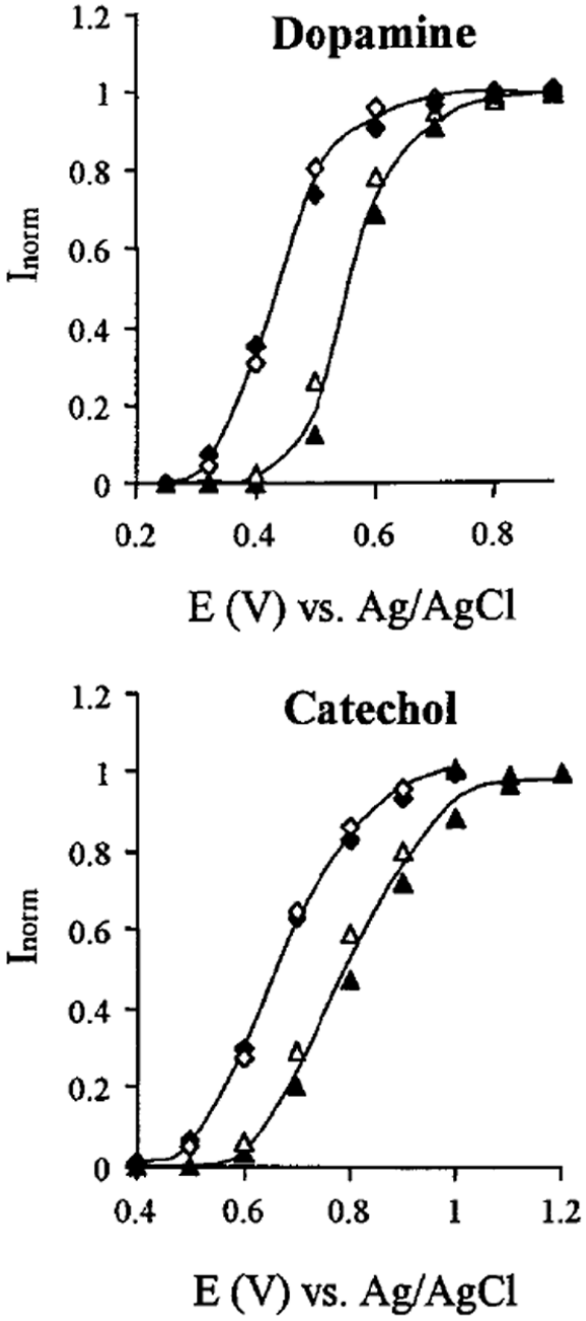

Figure 4.

HDVs recorded for dopamine and catechol using separation voltages of 15 (◆,◇) and 30 kV (▲,△) using end-column detection without a decoupler. Two HDVs were recorded at each separation voltage; the open and closed symbols represent different trials. Conditions: analyte concentrations, 40 μM; buffer, 25 mM phosphate/Tris at pH 6.5; CE currents, 0.7 μA at 15 kV and 1.5 μA at 30 kV; capillary-to-electrode distance, 20 μm.

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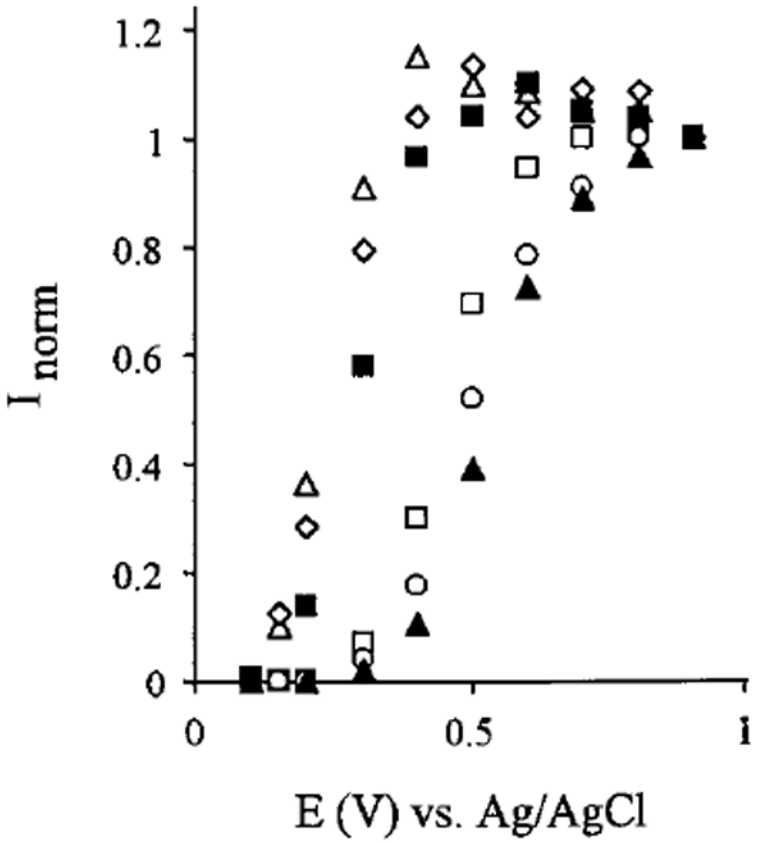

Figure 5.
HDVs recorded for dopamine and catechol using a capillary with a fracture decoupler at separation voltages of 15 and 30 kV and using pressure-driven flow (no applied CE voltage). Symbols: dopamine in the fracture system at 15 kV (△), dopamine in the fracture system at 30 kV (◇), dopamine using pressure-driven flow (■), catechol in the fracture system at 15 kV (□), catechol in the fracture system at 30 kV (○), and catechol using pressure-driven flow (▲). Conditions: analyte concentrations, 40 μM; buffer, 25 mM phosphate/Tris at pH 6.5; capillary-to-electrode distance, 20 μm; CE currents (in the fracture system), 0.7 μA at 15 kV and 1.6 μA at 30 kV.

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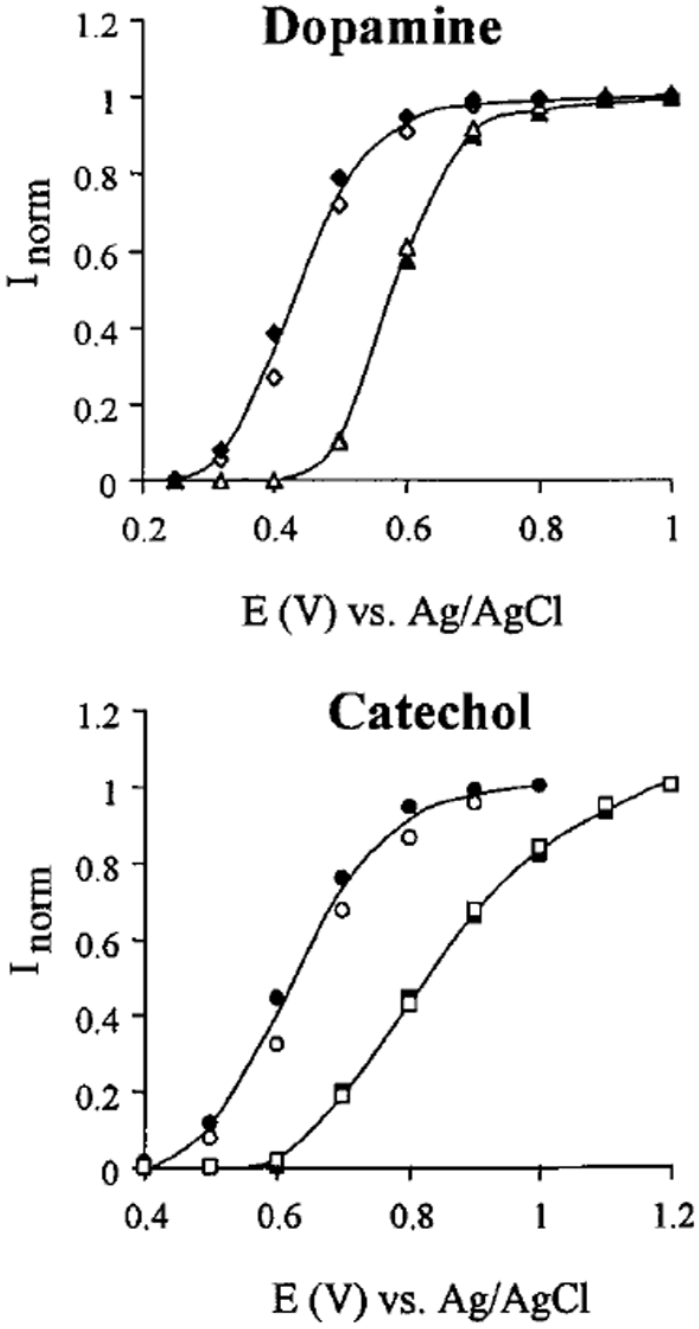

Figure 6.

HDVs recorded for dopamine and catechol at a constant electrophoretic current. An electrophoretic current of 1.6 μA was obtained by using an 11-kV separation voltage in combination with a 100 mM phosphate/Tris buffer, or an applied voltage of 30 kV in combination with a 25 mM phosphate/Tris buffer. Symbols: dopamine (◆,◇) and catechol (●,○) at 11 kV/100 mM buffer, dopamine (▲,△) and catechol (■,□) at 30 kV/25 mM buffer. Two HDVs were recorded at each condition; the open and closed symbols represent the different trials. Conditions: analyte concentration, 40 μM; capillary-to-electrode distance, 20 μm.

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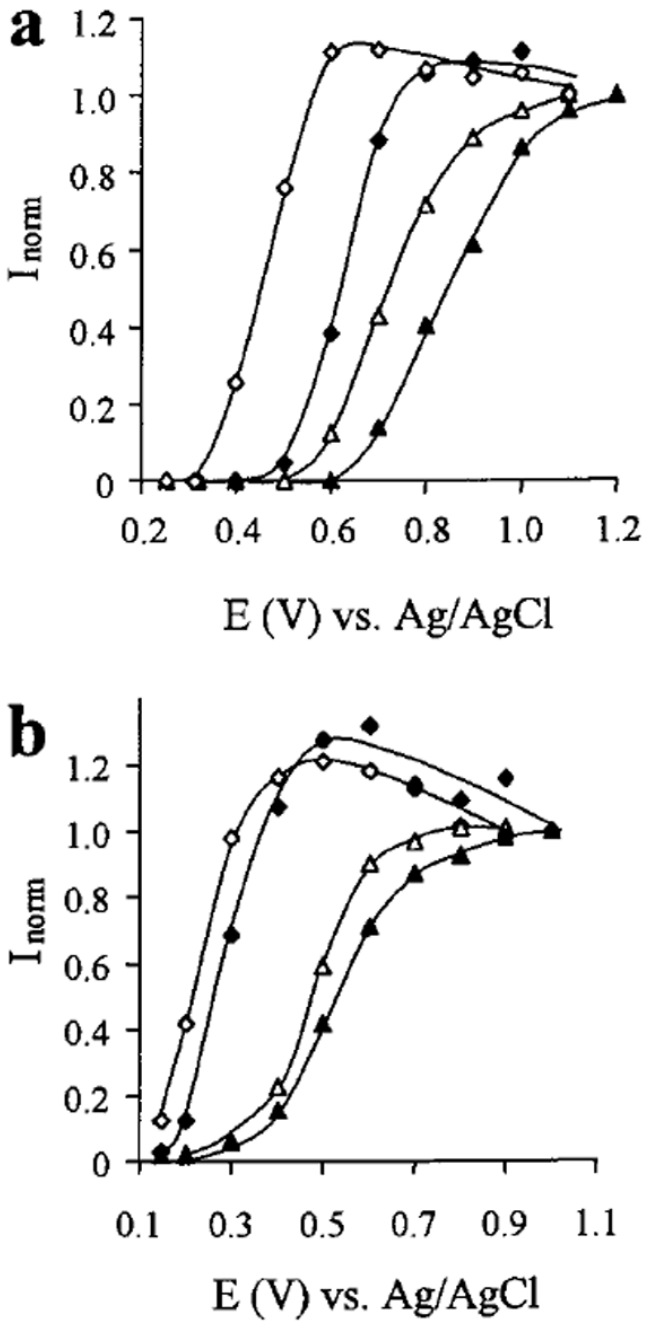

Figure 7.

HDVs recorded for dopamine and catechol using 10 and 100 mM phosphate/Tris buffers without (a) and with (b) a fracture decoupler. Symbols: dopamine (◇) and catechol (△) with a 10 mM buffer, dopamine (◆) and catechol (▲) with a 100 mM buffer. Conditions: analyte concentration, 30 μM; CE voltage, 30 kV; CE currents, 0.6 μA with 10 mM buffer and 5.3 μA with 100 mM buffer; capillary-to-electrode distance, 20 μm.