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The Use of a 3D-Printed Microfluidic Device and Pressure Mobilization for Integrating Capillary Electrophoresis with Electrochemical Detection

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Abstract

Capillary electrophoresis coupled with electrochemical detection can be a powerful analysis tool; however, previous methods developed to integrate these two techniques can often times be fragile and have alignment issues such that there are no commercially available approaches. In this paper, we present the use of a 3D-printed Wall-Jet Electrode device for integrating capillary electrophoresis with electrochemical detection. A pressure mobilization step was also utilized to further reduce noise by allowing the electrophoresis separation step to continue only until the first analyte was close to elution. Then, the separation voltage was terminated and pressure-based flow was used for elution of the analyte bands onto the electrode surface with a wall-jet configuration. It is shown that the pressure-based elution is beneficial for the reduction of baseline noise and elimination of field effects. A mixture of catecholamines were separated to demonstrate effectiveness of the system. In addition, the system was coupled with a Beckman Coulter commercial capillary electrophoresis instrument in a straightforward manner. The system was also shown to be effective in separations done with a high ionic strength physiological buffer. This 3D printing approach can be used by researchers to utilize electrochemical detection on commercial capillary electrophoresis systems by downloading the provided STL and/or CAD files.

Introduction

Capillary electrophoresis (CE) is an efficient analytical technique used for the separation of small analyte samples. Conventional CE has been widely utilized in a variety of impactful applications, including the completion of the Human Genome Project [1] and pharmaceutical analysis [2]. Additional applications of CE are continually being developed for analyses of proteins [3], forensic data [4], and clinical samples [5]. Novel devices that facilitate or improve current methods for CE can be applied to a wide variety of applications/disciplines.

One active area of research is the modes of detection for conventional CE. In commercially available CE instruments, UV-Vis absorbance is the most commonly used detection method

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Additional files include supplemental information as well as CAD and STL files.

Conflicts of interest

There are no conflicts of interest to declare.

due to the ease of implementation. A small portion of the polyamide coating is removed from the fused silica capillary so that light can be directed through the capillary (commonly termed on-capillary detection). However, the small inner diameters of most capillaries result in a very short pathlength, so limits of detection (LODs) tend to be relatively high [6, 7]. Additionally, this method is not desirable for analytes with small extinction coefficients. Very low LODs have been obtained using laser-induced fluorescence detection, commonly using on-capillary detection [8, 9]; however most analytes require derivatization before analysis. CE coupled to detection via mass spectroscopy (CE-MS) has been rapidly developing [10] and some commercial CE instruments come with accessories to facilitate coupling with MS directly. However, CE-MS can often be complicated and requires the use of relatively expensive mass spectrometers.

As an alternative to the detection methods offered by commercial instruments, electrochemical (EC) detection can also be integrated with CE systems. Amperometry allows selective detection since the potential can be adjusted for a given analyte. In addition, potentiostats are relatively inexpensive. An important aspect of using this detection mode is how the EC detection system is isolated from the electrophoretic high voltage (typically 25–30 kV). Previously, this has been achieved with two different detection schemes – off-column detection and end-column detection. These topics have been extensively reviewed [11]. In off-column detection, the capillary is grounded by a decoupler before reaching the detection electrode. In the first applications of CE-EC, off-column detection was utilized by introducing a small fracture in the capillary and allowing the system to ground through the fracture. With sufficient electroosmotic flow and a short detection capillary, the analyte can be pushed past the fracture to the detection electrode while maintaining separation efficiency [12, 13]. Various modifications to off-column detection have since been employed. One such variation is the use of a palladium decoupler, which was found to significantly dissipate hydrogen, increasing the efficiency of the decoupler [14]. Although off-column detection designs have been shown to produce the lowest limits of detection, successful execution of off-column detection is often difficult. Primarily, this is because the fracture introduced to the capillary can be quite fragile. Nafion can be cast over the fracture to improve stability while still allowing efficient grounding of the separation voltage [15, 16].

End-column detection is a popular alternative to off-column detection because the system is more robust. In end-column detection, the electrode is simply positioned outside the capillary outlet. In 25 μm i.d. capillaries or smaller, it has been found that the voltage drop across the capillary is great enough that interference with the detection circuit is minimal [17]; however, a shift in the half-wave potential is possible [18]. This configuration has been used for high-throughput separations [19], analysis of insecticides [20], and quantitation of DNA damage [21].

A common difficulty in the integration of electrodes with conventional CE (with either approach) is the alignment of the capillary with the electrode. One approach is to integrate the decoupler and the working electrode into a microchip device [22, 23]. In this design, a decoupler is placed in a microchip with the working electrode integrated further down the separation channel. This configuration is similar to traditional off-column detection but reduces the amount of band broadening while ensuring accurate alignment and a robust

interface. Additionally, when electrodes are embedded in the microchip, the electrodes can be easily polished and reused. There have been many publications on electrochemical detectors for microchip CE [24, 25]; however, conventional CE remains popular due to the high separation efficiencies and ability to inject from vials or 96-well plates.

Recently, the use of 3D-printing has become widely utilized. In the field of microfluidics, devices with complex geometries that were previously impossible to fabricate using 2D microchips are now possible to create using digital design and printing layer-by-layer [26–28]. Additionally, 3D-printed devices are highly reproducible and designs can be rapidly shared between labs. The cost and time of fabrication is significantly lowered in comparison to other designs, and the resulting models are rugged and easy to work with. Previously, the use of a 3D-printed Wall-Jet Electrode (WJE) for fluid-based flow injection analysis was shown to be an efficient analytical detection device [29]. In a WJE, the incoming flow impinges on the working electrode perpendicularly, and the flow then spreads out radially into outlet channels. This improves mass transport of analyte at the electrode surface and increases the sensitivity.

In this paper, we show the use of a modified 3D-printed WJE for use in CE-EC systems. The electrophoretic separation takes place across a capillary integrated into a WJE device. After the separation, the analytes are eluted using pressure, leading to a decrease in baseline noise in comparison to traditional end-column detection. A major advantage of 3D-printing is that it facilitates centering of the capillary with the electrode, which is critical for the design. This system is robust, versatile, and can also be integrated with a commercial Beckman Coulter P/ACE MDQ instrument. We fully characterize the device and investigate variables that affect resolution and the ability to inject high ionic strength samples. Importantly, we include all of the STL and CAD files so that others can use this approach on commercial CE instruments.

Experimental

Materials and Methods

The following chemicals and materials were used as received: 5-minute epoxy (Permatex, Harford, CT, USA); catechol, 3,4-dihydroxyphenylacetic acid, dopamine, norepinephrine, Hank's balanced salt solution (HBSS), sodium dodecyl sulfate, TES sodium salt, boric acid, fluorescein, fluorescein isothiocyanate-dextran, potassium dicyanoaurate, sodium carbonate, (Sigma Aldrich, St. Louis, MO, USA); 500 μm Pt wire, 100 μm gold wire (Alfa Aesar, Ward Hill, MA, USA); copper electrical wire, soldering wire and heat shrink tubes (RadioShack); FingerTight fitting F-120 $\times$, capillary tubing connectors F-230 and Super Flangeless Fitting P-131 (IDEX Health & Science, Oak Harbor, WA); EL30P1.5 High Voltage Power Supply (Glassman High Voltage, Inc., High Bridge, NJ, USA) or LabSmith HVS448 3000V High Voltage Sequencer (LabSmith, Livermore, CA USA); 25 μm i.d. and 50 μm i.d. capillary (Polymicro Technologies, Phoenix, AZ, USA); Streampix Digital Video Recording software (Norpix, Inc., Montreal, Canada); Stratasys Objet Eden 260 V 3D Printer (Edina, MN, USA); with Full Cure 720 model material and Full Cure 705 support material (Stratasys, Edina, MN, USA); Stratasys Idea Series Mojo 3D Printer (Eden Prairie, MN, USA); with ivory P430 ABS model material and SR-30 soluble support material (Stratasys, Ltd., Edina,

MN, USA); BAS electrode polishing kit (Bioanalytical Systems Inc, West Lafayette, IN, USA); CH Instruments potentiostat (Austin, TX, USA); P/ACE MDQ instrument with UV Detection Module and External Detector Adaptor kit (Beckman Coulter, Fullerton, CA, USA), 32 Karat 8.0 software (Beckman Coulter, Fullerton, CA, USA); Autodesk Inventor Professional 2015 (San Rafael, CA, USA); MagicPlot Student 2016 (Saint Petersburg, Russia); Chromperfect (Justice Innovations, Inc., Denville, NJ, USA).

Fabrication of 3D-Printed Device

The 3D-printed devices utilized in this study were designed using Autodesk Inventor Professional 2015. The completed designs were saved in an .STL format, and were printed on a high resolution Stratasys Objet Eden 260 V 3D Printer. The material selected for this design was Full Cure 720, a semi-transparent material, and the support material was Full Cure 705. The composition of the Full Cure 720 material is propriety, but it contains approximately 10–30% isobornyl acrylate, 10–30% acrylic monomer, 15–30% acrylate oligomer, 0.1–1% photo initiator [30]. The supporting material was removed manually and with the use of high pressure water. An image of the design can be found in the supplemental information (Fig. S-1).

Fabrication of Electrodes

The fabrication of epoxy-embedded electrodes in IDEX Fangeless fittings has been previously described [29]. In this study, a 100 μm diameter gold wire was utilized as the working electrode. First, the wire is connected to a piece of copper electrical wire using colloidal silver and soldering wire. The connection is then enclosed in heat shrink tubing for insulation. To ensure electrode centering, before embedding the electrode into the fitting, the wire is threaded through a 3D-printed disk. 3D-printed disks (3.2 mm diameter, thickness varying between 0.5 – 1.5 mm, 0.25 mm hole size) fit snugly inside the fitting and center the electrode. 5-minute epoxy was used to fill the base of the fitting to hold the wire in place. Then, epoxy was applied on top of the electrode surface and allowed to cure. The electrode was then polished first by wet polishing until the epoxy surface was even with the fitting, and then with the BAS electrode polishing kit.

A gold pillar was produced on the surface of the 100 μm gold wire via an electrodeposition process. The solution used for the electrodeposition was composed of 50 mM potassium dicyanoaurate and 0.1 M sodium carbonate. A small amount of this solution was added to a small beaker such that the gold electrode, platinum auxiliary electrode, and Ag/AgCl reference electrode were submerged. -1.2 V was applied to the gold electrode for 2150 s, then the solution was stirred, and the reference electrode was switched out, followed by another 2150 s interval of -1.2 V . This deposition process produced a pillar approximately 20–30 μm .

Configuration of 3D-printed WJE Device for Capillary Electrophoresis

The 3D-printed WJE system was designed so that with a few short steps it can be used to integrate electrophoretic separation with electrochemical detection using end-capillary detection and pressure-assisted mobilization. In the set-up, first the epoxy-embedded working electrode was fit into the device. Next, the capillary was threaded through a

capillary tubing connector and F-120× Finger Tight fitting. The capillary must be aligned as close as possible to the working electrode, so before the fitting was secured, the capillary was pushed across the outlet channel so that it was nearly flush with the working electrode (Fig. 1C). Then the fitting was tightened to secure the capillary's position. Platinum wires were placed in each reservoir and the capillary inlet was inserted through a septum in a vial. To ensure that the inlet vial and buffer reservoirs had the same solution level, the inlet vial was fitted into another 3D-printed device that was placed on the same surface as the WJE device (Fig. 1A).

The system was flushed with 25 mM boric acid buffer at pH 9.2, and samples were made in boric acid. Pressure injections were completed using LabView or by manual injection. The analytes in the sample were separated electrophoretically by applying a high separation voltage via a platinum electrode at the buffer inlet, and platinum wires in the two buffer reservoirs served as the ground for the system. For fluorescence studies, the device was imaged under a microscope. Streampix Digital Video Recording software was used to record injections and to quantify the fluorescent plugs. A window was manually selected at the approximate position of the electrode, and the application plots the number of white-colored pixels over time.

When electrochemical detection was used, first an electrophoretic separation was performed as described above. Once the voltage was applied for the desired amount of time, the voltage was switched off. The platinum electrodes that served as the ground during the separation were attached to the potentiostat to serve as the pseudo-reference in EC detection. The working electrode was also connected to the potentiostat, and the amperometric detection was then turned on and allowed to equilibrate for several seconds. Then, pressure was applied to elute the analytes while amperometric detection was performed at +0.9V vs a platinum pseudo-reference.

The time needed for the electrophoretic separation was determined by estimating the migration time of the fastest analyte. This was done by first determining the time needed for elution with pressure alone. Then, voltage was applied for a certain amount of time, after which mobilization by the same pressure was completed. The difference between the two times was then determined and the electrophoretic velocity for the fastest eluting analyte calculated. Small adjustments to the separation time were then made to ensure the first analyte eluted 10–30 seconds after the pressure mobilization was initiated.

Configuration of Commercial CE System with 3D-Printed Device

The experiments completed on a commercial CE system were done using a Beckman Coulter P/ACE MDQ instrument. All studies using absorbance detection were done using Beckman Coulter's standard operating procedure. Measurements were completed using the UV detector module and a 50 μ m i.d. capillary that was 110 cm in length with 100 cm to the detection window. For coupling with the 3D-printed device, the External Detector Adapter for the P/ACE MDQ instrument was set up as indicated by the user guide. A 50 μ m i.d. capillary of 100 cm in length exited the P/ACE MDQ instrument and connected to the EC system (Fig. 2). In both set-ups, analyses were done on dopamine, norepinephrine, catechol, and 3,4-dihydroxyphenylacetic acid injections in a 25 mM boric acid buffer at pH 9.2. For

studies in high ionic strength buffer, dopamine and norepinephrine samples were prepared in Hank's balanced salt solution and a TES/SDS separation buffer system was used (pH 7.4).

Methods were programmed using 32 Karat 8.0 software. For experiments using UV detection, the CE instrument performed the injection, separation, and analysis as indicated in the method. Data was exported from the instrument in ASCII format, and was analyzed in MagicPlot Student 2016 and Chromperfect. When coupling with EC detection, the methods were programmed in External Detector Adapter (EDA) mode. The CE instrument performed a pressure injection, then applied the separation voltage across the capillary for the set amount of time. During the electrophoretic separation, the system was grounded via two platinum wires in the reservoirs of the 3D-printed device. After the voltage was complete, an alarm and a pause were programmed into the method, signaling that the system was ready to be switched to EC detection mode. The platinum wires were detached from the ground and connected to the potentiostat to serve as the pseudo-reference. A 100 μm gold electrode with a gold pillar was used as the detection electrode, and during the pause in the program it was also connected to the potentiostat. The EC system was then turned on and allowed to equilibrate for several seconds before the program was resumed. The CE instrument then applied pressure for elution and measurements were collected on the CH Instruments potentiostat. The data was analyzed in Chromperfect and displayed here in MagicPlot Student 2016 or Microsoft Excel. The time needed for the electrophoresis step was done in a similar manner to the home-built system described above; however, the calculation was facilitated by the presence of absorbance window 10 cm from the capillary inlet (per the installation of the external detector adapter). The total time for the separation voltage was calculated as a multiple of the peak appearing at 10 cm, depending on the total capillary length. In a similar fashion to the home-built system, small adjustments to the separation time were made to ensure the first analyte eluted 10–30 seconds after the pressure mobilization was initiated.

Results and Discussion

Field Effects Analysis

In this paper, we present the use of a 3D printed interface for CE and electrochemical detection that also uses a pressure mobilization step. The reason for using pressure elution rather than simultaneous CE-EC was an attempt to avoid interference between the high voltage for separation with the electric potential used for detection. Previously, it has been reported that when field effects from the high voltage interfere with electrochemical detection, there is a positive shift on the half-wave potential of the HDV plot [18, 31]. After a defined electrophoretic separation time, pressure (optimization of value discussed below) is used to push the separated bands from the capillary and onto the detection electrode. HDV plots were obtained in order to determine whether field effects dissipated after termination of the separation voltage. Catechol injections were performed, followed by either electrophoresis then pressure elution, or by pressure elution only (flow injection analysis) (see data in Fig. S-2 in supplemental information). The results of the HDV plots indicate approximately the same inflection and plateau points for both curves. This indicates that

residual field effects were significantly dissipated after electrophoresis, and so analyses were performed under the assumption that the system was free of field effects.

Baseline Electrochemical Noise Comparison

In end-column detection, it has been shown that in smaller i.d. capillaries, the voltage drop across the capillary is greater [17]. However, even in capillaries as small as 25 μm , the high voltage can have an effect on the detection circuitry. The increase is not solely an effect of the high voltage, but also of the distance between the working electrode and the capillary outlet [18, 31]. The greater the distance between the electrode and the capillary, the less the electrode is affected by the separation voltage. We analyzed these trends by obtaining plots of the baseline noise level under varying conditions.

In the study of baseline noise, the expected trends were obtained (Fig. 3). For all conditions, the baseline noise level was lower for the 25 μm i.d. capillary compared to the 50 μm i.d. capillary (y-axis is the same scale bar). The effect of the high voltage on the working electrode decreased with the capillary 250 μm from the electrode compared to having the capillary flush with the electrode. The level of baseline noise was also significantly reduced by removing the high voltage source. In both the 50 μm and 25 μm i.d. capillary, the lowest noise level was observed when using pressure elution.

These results clearly demonstrate the benefit of separating the processes of CE and EC. When utilizing EC with pressure rather than simultaneously with CE, the baseline noise level is greatly lowered. Since the processes can be isolated from one another, an additional benefit is that the EC detection system is protected from improper grounding that can occur with simultaneous CE-EC.

Trends in Elution Pressure and Resolution

After it was determined that using pressure elution for EC detection after CE separation was beneficial, the trend between resolution and elution pressure was examined. In this study, the separation of fluorescein and fluorescein isothiocyanate-dextran (FITC-dextran) was analyzed (with fluorescence detection) to determine the flow pattern within the devices and to study the effects of elution pressure. Data was collected by imaging through the transparent 3D-printed WJE device.

Through analysis of pressure-based flow injections, it was determined that peaks are narrower and more resolved with the capillary outlet in close proximity to the detection electrode (pushed flush when tightening fitting). It is estimated that the capillary outlet and the electrode are only several microns apart, but under a light microscope the edge of the capillary cannot be visualized since it is so close to the wall of the channel (and the imaging through the thick 3D printed device is not optimal). The fluorescent plugs rapidly became more diffuse as they eluted in the outlet channel. Additionally, the fluorescein was cleared more rapidly at the capillary outlet. Due to this analysis, the WJE device was designed to allow the manual adjustment of capillary positioning in the outlet channel. When the device was assembled, the capillary was manually pushed through the device until it was positioned flush with the electrode, then the PEEK fitting was tightened to secure it in place.

To determine trends in elution pressure, injections of fluorescein and FITC-dextran were performed on a 15 cm capillary (data shown in Fig. S-3 in supplemental information). First, the separation was completed electrophoretically and the elution time was determined to be approximately 200 s. In pressure elution trials, the high voltage was applied for 190 s, after which the pressure was applied. After the voltage was switched off, the leads for electrochemical detection were manually connected in the course of a few seconds, followed by the application of the desired pressure. While this manual method of switching electrode leads was used here, one can imagine using more sophisticated electronic switches to perform this function in a more automated fashion.

A possible issue in this procedure was broadening of the bands within the capillary since the analytes remain stagnant in the column during the switch between the electrophoretic separation and the connection of electrochemical detection. Due to this concern, the effect of higher pressure elution was investigated. Although peaks were narrower at high pressure, the resolution of the separation was found to increase with decreasing elution pressure. A graph of this trend can be seen in Fig. S-3. It was hypothesized that this trend was due to the more dramatic and rapid onset of a parabolic flow profile at higher pressures, leading to closer overlap between analyte bands.

Trends in Field Strength

Another variable that affects resolution is the field strength of the separation. Across a 30 cm capillary, field strengths of 150 V/cm, 250 V/cm, and 350 V/cm were tested in the separation of 25 μ M catechol and dopamine. This experiment was done by using a 1 s, 10 psi injection, followed by a varying electrophoresis separation step. For each separate field strength, the separation time was adjusted such that the dopamine peak eluted approximately 35 s after the application of pressure (so that the analytes were on the capillary for the same amount of time). 150 V/cm was applied for 420 s, 250 V/cm was applied for 210 s, and 350 V/cm was applied for 135 s, followed by mobilization with 1 psi pressure. It has been previously reported that the separation efficiency is directly proportional to the voltage applied in capillary zone electrophoresis [32]. The same trends were observed in this work, as the field strength increased, the resolution of the separation also increased (Fig. 4).

Integration of 3D-Printed Device with Commercial CE Instrument

The aforementioned studies utilized home-built systems with solenoid-based pressure systems and high voltage power supplies. As described previously, currently no commercial CE instruments are sold with EC detectors. In this study, we attempted to show that this device could be used in conjunction with a Beckman Coulter P/ACE instrument. Additionally, we studied whether the separation efficiency or sensitivity would be sacrificed by using this method. The separation of 50 μ M dopamine (DA), norepinephrine (NE), catechol (Cat), and 3,4-dihydroxyphenylacetic acid (DOPAC) was performed using the electrophoresis with on column UV detection as well as using the method described above for coupling with EC. Both methods utilized a 50 μ m i.d. capillary, and both experienced the separation across 100 cm of capillary length. To compensate for the fact that on-column UV detection requires an extra 10 cm past the detector, the voltage was adjusted such that a field strength of 270 V/cm was applied for both on-column UV detection and CE-EC.

The results of this comparison indicated that loss of separation resolution occurred when using end-capillary and pressure-based EC detection following CE in comparison to on-column UV detection using 200 nm light. However, the resulting separation was still acceptable for the CE-EC configuration, and the separation was obtained in less time. The resolution of the dopamine/norepinephrine separation was 1.6 for the EC detection and 3.8 for the UV detection system (Fig. 5).

Theoretical plates were also compared for each method of detection. The migration time used in the theoretical plate calculation for UV detection was the total amount of time that the analyte migrated in the capillary. For the entirety of the time reported, the system was under a field strength of 270 V/cm. In the EC detection calculations, voltage was only applied for 480 s, then there was a short pause, followed by pressure elution. To obtain the most accurate plate calculation, the migration time used was equal to 480 s of electrophoresis plus the elution time of the analyte under pressure. The switch from CE to EC mode was completed as quickly as possible to minimize band broadening from diffusion during the switch.

Due to the difference in the time scales, there is not a large difference or a clear trend in the theoretical plate calculations between the CE-EC and the UV detection methods (Table 1). At the same time, it is clear that the on-column UV detection led to better resolution. With the CE-UV method, the analytes were not eluted with pressure-based flow, resulting in the neutral Cat and negatively charged DOPAC remaining on-column for a much greater amount of time (as compared to the CE-EC method with pressure elution). Since these analytes remained on-column longer, they experienced larger peak widths at half-height. These values, along with the longer time values, made the theoretical plate calculations of both methods comparable.

In addition, a calibration curve was obtained for varied concentrations of dopamine. This experiment was performed on the P/ACE MDQ commercial CE instrument to obtain the most reproducible injections possible. Pressure injections of varying concentrations of dopamine were performed for 10 s at 8 psi. Voltage was applied for 480 s at 270 V/cm, followed again by elution pressure of 8 psi. The results obtained from this calibration curve indicated acceptable linearity ($R^2 = 0.9992$) and sensitivity (387 pA/ μ M). The limit of detection (LOD) for dopamine was 350 nM. The calibration curve is shown in the supplemental information Figure S-4.

Separations in High Ionic Strength Buffer

Microfluidic devices are powerful tools for cellular analyses, since the minimization of biological systems allows for greater control of biological variables and analysis can be performed on small sample sizes [33]. For this reason, we found it important to determine whether the 3D-printed detection system could be utilized for the separation and detection of catechol derivatives in a physiological buffer. When performing a separation in a high ionic strength buffer, the separation becomes more difficult due to the principle of de-stacking. Electroosmotic flow (EOF) is inversely proportional to the conductivity of the buffer. Therefore, injecting samples in a high ionic strength buffer lowers the effective EOF across the analyte plug. The ions in the plug migrate slowly in the sample zone. When the analyte

reaches the lower ionic strength background buffer, their migration speed increases which leads to band broadening. Several approaches have been described to mediate this issue with on-capillary pH-mediated acid stacking [34, 35].

The physiological cell buffer Hank's balanced salt solution (HBSS) was selected for this study, and the separation buffer was 25 mM TES sodium salt. In order to increase the resolution, 25 mM SDS was also added to the separation buffer. The results of this study can be seen in Fig. 6.

The DA and NE sample used in Fig. 6A was prepared in the same buffer utilized in the separation, so no stacking or de-stacking effects influenced the separation. The calculated resolution for this condition was 1.6. In Figure 6B, the DA and NE were prepared in the physiological buffer HBSS, which contains about 150 mM of several biological salts. Since HBSS has a much higher ionic strength than the 25 mM TES/25 mM SDS separation buffer, separations of samples prepared in this manner are subject to de-stacking effects. This was observed in the separation shown in Figure 6B, as the calculated resolution was 0.9. Despite the de-stacking effects and the pressure-induced band broadening, it was possible to obtain resolution such that separate quantitation of DA and NE is possible.

Conclusion

This work demonstrated the successful modification of a 3D-printed Wall-Jet Electrode device for capillary electrophoresis and electrochemical detection. The system was robust, due to the alignment being facilitated by 3D-printing, and the use of pressure-based flow for elution reduced the interference of field effects and improved the level of baseline noise. Additionally, this system was compatible with a Beckman Coulter commercial CE instrument, further facilitating the use of this device. A mixture of four catecholamines were efficiently separated by the system in conjunction with the commercial CE instrument. We have included all .STL and .CAD files for any interested researchers to utilize.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Configuration of experimental set-up. A) Complete set-up of detection device with manual injection system B) CAD design of the 3D-printed WJE device, showing the position of the capillary inlet and the 100 μm gold working electrode. C) Alignment of 50 μm i.d. capillary with 100 μm gold electrode (29 μm gold pillar). D) Fluorescein plug exiting capillary, flow can be seen diverging at the electrode. Fluorescein appears to be outside the limits of the channel due to imaging through the translucent Full Cure 720 material.

Figure 2. Set-up of 3D-printed detection device coupled with commercial capillary electrophoresis instrument. The 3D-printed device can be coupled to the commercial instrument for injections and electrophoretic separation, and then connected to the potentiostat for electrochemical detection.

Figure 3.

Comparison of baseline noise in a (A) 50 μm i.d. and (B) 25 μm i.d. capillary. In each trial, the WJE system was loaded with 25 mM boric acid buffer at pH 9.2. The capillaries used were 33 cm. Platinum wires immersed in the device's reservoirs simultaneously served as the electrical ground and the pseudo-reference electrode. With the smaller 25 μm i.d. capillary, baseline noise was reduced. Additionally, the noise level decreased with distance to the electrode. Without any interference from high voltage, the noise was lowest in both capillaries when using pressure mobilization.

Figure 4.

Comparison of resolution for varying field strengths during electrophoretic separation of dopamine and norepinephrine on a 33 cm capillary. Pressure injections of 25 μ M catechol and dopamine were applied for 10 seconds and 1 psi, followed by electrophoresis of variable field strength and then elution by 1 psi pressure. The results indicate that an increase in field strength results in increased separation efficiency. The electropherograms were offset manually to facilitate visual comparison, so the change in current is reported.

Figure 5.

Comparison of the separation of dopamine, norepinephrine, catechol, and 3,4-dihydroxyphenylacetic acid using (A) pressure elution and electrochemical detection and (B) electrophoresis and UV detection. Pressure injections of 50 μ M DA, NE, Cat, and DOPAC were performed for 4 seconds at 4 psi followed by 480 seconds of electrophoresis at 270 V/cm. The capillary utilized for the electrochemical detection was 100 cm, and the capillary utilized for the UV detection was 110 cm with 100 cm to window. Electrochemical measurements were carried out at +0.9 V vs. platinum pseudo-reference.

Figure 6.

The separation of dopamine (DA) and norepinephrine (NE) prepared in (A) 25 mM TES sodium salt and 25 mM SDS (B) Hank's balanced salt solution (HBSS) carried out on a 100 cm 50 μm i.d. fused silica capillary are shown. (A) 100 μM DA and NE samples were prepared in a 25 mM TES sodium salt and 25 mM SDS buffer at pH 7.4, and the separation was carried out in 25 mM TES sodium salt with 25 mM SDS. Pressure injections were performed for 10 s at 8 psi. Voltage with a field strength of 270 V/cm was applied for 10 min followed by 8 psi pressure mobilization. Electrochemical measurements were carried out at +0.9 V vs. pseudo platinum reference. (B) 100 μM DA and NE samples were prepared in HBSS and the separation was carried out in a 25 mM TES sodium salt and 25 mM SDS buffer at pH 7.4. The same separation conditions were utilized.

Table 1.

Width at half-height ($W_{1/2}$) and theoretical plate calculations for dopamine (DA), norepinephrine (NE), catechol (Cat) and 3,4-dihydroxyphenylacetic acid (DOPAC).

	DA (EChem)	DA (UV)	NE (EChem)	NE (UV)	Cat (EChem)	Cat (UV)	DOPAC (EChem)	DOPAC (UV)
$W_{1/2}$ (s)	4.75	4.85	5.69	5.82	5.46	7.46	6.94	10.78
Plates	65,750	54,600	48,700	45,000	62,900	53,550	43,900	49,800