Published in final edited form as:

Electrophoresis. 2012 September ; 33(17): 2725–2732. doi:10.1002/elps.201200113.

A parallel dual-electrode detector for capillary electrophoresis

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Abstract

An approach to on-capillary dual-electrode detection for CE using a parallel electrode configuration has been developed. The parallel configuration provides two operating modes. In the first mode, one working electrode is held at an oxidizing potential and the second working electrode is held at a reducing potential. This results in redox cycling of analytes between the oxidized and reduced forms, enhancing sensitivity compared to single-electrode detection. In the second mode, both working electrodes are held at different oxidizing potentials. This mode provides electrochemical characterization of electrophoretic peaks. In the redox cycling mode, signal enhancement of up to twofold was observed for the dual-electrode detection of phenolic acid standards compared to single-electrode detection. Variation in response of less than 10% from electrode to electrode was determined (at a concentration of 60 nM) indicating reproducible fabrication. LODs were determined to be as low as 5.0 nM for dual-electrode configuration. Using the dual-potential mode peak identification of targeted phenolic acids in whiskey samples were confirmed based on both migration time and current ratios.

Keywords

Amperometric detection; Capillary electrophoresis; Dual-electrode

1 Introduction

CE has been coupled frequently to UV absorbance and fluorescence detectors, with LODs in the low micromolar range ($\sim 0.1 \mu\text{M}$) and low nanomolar range, respectively. Fluorescence detection typically requires derivatization, resulting in more complex and less reproducible analyses. Several important neurotransmitters, along with many other biological compounds are inherently electroactive, making electrochemical detection coupled to CE (CE-EC) an attractive alternative to fluorescence. Additionally, subnanomolar LODs comparable to fluorescence can be achieved at a fraction of the cost [1,2]. Integration is relatively easy and lends itself well to miniaturization. Furthermore, excellent selectivity in biological matrices is easily achieved using working potentials selective for the analytes of interest. In addition, large linear dynamic ranges can also be attained with EC detection [3, 4]. There have been several approaches to integrating CE with EC detection previously reported in the literature.

On-capillary detection has generally been described as the working electrode (WE) being on or inside the capillary [5]. This mode of detection is desirable because there is minimal loss of analyte signal due to diffusion away from the electrode surface, thus providing greater

sensitivity. Typically, this approach involves isolating the separation current from the current of the WE, which has most often been accomplished using an electric decoupler. One of the first decouplers reported by Wallingford and Ewing, involved fracturing the capillary in to two pieces, which were linked using a porous glass sleeve creating a joint [6]. This concept evolved into a bare fracture, whereby the capillary pieces are not joined by another material, but instead are immersed in run buffer and analytes are moved toward the detector by EOF generated from the separation voltage [7]. The idea of the electric decoupler was carried out further with the addition of a Nafion polymer coating [8, 9]. In this case, the polymer was used to maintain the structure of the capillary and minimize loss or exchange of analyte through the fracture, thus improving detection limits. Hu et al. used hydrofluoric acid (HF) to create an etched joint in the capillary [10]. More recently, Osbourn and Lunte developed a decoupler using cellulose acetate polymer [11]. Instead of removing the polyamide coating and HF etching down the walls of the capillary as Hu et al. demonstrated, laser ablation was used to create holes in the capillary that were then covered using the previously mentioned polymer. Although these approaches eliminated challenges such as electrode alignment and reproducibility of electrode positioning, fabrication of decouplers pose challenges and their fragile nature decreases the robustness of the system. A more robust approach to on-capillary EC detection, when compared to conventional CE systems, is through the use of microchips [12-18]. The most commonly used approach for these systems is accomplished by placing a large palladium electrode upstream from the WE [13, 14]. The palladium electrode absorbs and dissipates hydrogen produced at the upstream electrophoretic cathode, decoupling the devices at field strengths up to 1200 V/cm [13].

In end-column detection, the electrode is placed outside the capillary and aligned with the outlet [5]. This detection scheme is easily fabricated for CE, as it does not require an electrical decoupler. Instead the current produced from the separation is dissipated to a degree from the WE by using capillaries with small inner diameters (5–25 μm) [19-23]. Small inner diameters coupled with long lengths are adequate to prevent electrophoretic current from coming back through the ground of the detector, which can cause an increase in background noise. Issues with the alignment of the electrode at the outlet of the capillary result in both decreased sensitivity and band broadening due to diffusion of the analyte away from the electrode surface. To improve end-column detection, Ewing and co-workers employed HF etching, whereby the inner walls of the outlet were dissolved and the inner diameter of the capillary was opened sufficiently to insert a micro-WE [21]. While this leads to improved sensitivity, due to less diffusion of the analyte away from the surface of the electrode, the separation current was shown to not be completely shielded from the current of the WE resulting in shifts in apparent peak potentials [24,25]. End-column detection has also been widely employed in microchip systems by placing the WE at the outlet of the capillary, typically in the waste reservoir [26-31]. These systems experience the same issues as conventional systems employing end-column detection: reproducibility with electrode alignment and distance, diffusion of the analyte away from the electrode surface, and increased background noise at the detector (due to some residual field effects produced from the electrophoretic current).

Although the previously described detection schemes adequately interfaced CE with EC detection, the use of dual-electrode detection for CE can improve the selectivity and sensitivity compared to single-electrode detection. An initial application of a dual-electrode detection scheme for CE was described for the determination of cysteine and cystine [32]. Lin et al. utilized a series configuration with the reduction electrode placed upstream from the oxidation electrode in an end-column format, LODs were reported to be 5 μM . Zhong and Lunte reported a dual-Au/Hg electrode in a series configuration in which a tubular electrode was constructed and placed upstream from a macrodisk electrode aligned with the outlet of the capillary [33]. The LOD for this system was determined to be 0.5 μM . The

improvement in LODs can be in-part explained by the larger surface areas (125 μm) that were employed, which improved the amount of analyte detected at the downstream electrode. Holland and Lunte demonstrated another integrated approach using Pt wire electrodes. In this system, one electrode was inserted into the separation capillary while the other was placed across the capillary outlet, the configuration offered slight improvement in LOD (1 μM) compared to a previous report by Zhong and Lunte ~ [33,34]. Chen et al. reported a parallel-opposed dual-electrode configuration in order to improve sensitivity over conventional CE-EC systems through redox cycling. Using this configuration, 12 nM detection limits were obtained [35, 36]. Microchip systems have also employed dual-electrode detection schemes, both in-capillary and end-capillary [12-14, 37-41]. While these devices allow for rapid analysis (seconds time scale), improved temporal resolution and ease of integration into automated lab-on-a-chip systems, they are plagued by diminished resolution and lower efficiencies, resulting in inferior detection limits ($>0.2 \mu\text{M}$) when compared to conventional CE systems that are decoupled.

The purpose of this research was to develop an analytical detection scheme that employed a dual-parallel detection configuration that could easily be coupled to CE. Using this detection scheme issues such as diminished selectivity in complex biological samples can be improved (over conventional single-electrode detection schemes), while also enhancing sensitivity. This dual-electrode design allowed for two different detection modes to be used: a redox cycling mode and a dual-potential mode. The redox cycling mode was used to enhance sensitivity and improve LODs compared to a single WE. Additionally, the dual-potential mode was used to selectively identify several different phenolic acids in whiskey samples. Using this mode migration times in conjunction with current, ratios were used as an orthogonal means to confirm peak identity. Furthermore, the redox cycling mode was used to confirm peak identity based on the chemical reversibility of the phenolic acids.

2 Materials and methods

Isopropanol, methanol, HF, hydrochloric acid, and sodium hydroxide were obtained from Fisher Scientific (Pittsburgh, PA, USA). Sodium bicarbonate, sodium tetraborate decahydrate, tetradecyltrimethylammonium bromide (TTAB), 3,4-dihydroxybenzoic acid (protocatechuic acid, 3,4-DHBA), 4-hydroxybenzoic acid (4-HBA), ferulic acid, gallic acid, gentisic acid (GA), *p*-coumaric acid, syringic acid (SA), vanillic acid, and 3,4-dihydroxyphenylacetic acid (3,4-DHPA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals were reagent grade and used as received.

The dual-parallel electrode fabrication was based on the design of the single carbon fiber (CF) electrode described previously by our lab [9]. The modification to this design was the addition of the second 33 μm CF to the outside of the original electrode as shown in Fig. 1. Briefly, two single 33 μm CF (AVCO Specialty Materials, Lowell, MA, USA) electrodes were fabricated by threading the CF through fused silica capillary (105 μm od, 40 μm id) leaving approximately 2 mm of CF exposed at both ends of the capillary. One of the fused silica capillary pieces was flame etched to remove the polyamide coating. This was done to ensure that the electrodes could be inserted into the opening of the capillary. One end of the exposed CF was inserted into a 23 gauge 1 inch length needle, which had an opening bored into the side of the needle. Each single CF was inserted into the needle until it reached the opening in the side of the needle. A drop of colloidal silver paste (Ted Pella, Redding, CA, USA) was then spread over the opening in the needle in order to make the electrical connection for the WE. The two single electrodes were then UV glued together, making sure the tips of the electrodes were in alignment. The combined electrodes were then fed through a 1.5–2.0 cm length piece of fused silica capillary (658 μm od, 537 μm id) for rigidity and support. Super glue was used to affix the electrodes to the support. Electrodes were trimmed

using tissue scissors to a length of 1600 μm ($\pm 12 \mu\text{m}$) and an average width of 55 μm (9.0 μm). Figure 1A–D illustrates this process completely.

Electrophoresis was performed using a high voltage (0–30 kV) power supply (Spellman High Voltage, Hauppauge, NY, USA). The cathodic end of the capillary was isolated in a Plexiglas box containing an interlock for the user safety. Fused silica capillaries (Polymicro Technologies, Phoenix, AZ, USA), 25 μm id and 80 cm in length, were used for the separation of the phenolic acids. The capillary was placed into the EC cell which contained the BGE and served as the anodic reservoir (separations were run in reverse polarity).

In order for the dual-parallel electrode to be inserted into the separation capillary, the outlet of the capillary was modified using HF acid, a concept inspired by work described previously by Ewing and co-workers [21]. Briefly, HF etching was done by submerging the outlet end of the capillary into 49% HF acid for a period of 100 min. This resulted in an opening ($\sim 230 \mu\text{m}$) sufficient for insertion of the dual-parallel electrode. Furthermore, with this etch time, a maximum insertion depth of $\sim 660 \mu\text{m}$ was possible.

Prior to use electrodes were rinsed with MeOH and nanopure water. A platinum auxiliary electrode and an Ag/AgCl reference electrode (Bioanalytical Systems, West Lafayette, IN, USA) were used. The CF WEs were controlled by two LC-4C potentiostats (Bioanalytical Systems). All applied potentials reported were versus an Ag/AgCl reference electrode. Data acquisition was performed by Chrom&Spec software (Ampersand International, Beachwood, OH, USA) at a frequency of 10 Hz per channel. Electrodes used for the validation of this system experienced no electrode fouling even after repeated use of up to 1 month. Furthermore, no additional cleaning was needed other than the initial rinse with MeOH previously mentioned.

All phenolic acid solutions were prepared using 1% (v/v) perchloric acid (0.1 M) in nanopure water (Millipore, Billerica, MA, USA). All samples were injected electrokinetically (-12 kV , 2 s) onto the capillary. The BGE for this research was 75 mM sodium tetraborate, 0.5 mM TTAB, and pH adjusted using concentrated HCl to 8.4. Analytes were baseline resolved in less than 25 min using a separation voltage of -12 kV . The dual-electrodes were inserted to a depth of 374 μm ($\pm 19 \mu\text{m}$). The oxidation and reduction potentials used to detect the phenolic acids were +800 mV and -200 mV versus Ag/AgCl, respectively.

Whiskey samples were diluted 1:1 with 1% (v/v) perchloric acid (0.1 M) and spiked with a 250 nM 4-HBA standard. Prior to dilution, the whiskey was filtered through 0.22 μm sized Millex GP syringe filter. Samples were then injected directly onto the capillary for analysis. The sample was injected as previously described. The separation voltage was -10 kV for the analysis of whiskey samples, in order to minimize co-migration of various phenolic acids contained in the sample. In order to account for the variability in migration times, all elution times were normalized to the migration time of 4-HBA, which was used as an internal standard.

A mixture of four phenolic acids, possessing different electrochemical properties (3,4-DHBA, 4-HBA, SA, and GA) were used to validate the dual-electrode design. Both SA and GA are relatively easily oxidized while 3,4-DHBA and 4-HBA oxidize at higher potentials. While both GA and 3,4-DHBA are chemically reversible, SA and 4-HBA are chemically irreversible. Electrophoretically assisted hydrodynamic voltam-magrams (HDVs) were generated to optimize WE potentials for the oxidation and reduction of the analytes of interest. An oxidation potential of +800 mV versus Ag/AgCl was determined to be on the current plateau for the more difficultly oxidized phenolic acids 3,4-DHBA and 4-HBA. A reduction potential of -200 mV versus Ag/AgCl was found to be sufficient for the reduction

of GA and 3,4-DHBA. It should be noted that the surfaces of CFs vary from electrode to electrode; therefore, it was important to establish proper working potentials for each set of electrodes. This was done by assessing the peak heights for the current ratios at 500 mV/800 mV and 200 mV/+800 mV. Responses that were within 0.1–0.2 of the initial HDV generate were determined to be acceptable. The same assessment was also used to establish the reduction potential for each set of electrodes.

3 Results and discussion

3.1 Redox cycling mode

To test the ability of the parallel dual-electrode system for redox cycling, one electrode (WE_1) was held at +800 mV and the second electrode (WE_2) was held at a potential of –200 mV versus Ag/AgCl. Figure 2 shows that with both electrodes on, responses were observed at both electrodes for the reduced phenolic acids injected. Since the phenolic acids are in their reduced forms, no response would be observed at WE_2 if the phenolic acids were not first oxidized at WE_1 . This lack of response at WE_2 was observed when WE_1 was turned off (data not shown). With only WE_1 on (i.e., single-electrode mode), the response detected was smaller than in the dual-electrode mode. This demonstrated that complete redox cycling was occurring in the parallel dual-electrode, as shown in Fig. 3.

Table 1 summarizes the data obtained using the parallel dual-electrode in redox cycling mode. Initially, the collection efficiencies were calculated for each analyte in order to determine what percentage of analyte was being reduced at the second electrode. This was done by dividing the peak height at WE_2 by the peak height at WE_1 . The calculated values varied from 8 to 62% collection efficiency for 4-HBA and GA, respectively. The variability in collection efficiencies were directly correlated to chemical reversibility. As previously mentioned, the chemical reaction for 4-HBA is irreversible, involving a one electron, one proton transfer, resulting in the formation of a highly reactive free radical, SA follows the same mechanism. Conversely, the chemical reactions for 3,4-DHBA and GA are reversible going through a two electron, two proton transfer resulting in the formation of quinone moieties which can be reduced back to their respective quinols. In addition to the collection efficiencies, signal enhancement for each analyte was also determined. The signal enhancement was calculated by dividing the slope of the curve from a linear regression analysis of WE_1 in redox cycling mode, by the slope of WE_1 when only a single electrode was used. The same trends observed for collection efficiencies were also observed for the signal enhancements. That is, approximately a twofold increase in signal was determined for GA, while there was virtually no signal enhancement observed for 4-HBA. Furthermore, a linear trend was observed for the parallel dual-electrode, at both electrodes, of over two orders of magnitude (100 nM–4.0 nM), with correlation coefficients of 0.92 and 0.99, respectively. The poor linearity was only observed at WE_2 for 3,4-DHBA, this was attributed to the intensity of the slope in baseline at this point in the electropharogram. Since, WE_2 will not be used for quantification in this analysis, the poor linearity reported was not of great concern.

The reproducibility of the dual-electrode fabrication was investigated by comparing the sensitivities of three different dual-electrode sets. This was done by using linear regression analysis for one electrode set, with the sensitivities from subsequent sets of electrodes being compared at a concentration of 60 nM. The values shown in Table 2 represent the relative standard deviations for the determined peak heights between the three different sets of electrodes. No significant deviation was observed for 3,4-DHBA, 4-HBA, SA, or GA using this method of analysis at WE_1 . The deviation at WE_2 for 4-HBA was slightly higher compared to the other analytes. This was attributed to the small response observed and subsequently the reproducibility in the integration at WE_2 for 4-HBA compared to the other

analytes. There was minimal variability within the fabrication process, which did not significantly alter the response from electrode to electrode. It should be noted that while there was minimal variability in response from electrode to electrode, the amount of redox cycling observed is directly related to the working potential applied. Brief HDVs should be run for each new set of electrodes to ensure that there is minimal variability in signal enhancements and collection efficiencies. Additionally, the reproducibility between injections was also determined ($n = 8$) by comparing peak heights. The data shown in Table 2 represent the relative standard deviations in the peak heights for both electrodes at a concentration of $1.0 \mu\text{M}$. It was determined that there was less than 10% variation in peak heights between injections.

3.2 Dual-potential mode

The dual-potential mode is a selective configuration that is useful when analyzing complex biological matrices. Current ratios can be obtained in this configuration and used in conjunction with migration times to identify sample components. In Fig. 4, WE_1 was held at a potential of +800 mV, while WE_2 was held at a potential of +500 mV versus Ag/AgCl. At a potential of +500 mV approximately +35% of the current for 3,4-DHBA and 4-HBA was observed relative to +800 mV, while approximately 85% of the current was observed for SA and GA. The ratio of the current response at these two potentials ($i_{+500 \text{ mV}}/i_{+800 \text{ mV}}$) was characteristic of each compound and could therefore be used in confirming the identity of peaks in actual samples. This current ratio in essence represents a two-point voltammogram. As a compound's electro-chemical characteristics are orthogonal to its electrophoretic migration characteristics, using both properties to identify peaks in complex samples provides significantly higher confidence than using migration alone.

3.3 Whiskey analysis

Analysis of a whiskey sample was used to demonstrate the utility of the parallel dual-electrode for the analysis of complex samples. For this analysis, several additional phenolic acid standards were characterized by CE with dual-electrode detection. Analytes were selected based on a previous report by Roston and Kissinger which profiled several different spirits by LC-EC [42]. In order to minimize co-migration of these additional phenolic acids, the separation voltage was decreased to -10 kV . The migration and electrochemical properties of all of the standards tested are shown in Table 3.

The initial analysis used the dual-potential mode with one electrode held at +800 mV and the other held at +500 mV versus Ag/AgCl. Nine peaks were identified from these electropherograms, as shown in Fig. 5. Preliminary peak identities were made based on comparing the migration times of the peaks in the whiskey sample to those of the phenolic acid standards. It should be noted that migration time in CE is a poor indication of peak identity. For this system intraday and interday variability with respect to migration were found to be 0.5 and 1.25 min, respectively. In order to minimize variability in migration time, 4-HBA was used as an internal standard. As shown in Fig. 5, 4-HBA migrates between Peaks 2 and 3 and with no response observed from the whiskey sample between these two peaks, resulting in no issue of interference. Based on migration time alone, Peak 1 was tentatively identified as 3,4-DHBA or 3,4-DHPA, Peak 2 as 3,4-DHPA or gallic acid, Peak 3 as vanillic acid, Peak 5 as either p-coumaric acid or SA, Peak 6 as either p-coumaric acid or ferulic acid, and Peak 7 as GA. The migration time for Peak 8 correlated with that of GA as well, while Peak 9 did not match the migration times of any of the available standards, making identification of Peak 9 impossible.

The whiskey sample was then spiked with standards to more accurately make migration-based peak identity assignments. Based on linear regression analysis, peak concentrations in

the whiskey sample were roughly established. Analytes were then spiked in at concentrations that would effectively double the peak height of the corresponding peak of interest. These spiking experiments overcome the migration variability inherent in CE and allowed migration-based identification of Peak 1 as 3,4-DHBA, Peak 2 as gallic acid, Peak 3 as vanillic acid, Peak 5 as SA, Peak 6 as *p*-coumaric acid, Peak 7 as ferulic acid, and Peak 8 as GA.

To further confirm the peak identities, as determined by spiking, the electrochemical properties of each peak in the whiskey sample were compared to the corresponding standard compound. Electrochemical characterization is orthogonal to the migration-based characterization of the peaks in the sample and significantly enhances the confidence in peak identifications, particularly in complex samples such as represented by whiskey. Three different current ratios were used to characterize each peak in the whiskey sample. In the dual-potential mode, the current ratios determined at +500 mV versus +800 mV and at +200 mV versus +800 mV provide quantitative assessment of the ease of oxidation of each peak, shown in Figs. 5 and 6. In the redox cycling mode, the ratio of the currents obtained at -250 mV versus +800 provides assessment of the reversibility of the oxidation of each peak in the sample, as seen in Fig. 7. The current ratios obtained for both the peaks in the whiskey samples and standard compounds are listed in Table 3. Based on both the oxidation potential ($i_{+500 \text{ mV}}/i_{+800 \text{ mV}}$, dual-potential mode) and reversibility ($i_{-250 \text{ mV}}/i_{+800 \text{ mV}}$, redox cycling mode), Peak 1 was confirmed as 3,4-DHBA. For Peak 2, both the oxidation potential and reversibility matched that of gallic acid, confirming this identification. It should be noted that in this case the oxidation of gallic acid is irreversible and therefore no response was observed at -250 mV versus Ag/AgCl for either Peak 2 or the gallic acid standard. Using both the dual-potential mode as well as the redox cycling mode, Peaks 3 and 5 were confirmed as vanillic acid and SA, respectively. Peak 6 was present at low concentration (~10 nM), making the integration of Peak 6 difficult to reproduce from injection to injection. However, even with this difficulty, the electro-chemistry correlated well with that of the *p*-coumaric acid standard. With the spiking analysis performed at both 20 and 40 nM for *p*-coumaric acid current ratios of 0.71 and 0.71 were obtained in dual-potential mode, respectively. This then confirmed Peak 6 as *p*-coumaric acid. While Peak 7 was found to co-migrate with ferulic acid, the electrochemical characterization of the peak in the whiskey sample did not match that of the standard compound. This indicates that either the peak in the whiskey sample was misidentified based on migration or that there was a co-migrating interference. In either case, this peak would be misidentified and an incorrect concentration determined without the electrochemical characterization. Finally, the electrochemical characterization of Peak 8 also showed this was not GA, the compound initially identified based on co-migration.

4 Concluding remarks

Electrochemistry provides a powerful detection technique for CE. Using two WEs provides enhancement in selectivity, detection limits, and peak identification. Aligning the electrodes in a parallel configuration provides new operating opportunities not available in the more common series configuration.

Acknowledgments

This work was support by a grant from the National Institutes of Health R01-NS066466.

Abbreviations

CF	carbon fiber
3,4-DHBA	3,4-dihydroxybenzoic acid
3,4-DHPA	3,4-dihydroxyphenylacetic acid
EC	electrochemical
GA	gentisic acid
4-HBA	4-hydroxybenzoic acid
HDVs	hydrodynamic voltammograms
HF	hydrofluoric acid
SA	syringic acid
TTAB	tetradecyltrimethylammonium bromide
WE	working electrode

5 References

- [1]. Weng Q, Xu G, Yuan K, Tang P. J. Chromatogr., B Anal. Technol. Biomed. Life Sci. 2006; 835:55–61.
- [2]. Xu J-J, Wang A-J, Chen H-Y. TrAC, Trends Anal. Chem. 2007; 26:125–132.
- [3]. Du Y, Wang E. J. Sep. Sci. 2007; 30:875–890. [PubMed: 17536733]
- [4]. Kong Y, Chen H, Wang Y, Soper SA. Electrophoresis. 2006; 27:2940–2950. [PubMed: 16688700]
- [5]. Lunte, SM.; Martin, RS.; Lunte, CE. Electroanal. Methods Biol. Mater. Marcel Dekker Inc.; New York: 2002. p. 461–490.
- [6]. Wallingford RA, Ewing AG. Anal. Chem. 1987; 59:1762–1766. [PubMed: 3631501]
- [7]. Linhares MC, Kissinger PT. Anal. Chem. 1991; 63:2076–2078.
- [8]. O'Shea TJ, Greenhagen RD, Lunte SM, Lunte CE, Smyth MR, Radzik DM, Watanabe N. J. Chromatogr. 1992; 593:305–312.
- [9]. Park S, Lunte SM, Lunte CE. Anal. Chem. 1995; 67:911–918.
- [10]. Hu S, Wang Z-L, Li P.-b. Cheng J.-k. Anal. Chem. 1997; 69:264–267.
- [11]. Osbourn DM, Lunte CE. Anal. Chem. 2001; 73:5961–5964. [PubMed: 11791566]
- [12]. Castano-Alvarez M, Fernandez-Abedul MT, Costa-Garcia A. J. Chromatogr., A. 2006; 1109:291–299. [PubMed: 16472530]
- [13]. Mecker LC, Martin RS. Electrophoresis. 2006; 27:5032–5042. [PubMed: 17096314]
- [14]. Lai C-CJ, Chen C.-h. Ko F-H. J. Chromatogr., A. 2004; 1023:143–150. [PubMed: 14760858]
- [15]. Chen C, Hahn JH. Anal. Chem. (Washington, DC, U. S.). 2007; 79:7182–7186.
- [16]. Dawoud AA, Kawaguchi T, Jankowiak R. Electrochem. Commun. 2007; 9:1536–1541.
- [17]. Gunasekara DB, Hulvey MK, Lunte SM. Electrophoresis. 2011; 32:832–837. [PubMed: 21437918]
- [18]. Guan Q, Henry CS. Electrophoresis. 2009; 30:3339–3346. [PubMed: 19802848]
- [19]. Huang X, Zare RN, Sloss S, Ewing AG. Anal. Chem. 1991; 63:189–192. [PubMed: 1812795]
- [20]. Sloss S, Ewing AG. Anal. Chem. 1993; 65:577–581.
- [21]. Powell PR, Woods LA, Ewing AG. J. Sep. Sci. 2005; 28:2540–2545. [PubMed: 16405186]
- [22]. Woods LA, Ewing AG. ChemPhysChem. 2003; 4:207–211. [PubMed: 12619421]
- [23]. Qian J, Wu Y, Yang H, Michael AC. Anal. Chem. 1999; 71:4486–4492. [PubMed: 10546530]
- [24]. Matysik F-M. J. Chromatogr., A. 1996; 742:229–234.
- [25]. Wallenborg SR, Nyholm L, Lunte CE. Anal. Chem. 1999; 71:544–549. [PubMed: 9989376]
- [26]. Pumera M, Merkoci A, Alegret S. Electrophoresis. 2007; 28:1274–1280. [PubMed: 17366488]

- [27]. Wu Y-Y, Qu F, Lin J-M. Chin. J. Chem. 2005; 23:155–159.
- [28]. Wang Y, Chen H, He Q, Soper SA. Electrophoresis. 2008; 29:1881–1888. [PubMed: 18393335]
- [29]. Wu Y, Lin J-M, Su R, Qu F, Cai Z. Talanta. 2004; 64:338–344. [PubMed: 18969609]
- [30]. Wang J, Pumera M. Anal. Chem. 2002; 74:5919–5923. [PubMed: 12498184]
- [31]. Vazquez M, Frankenfeld C, Coltro WKT, Carrilho E, Diamond D, Lunte SM. Analyst (Cambridge, U. K.). 2010; 135:96–103.
- [32]. Lin BL, Colon LA, Zare RN. J. Chromatogr., A. 1994; 680:263–270.
- [33]. Zhong M, Lunte SM. Anal. Chem. 1999; 71:251–255. [PubMed: 9921132]
- [34]. Holland LA, Lunte SM. Anal. Chem. 1999; 71:407–412. [PubMed: 9949730]
- [35]. Chen D-C, Chang S-S, Chen C-H. Anal. Chem. 1999; 71:3200–3205. [PubMed: 21662911]
- [36]. Chen DC, Zhan DZ, Cheng CW, Liu AC, Chen CH. J. Chromatogr., B Biomed. Sci. Appl. 2001; 750:33–39. [PubMed: 11204221]
- [37]. Du F, Fung YS. Electrophoresis. 2010; 31:2192–2199. [PubMed: 20593393]
- [38]. Martin RS, Gawron AJ, Fogarty BA, Regan FB, Dempsey E, Lunte SM. Analyst (Cambridge, U. K.). 2001; 126:277–280.
- [39]. Martin RS, Gawron AJ, Lunte SM, Henry CS. Anal. Chem. 2000; 72:3196–3202. [PubMed: 10939387]
- [40]. Fischer DJ, Vandaveer WRIV, Grigsby RJ, Lunte SM. Electroanalysis. 2005; 17:1153–1159.
- [41]. Pai RS, Walsh KM, Crain MM, Roussel TJ Jr. Jackson DJ, Baldwin RP, Keynton RS, Naber JF. Anal. Chem. (Washington, DC, U. S.). 2009; 81:4762–4769.
- [42]. Roston DA, Kissinger PT. Anal. Chem. 1981; 53:1695–1699. [PubMed: 7283161]

Figure 1.

Dual-parallel CF electrodes inserted $\sim 370\ \mu\text{m}$ into the end of an HF etched capillary.

Fabrication of dual-electrode: (A) fused silica capillary ($40\ \mu\text{m}$ id) with $33\ \mu\text{m}$ CF exposed at both ends; 23 gauge 1 inch length needle with hole bored in side. (B) assembled β single CF microelectrode with colloidal silver paste on the bored hole of the needle. (C) individual single CF microelectrodes UV glued together. (D) fully assembled CF parallel dual-electrode.

Figure 2.

Parallel dual-electrode in redox cycling mode. Response from a $1.0 \mu\text{M}$ mixture of phenolic acids: (A) 3,4-DHBA, (B) 4-HBA, (C) SA, and (D) GA; only $WE_{1(+800\text{ mV})}$ (---); both $WE_{1(+800\text{ mV})}$ and $WE_{2(-200\text{ mV})}$ (—). Separation conditions: -12 kV, 75 mM sodium tetraborate, 0.5 mM TTAB, pH 8.4 . Injection: -12 kV, 2 s

Figure 3.
Electrochemical mechanism for redox cycling mode.

Figure 4. Response using the parallel dual-electrode in dual-potential mode. Standards were $1.0 \mu\text{M}$. Applied electrode potential: $WE_1 = +800 \text{ mV}$, $WE_2 = +500 \text{ mV}$. Separation conditions: -12 kV , 75 mM sodium tetraborate, 0.5 mM TTAB, $\text{pH } 8.4$. Injection: -12 kV , 2 s . Peak identification: (A) 3,4-DHBA, (B) 4-HBA, (C) SA, and (D) GA.

Figure 5.

Response at the dual-electrode for a whiskey sample diluted 1:1 (v/v) with 1% in (0.1 M) HClO_4 spiked with 250 nM 4-HBA. Electrodes were used in dual-potential mode with $\text{WE}_1 = +800$ mV and $\text{WE}_2 = +500$ mV. Peaks were identified based on migration time and current ratios using peak height. Separation conditions: -10 kV, 75 mM sodium tetraborate, 0.5 mM TTAB, pH 8.4. Injection: -12 kV, 2 s. Peak numbers related to Table 3.

Figure 6. Same conditions as mentioned in Fig. 5, except working potential applied to $WE_2 = +200 \text{ mV}$. Peak numbers related to Table 3.

Figure 7.

Response at the dual-electrode for a whiskey sample. Electrodes were used in redox cycling mode with $WE_1 = +800 \text{ mV}$ and $WE_2 = -250 \text{ mV}$. Separation conditions were the same as for those reported in Fig.5.

Data summarizing the signal enhancement and collection efficiencies for the parallel dual-electrode in redox cycling mode

Table 1

	Sensitivity single electrode +800 mV (fA/nM)	Collection efficiency	Signal enhancement	Sensitivity redox cycling mode, w ₁ , +800 mV (fA/nM)	Sensitivity redox cycling mode, w ₂ , -200 mV (fA/nM)
3,4-DHBA	550 ± 10	31 ± 9%	1.35	730 ± 30	260 ± 50
4-HBA	1230 ± 50	6.2 ± 0%	1.05	1300 ± 80	115 ± 5
SA	920 ± 40	15 ± 4%	1.12	1030 ± 50	180 ± 20
GA	530 ± 40	62 ± 20%	1.72	890 ± 50	560 ± 20

SDs reported based on peak heights (*n* = 5).

Table 2

Data summarizing injection reproducibility and reproducibility of electrode fabrication

	Injection reproducibility, WE ₁ (%)	Injection reproducibility, WE ₂ (%)	Electrode fabrication reproducibility, WE ₁ (%)	Electrode fabrication reproducibility, WE ₂ (%)
3,4-DHBA	9.8	9.8	10	10
4-HBA	7.8	14	8.8	12
SA	8.8	12	4.5	4.3
GA	9.4	6.2	0.20	0.30

RSD reported, based on peak height.

Table 3

Analysis of migration times and current ratios using dual-potential mode and redox cycling mode for standards and unknown peaks in the whiskey sample

	Time (min)	<i>i</i> ratio _(+500 mV/+800 mV)	<i>i</i> ratio _(+200 mV/+800 mV)	<i>i</i> ratio _(-250 mV/+800 mV)
3,4-DHBA	18.0 ± 0.2	0.21 ± 0.03	ND	0.31 ± 0.09
Peak 1	18.2 ± 0.3	0.34 ± 0.04	0.052 ± 0.003	0.2 ± 0.1
3,4-DHPA	18.5 ± 0.1	0.95 ± 0.05	0.04 ± 0.01	0.54 ± 0.03
Peak 2	18.6 ± 0.2	0.97 ± 0.03	0.09 ± 0.01	NR
Gallic acid	18.65 ± 0.03	1.15 ± 0.09	0.12 ± 0.03	NR
4-HBA	20.2 ± 0.0	0.33 ± 0.04	ND	0.062 ± 0.004
Vanillic acid	20.9 ± 0.1	1.14 ± 0.07	0.023 ± 0.001	0.26 ± 0.04
Peak 3	21.0 ± 0.4	1.16 ± 0.04	0.25 ± 0.01	0.31 ± 0.01
Peak 4	21.3 ± 0.1	1.4 ± 0.1	BLOD	BLOD
Peak 5	21.6 ± 0.1	1.10 ± 0.04	0.3 ± 0.1	0.19 ± 0.01
SA	21.7 ± 0.2	1.06 ± 0.04	0.230 ± 0.006	0.15 ± 0.04
p-Coumaric acid	21.7 ± 0.0	0.69 ± 0.06	0.005 ± 0.001	NR
Peak 6	22.1 ± 0.3	0.85 ± 0.07	0.04 ± 0.02	BLOD
Ferulic acid	22.3 ± 0.1	1.30 ± 0.08	0.32 ± 0.05	0.25 ± 0.01
Peak 7	22.8 ± 0.3	0.74 ± 0.01	0.09 ± 0.02	0.14 ± 0.01
GA	22.9 ± 0.3	1.71 ± 0.08	0.93 ± 0.05	0.6 ± 0.2
Peak 8	23.3 ± 0.3	0.93 ± 0.07	0.14 ± 0.02	0.03 ± 0.01
Peak 9	24.9 ± 0.5	ND	ND	ND

Separation conditions are the same as those reported in Fig. 5.

ND, not detected. BLOD, below LOD. NR, not reversible.