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Electroosmotic flow and its contribution to iontophoretic delivery

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Abstract

Iontophoresis is the movement of charged molecules in solution under applied current using pulled multi-barrel glass capillaries drawn to a sharp tip. The technique is generally non-quantitative, and to address this, we have characterized the ejection of charged and neutral species using carbon-fiber electrodes attached to iontophoretic barrels. Our results show that observed ejections are due to the sum of iontophoretic and electroosmotic forces. Using the neutral, electroactive molecule 2-(4-nitrophenoxy) ethanol (NPE), which is only transported by electroosmotic flow (EOF), a positive correlation between the amount ejected and the diameter of each barrel's tip was found. In addition, using various charged and neutral electroactive compounds we found that, when each compound is paired with the EOF marker, the percentage of the ejection due to EOF remains constant. This percentage varies for each pair of compounds, and the differences in mobility are positively correlated to differences in electrophoretic mobility. Overall, the results show that capillary electrophoresis (CE) can be used to predict the percentage of ejection that will be due to EOF. With this information, quantitative iontophoresis is possible for electrochemically inactive drugs by using NPE as a marker for EOF.

Introduction

Iontophoresis is a technique in which substances are ejected from a micropipette under the influence of an electric field. It is popular among neuroscientists as it can be used for the delivery of specific drugs to highly localized regions of the brain without disrupting ongoing behaviors, a likely consequence of administering the drugs systemically[1-11]. Despite the advantages of this approach, it is difficult to determine the amount of drug ejected, and thus the technique is typically considered to be non-quantitative[12, 13]. Ejections have been evaluated by correlating the potency of a drug (as determined by other preparations) to various ejection currents[14]. Although this methodology can produce adequate dose-response curves, it assumes that the drug acts with similar potency in the brain of an intact animal as in other preparations such as brain slices and cell cultures. This assumption is likely not valid due to the complex chemistry of the brain.

During iontophoresis, ejection of charged substances is a consequence of two processes: electroosmosis and migration[15]. As such, the observed rate of ejection is governed by the observed linear velocity (v_{obs}) that is defined by

$$v_{obs} = v_{eo} + v_{ep} \quad (1)$$

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where v_{eo} is the velocity of electroosmotic flow and v_{ep} is the rate of migration. Each of these velocities is further dependent on the individual mobilities as defined by:

$$v_{\mathrm{eo}}=\frac{-\varepsilon\zeta}{\eta}\mathrm{E}=\mu_{\mathrm{eo}}\mathrm{E} \tag{2}$$

$$v_{\mathrm{ep}}=\mu_{\mathrm{ep}}\mathrm{E} \tag{3}$$

where μ_{ep} and μ_{eo} are the mobilities of migration and electroosmosis, respectively, E is the applied electric-field, ε is the permittivity, ζ is the zeta-potential formed at the glass capillary-solution interface, and η is the solution viscosity.

The role of electroosmosis on the ejection of species from iontophoretic barrels has been controversial. Early experiments were unable to confirm a major contribution of electroosmosis[16]. In contrast, Szabadi and co-workers used radioactivity measurements to show that the neutral molecule glucose could be ejected iontophoretically with an efficiency that was 23% of the ejection of norepinephrine, a monovalent cation under their conditions[17]. Furthermore, electroosmosis has also been documented during transdermal iontophoresis[18-22].

When electroosmosis is ignored, ejection is due solely to migration and the number of moles (M) ejected is defined as:

$$M=\mathrm{n}\frac{iT}{zF} \tag{4}$$

where n is the transport number (a number empirically determined that describes the percentage of total current that the compound of interest carries), i is the applied current, T is the time, z is the charge, and F is Faraday's constant[23]. From this relationship, one expects that if the transport number is known, quantitative predictions of the amount ejected can be made by controlling the applied current and time. In practice, great variability exists in the amount ejected from barrel to barrel despite using the same applied current, time, and solution conditions. Consequently, a direct measurement of the amount ejected is necessary for quantitative analysis, since the relationship given in equation (4) is an incomplete description of mass transport during iontophoresis.

Numerous approaches have been used to quantify iontophoresis directly using techniques such as fluorescence, radioactivity, and electrochemistry[14, 16, 17, 24-36]. For example, ion-selective microelectrodes were used to monitor ejections of acetylcholine from nearby iontophoretic pipettes. This was done by measuring changes in potential at the ion-selective electrodes due to the introduction of acetylcholine to the bath solution. The study showed that the rate of acetylcholine ejection varied for different iontophoresis barrels, thus requiring calibration for each pipette barrel[29]. Purves did extensive work using a cationic fluorescent molecule, quinacrine, which showed similar results. He also showed that release from iontophoretic pipettes is time-dependent and is influenced by “the history of the pipette” – meaning it is affected by the retaining current applied previous to ejection[13, 29, 33-35]. Carbon-fiber microelectrodes have been used to effectively monitor the ejection of catecholamines in vitro and in vivo, but this technique is limited to electroactive substances[24-26].

In the present work, we further examined the role of electroosmosis in iontophoresis using carbon-fiber microelectrodes to monitor the ejection of electroactive compounds as described by Millar et al[24-26]. By using neutral and charged compounds with distinct electrochemical signals we monitored the rate of ejection for each separately. This approach allowed us to determine the relative contribution of electroosmosis to the ejection of various charged compounds. Our findings indicate that electroosmosis plays a significant role, accounting for over 30% of the total ejection observed for cationic compounds and 80% for anionic compounds. For each compound tested, the rate of electroosmotic flow (EOF) relative to the observed ejection remained constant, suggesting that the variability in the absolute amount ejected from different pipette barrels is due to changes in the amount of EOF present. Furthermore, differences in iontophoretic mobility were correlated with differences in electrophoretic mobility, as determined by capillary electrophoresis.

Experimental Section

Chemicals

Unless noted, all chemicals were purchased from Sigma-Aldrich (St. Louis, MO) and used as received. Solutions were prepared using doubly distilled deionized water (Megapure system, Corning, NY). A physiological buffer solution, pH 7.4, (15 mM TRIS, 126 mM NaCl, 2.5 mM KCl, 25 mM $NaHCO_3$, 2.4 mM $CaCl_2$, 1.2 mM NaH_2PO4, 1.2 mM $MgCl_2$, 2.0 mM Na_2SO_4) was used in all electrochemical experiments.

Iontophoresis Probes

Multi-barrel (3 or 5) probes were constructed by fusing a single-barrel glass capillary (A-M Systems, Carlsborg, WA) to a multi-barrel (2 or 4) glass capillary that contain filaments that aid in filling the barrel by capillary action (A-M Systems, Carlsborg, WA and Stoelting Co., Wood Dale IL). The single barrel was loaded with a carbon fiber (T-650, Thornel, Amoco Corp., Greenville, SC). The capillaries were bundled together with heat shrink and were tapered to a tip of about 1 μm in diameter using a micropipette puller (Narashige, Tokyo, Japan) with a two-step twist and pull process. The protruding carbon fiber was cut to a length between 30 and 50 μm. Before use, barrels containing the carbon fiber were backfilled with electrolyte (4 M potassium acetate, 150 mM potassium chloride) and fitted with wires for electrical contact. A silver/silver chloride electrode served as the reference. Empty barrels for iontophoresis were filled with analyte solutions prepared in 5 mM NaCl at pH 5.8. An electron micrograph and drawing of the assembly with 2 and 4 iontophoretic barrels is shown in Figure 1.

Data Acquisition

Cyclic voltammograms were acquired using data-acquisition hardware and local software written in LabVIEW (National Instruments, Austin, TX). The cyclic voltammetry waveform was generated and the voltammetric signal was acquired with an A/D, D/A board, the PCI-6052E (National Instruments). A PCI-6711E D/A board (National Instruments) was used to synchronize waveform application, data acquisition, and to trigger the iontophoretic current applied and the loop injector in the flow injection apparatus. The voltammetric waveform was input into a custom-built instrument for application to the electrochemical cell and current transduction (University of North Carolina at Chapel Hill, Department of Chemistry Electronics Facility). After collection, background subtraction, signal averaging, and digital filtering (low pass filtered at 1 kHz) were all done under software control.

For most experiments, a triangular waveform was applied with a scan rate of 200 V s^{-1} with a rest potential of -0.7 V versus Ag/AgCl between scans, an initial scan to -1.3 V, followed by an excursion to 1.0 V, and a scan to the rest potential. The scans were repeated every 100 ms. These parameters were chosen to maximize separation of peaks for the analytes being studied.

Iontophoresis Ejections

Multi-barrel iontophoresis probes with a carbon-fiber microelectrode (Figure 1) were used to eject and detect the analytes that were made up as 10-12 mM solutions in 5 mM NaCl at pH 5.8 unless otherwise noted. Positive currents (average of 5 to 40 nA) delivered by a constant current source designed for iontophoresis experiments (Neurophore, Harvard Apparatus, Holliston, MA) were used to eject the various analytes. For each barrel, an appropriate ejection current was determined by first performing 30 second test ejections until a signal sufficient for analysis was measured (average of -5 to -30 nA at the reduction potential of NPE). In some of the experiments, a retaining current of -5 nA was applied between ejections. For all remaining experiments, a current of 0 nA was applied between ejections.

Flow Injection Apparatus

The electrode was positioned at the outlet of a six-port rotary valve[37]. A loop injector was mounted on an actuator (Rheodyne model 7010 valve and 5701 actuator) that was used with a 12-V DC solenoid valve kit (Rheodyne, Rohnert Park, CA) to introduce the analyte to the surface of the electrode. The linear flow velocity (1.0 cm s^{-1}) was controlled with a syringe infusion pump (Harvard Apparatus model 940, Holliston, MA).

Special considerations were necessary because oxygen can be detected with the waveform used. Therefore, PEEK® tubing (Upchurch Scientific, Oak Harbor, WA) and glass syringes were used to limit oxygen interference, and all solutions were degassed with nitrogen before use.

HPLC Experiments

Iontophoretic ejections were made into a 500 μL volume of 0.1 N perchloric acid. HPLC injections (10 μL) were made onto a reverse phase column (C-18, 5 μm, 4.8 × 250 mm, Waters symmetry 300). The mobile phase (prepared in HPLC grade water) contained 0.1 M citric acid, 0.1 mM EDTA, and 1 mM hexyl sodium sulfate, pH 3.5 and 10% organic modifier (methanol). Analytes were detected with a thin-layer radial electrochemical flow cell (BASi, West Lafayette, IN USA), with the working electrode at 0.7 V vs. the Ag/AgCl reference. Data was collected using a custom written LabVIEW program (Courtesy of Professor James Jorgensen, UNC-CH). Peak areas were calculated using statistical moments regression (Igor Version 5.0). Differences in detection efficiency were normalized for by using a response ratio for each analyte.

Capillary Electrophoresis Experiments

These were performed using a HP[3D]CE system (Agilent Technologies) equipped with an on-column diode-array detector, an auto-sampler and a 30 kV power supply. CE Chemstation (Agilent Technologies) was used for CE control, data acquisition and handling. The separation was performed in a 50 μm fused silica capillary 96.0 cm in total length, and 87.5 cm to the UV detector. All experiments were carried out in cationic mode (the anode at the inlet and cathode at the outlet). Samples were run at a concentration of 2 mM in 17 mM PBS (phosphate buffered saline, made up of 0.25% monosodium phosphate and 0.04% disodium phosphate) with a pH of 5.8 to keep constant with the buffer conditions used for the iontophoresis experiments. UV detection was measured at 195 and 240 nm. The electrophoretic mobility was calculated as:

$$\mu_{ep}=\left(\frac{L}{t_r}\right)\left(\frac{L_t}{V}\right) \quad (5)$$

where L is the distance from the inlet to the detection point, t_r is the time required for the analyte to reach the detection point, V is the applied voltage, and L_t is the total length of the capillary[38].

Results and Discussion

Construction of carbon-fiber iontophoresis probes

Initially, probes were constructed using only multi-barrel glass by loading one barrel with a carbon fiber and then pulling the assembly to a fine tip. Although this approach provided adequate results, it had two disadvantages. First, multi-barrel glass comes either with or without filaments. The filaments are needed for backfilling solutions into the barrels for iontophoresis. However, when the carbon fiber is placed in one of these filament-containing barrels, it can reduce the quality of the carbon fiber- glass seal which will increase the magnitude of the cyclic voltammetric background current. Second, some of these probes exhibited electrical cross-talk between the iontophoresis barrels and the barrel containing the carbon-fiber. We believe this is due to the thin glass that separates the barrels, which when filled with ionic solutions, allows capacitive coupling.

To remedy these problems, we modified our construction techniques by using multi-barrel, filament glass for iontophoresis, and then fusing a separate single barrel capillary to it (without a filament) for electrochemical detection. This improved the carbon-fiber glass seal and electrical crosstalk was much less likely to be seen. This procedure allows fabrication of any number of configurations using 2, 3, and 4-barrel glass, and we have seen no difference in performance. Images of a 2 and 4-barrel capillary fused to single capillary containing a carbon-fiber microelectrode are shown in Figure 1.

Quantitative iontophoresis of electroactive compounds

We were able to monitor iontophoretic ejections in real-time by coupling iontophoresis with fast-scan cyclic voltammetry (Figure 1). Figure 2 shows consecutive ejections of 10 mM dopamine with an applied iontophoretic current of 10 nA and a retaining current of -5 nA. The ejections are fairly reproducible and show fast time responses. As seen from the cyclic voltammogram, the expected signature peak from the oxidation of dopamine occurs at around 0.6 V. After calibration, the oxidation current was converted to dopamine concentration, which shows that for 10 nA of iontophoretic current, the signal at the carbon-fiber surface is equivalent to that found in a 5 μM dopamine solution. In reality, the concentration along the electrode length varies with the concentration (10 mM) of dopamine within the barrel at the micropipette tip, to progressively lower concentrations down the length of the cylindrical electrode. This concentration profile approximates the dilution expected for ejection from a point source[39].

To characterize the role of electroosmosis in iontophoretic ejections, we chose a neutral, electroactive compound to serve as a marker for EOF. Considerations such as pK_a, solubility in water, and lack of voltammetric overlap with dopamine limited the options available. The pK_a determines whether the molecule is neutral at the pH values used during experimentation. Solubility in water is essential because it is the preferred solvent for making up drug solutions typically used in iontophoresis; other solvents can affect EOF and may also be toxic when used in biological experiments. The EOF marker must be voltammetrically different from dopamine so that the signals from each can be resolved. With all of these prerequisites in mind, we chose 2-(4-nitrophenoxy) ethanol as the EOF marker for the experiments performed with FSCV (Figure 3).

Figure 4 illustrates iontophoretic ejections of a mixture of dopamine and NPE at equal concentrations (10 mM). The pH of this solution is 5.8, at which NPE is neutral and dopamine

is a monocation. Ejection of both of these compounds confirms that EOF plays a role in iontophoresis since the only mechanism for ejection of NPE is electroosmosis. Much like the results for dopamine, ejections of NPE are reproducible and rapid. As seen from the cyclic voltammogram, the oxidation of dopamine can be seen at around 0.6 V, whereas the reduction of NPE can be seen at about -1.2 V, facilitating the quantification of both species. Because dopamine is charged, its ejection should be due to iontophoretic forces and electroosmosis; consistent with this expectation more dopamine (~14 μM) than NPE (~6 μM) is detected at the electrode surface.

Variability in iontophoresis

Although ejections are reproducible for a given barrel (demonstrated in Figures 2, 3, and 4), there is substantial variability when ejections from various barrels are compared to one another for a given iontophoretic current. The top panel of figure 5 shows NPE ejections from 5 different iontophoretic barrels containing the same solution (10 mM NPE). The apparent concentration recorded with the cylindrical electrode is linear with ejection current. The difference in the slope of the 5 lines indicates that each barrel is ejecting at a different rate.

Previous experiments by Bradley and Candy compared the amount of ejected cations for large and small diameter pipette tips[40]. Their results showed that the larger diameter pipettes ejected more for a fixed ejection current than small diameter pipettes. To examine whether this is the cause of variability in our experiments, the tip diameter of the iontophoretic barrels used was estimated from scanning electron microscope (SEM) images of the probes that were employed. One of the barrels contained a carbon fiber, while one of the other barrels was used to eject either dopamine or NPE. The concentration of dopamine or NPE detected during ejection followed similar behavior, and showed a correlation with the diameter of the ejection tip (Figure 5). Since the ejection of both neutral and charged species depends on the electric field (E) at the tip (equations 2 and 3), the variation likely arises because E is a function of the tip geometry. However, because we have not characterized the collection of ejected amounts with different dimensions of the iontophoretic barrel, a quantitative interpretation cannot be made. Even so, it is clear that the variability in tip dimensions leads to variability of the ejected amounts.

Quantification of electroosmosis and migration

The concentrations measured at the electrode surface are directly related to the flux at the iontophoretic tip and indicate the individual contributions from iontophoresis and electroosmosis. The pH of the solution in the barrel is critical because it will determine the charge of the compound in solution and the extent to which the silanol surface groups on the glass capillaries are ionized. To demonstrate the importance of the latter, the relative iontophoretic delivery of dopamine, a cation at pH values below pH ~7.4, and NPE, a marker for EOF, were compared from solutions of pH 5.8 and 4.0. Decreasing the pH increases protonation of the silanol groups and should decrease the ζ potential, thus decreasing the contribution of EOF. Consistent with this, the relative amount of dopamine ejected was greater at pH 4.0 due to the decreased transport of NPE by EOF.

To characterize the role of electroosmosis for other molecules, NPE was used as the marker for EOF at pH 5.8, and it was paired with a set of neutral and charged molecules that exhibit electrochemistry similar to that of dopamine shown in Figures 2 and 4. Acetaminophen (AP) and hydroquinone (HQ), which are both neutral at pH 5.8, traveled at the same speed as the EOF marker, NPE. As expected, charged molecules uric acid, norepinephrine, and dopamine, moved at rates that were significantly different from EOF. It might seem surprising that uric acid, an anion at the pH used, can be ejected with a positive current. This arises because electroosmosis effectively competes with the iontophoretic mobility that carries uric acid in the opposite direction. Norepinephrine and dopamine, which are cations, have transport rates

that are enhanced by their respective iontophoretic mobilities. Importantly, over a series of at least 5 barrels, the ratio between each compound tested and NPE remained reasonably constant as indicated by the standard deviations. That is, the fraction of the observed ejection due to EOF did not vary, despite fluctuations in the absolute amount of compounds ejected. Thus, consistent with the results in Figure 5B, variations in EOF arising from differences in the tip diameter are the origin of the variability in the amounts ejected of both neutral and charged compounds.

The differences in mobility observed in our iontophoresis experiments match differences in electrophoretic mobilities observed in published data[41] and are confirmed by our own experiments as demonstrated in figure 6. As such, it is possible to use electrophoretic mobility data in conjunction with iontophoresis data to make predictions of the rate of transport relative to EOF for compounds that are not electroactive, but that can be detected in the UV. Such compounds include sulpiride and cocaine, both of which are important drugs frequently studied in our laboratory. Such an approach has been used to characterize transdermal iontophoresis and the zeta potential of brain slices in rats [42, 43]. Although our experiments were run in the presence of 5 mM NaCl, electroosmosis is still an effective transport mechanism with much higher ionic strength.

Since iontophoretic ejections generate high, localized concentrations of analyte near the point of ejection, there was concern that quantitative measurements of the amount ejected may be convoluted due to differences in electrode response along its length. To ensure that this would not present a problem, we used HPLC as an independent measure to confirm the results obtained with our carbon-fiber iontophoresis probes. With HPLC we can separate a mixture post-ejection, and quantitatively determine how much was ejected by oxidizing at +0.7 V vs. Ag/AgCl. For these experiments, 10 minute iontophoretic ejections of AP and HQ were made into a known volume of perchloric acid (500 μL). Analysis of the mixture showed that the rate of transport for HQ relative to AP was 1.04 ± 0.21. This result is expected for two neutrals, and is in agreement with those obtained with the carbon-fiber iontophoresis probe measurements using NPE.

Effects of retaining currents

Traditionally, a retaining current is applied between ejections during iontophoresis to control for diffusive leakage. Given the tip size of our iontophoresis probes, we hypothesized that retaining current may not be necessary. To test this, we performed a series of dopamine ejections at various retaining currents. Initially the barrel was held at -10 nA for 6 minutes, and then the current was progressively increased to more positive currents and held at that current for another 6 minutes. During each of these ejection currents, the amount of dopamine and NPE ejected was monitored. If the electrodes were leaking between ejections, it is predicted that some ejection would be seen when the current was changed from -10 nA to -5 nA to -2 nA to 0 nA, since at each transition there would be less retaining current to control for leakage. As seen in Figure 7 this was not the case. In each of those transitions, no ejection was observed. Ejection was only observed when the current switched from 0 nA to 3 nA, indicating that no retention current is necessary, and holding at 0 nA is sufficient.

Considerations for *in vivo* use

Carbon-fiber microelectrodes have been routinely used to study fluctuations of catecholamines in the brain of anesthetized and freely-moving rats[44]. Carbon-fiber iontophoresis probes have also been used to deliver drugs to localized regions, and subsequently detect the changes induced in catecholamine release with electrochemistry[1]. However, quantitation of the amounts ejected has remained a challenge, except in the rare cases when the drugs used are electroactive. Since electroosmosis seems to be the transport mechanism most affected by the

variations present from barrel to barrel, it makes sense to use a marker for EOF as an indicator of the variability present. In addition, because the relationship between EOF and migration of charged compounds remains constant, the marker for EOF can be used as an internal standard to calibrate for the amounts of non-electroactive drugs ejected.

This methodology can be readily used *in vivo* with any electroactive neutral molecule, such as NPE, acetaminophen, or hydroquinone. It is important to keep in mind that the electrochemistry of some of these compounds may overlap with that of catecholamines. As such, if the experiment requires continuous ejection and detection of released cathecholamines, it would be advantageous to use NPE which does not overlap. Toxicity remains a concern, although given the localized nature of iontophoretic ejections and the effects of dilution, it is expected that this is minimal.

Conclusions

A long-standing disadvantage of iontophoresis has been its non-quantitative nature. Using carbon-fiber iontophoresis probes we have shown that the main challenge with quantitating iontophoretic ejections is the variability observed from barrel to barrel due to varied tip dimensions that affects electroosmosis. Using NPE, AP, or HQ as neutral markers for EOF, it is now possible to quantitate this variability. In addition, the positive correlation observed between electrophoretic mobility and iontophoretic mobility relative to our EOF markers enables us to extend this methodology and make quantitative predictions for compounds that are not electroactive, but that can be measured by other detection schemes used with CE such as UV or fluorescence. We plan to use this approach for quantitative iontophoresis applications in the study of neurotransmitter release and modulation in the brain of anesthetized and freely-moving rats.

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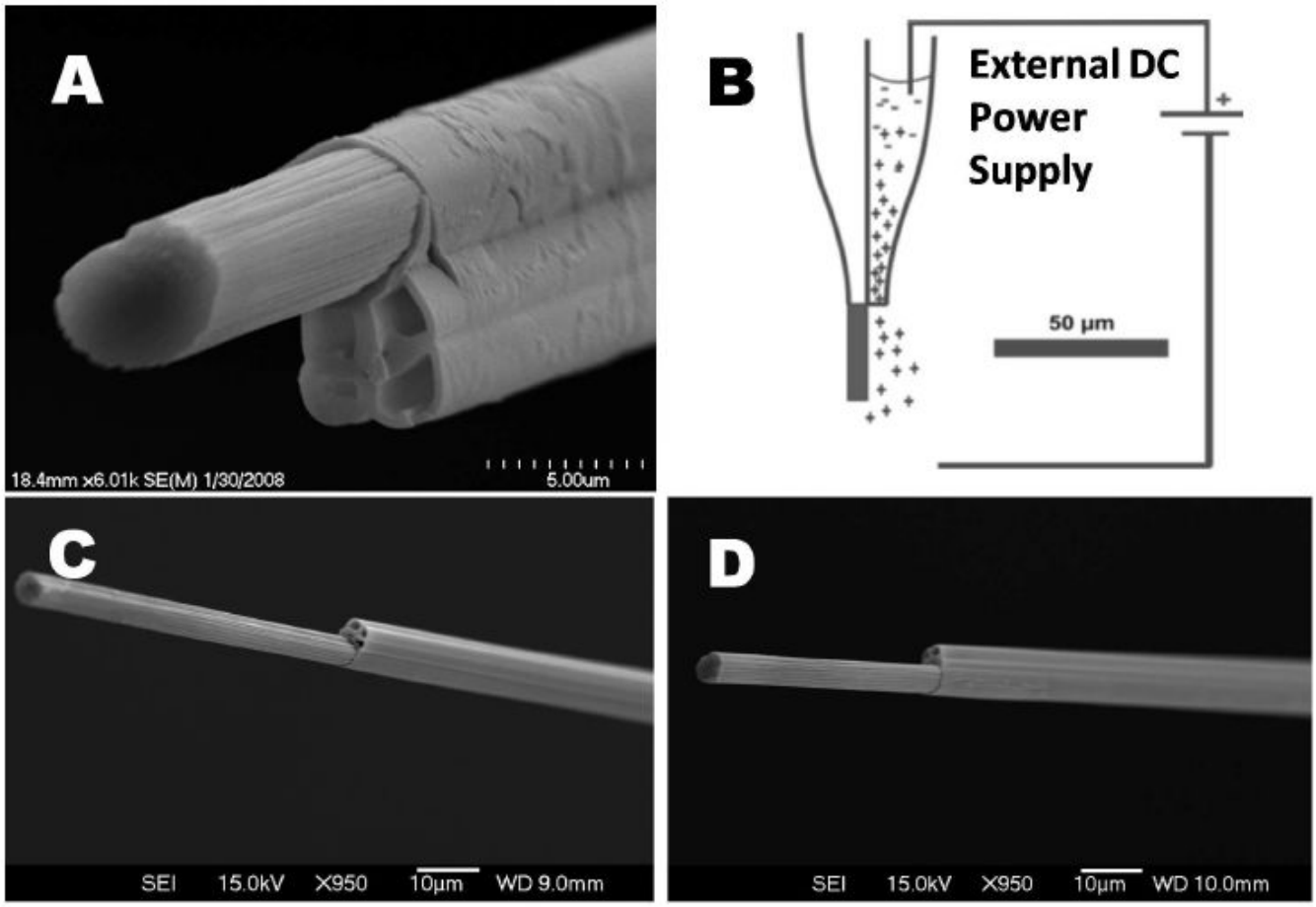

Figure 1.
SEM images and schematic of carbon-fiber iontophoresis probe. A) Close up showing the empty iontophoretic barrels of a 5-barrel probe. B) a DC power source is used to apply positive or negative current to solution-filled barrels for iontophoretic ejection. C-D) Carbon-fiber iontophoresis probes made by fusing a single barrel containing a carbon fiber to either 4 (shown in C) or 2 (shown in D) empty barrels.

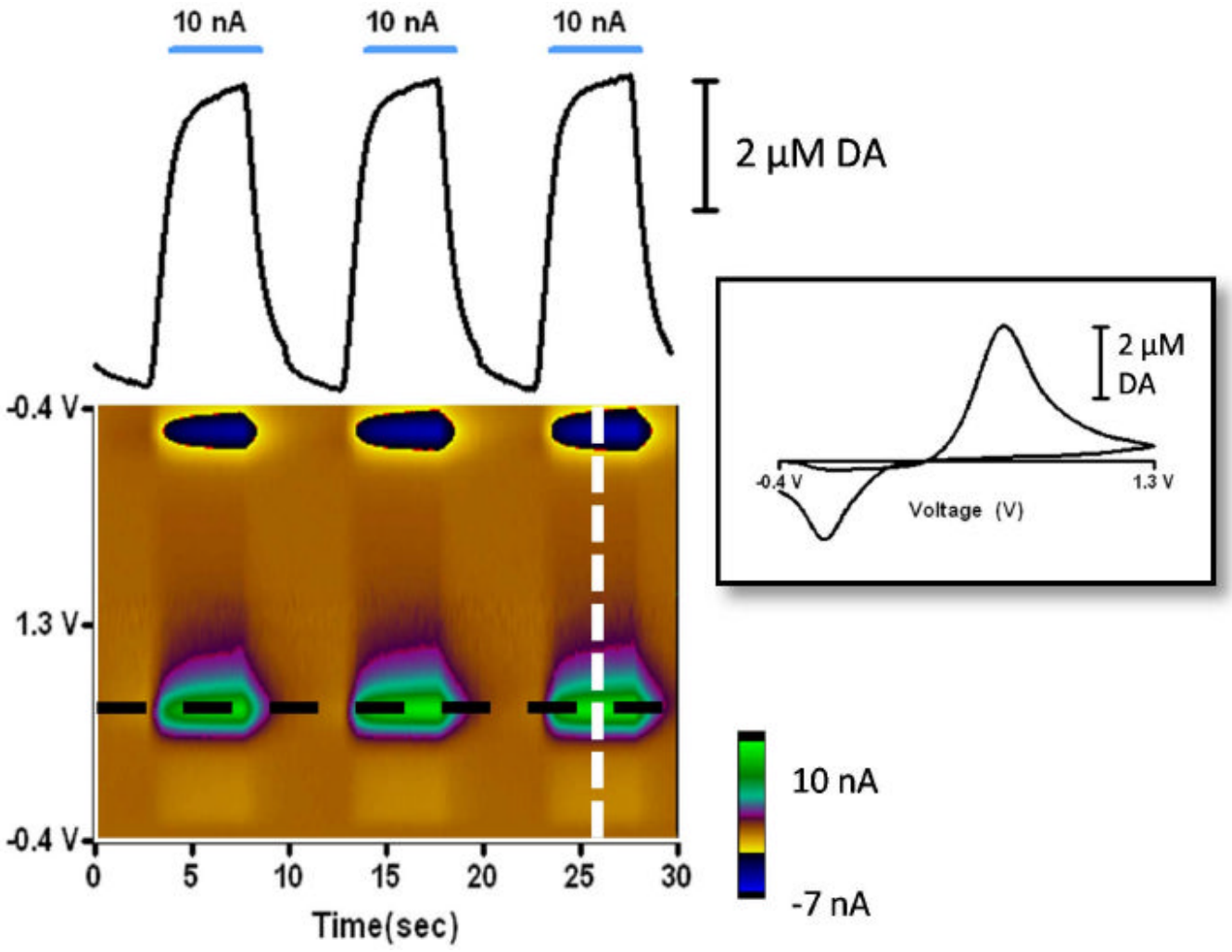

Figure 2.

Analysis of consecutive iontophoretic ejections of dopamine. Carbon-fiber iontophoresis probes were used to eject a solution of dopamine cations at pH 5.8 into a solution of TRIS buffer at pH 7.4. Upper left: Solid line represents the concentration of dopamine measured at its oxidation potential of 0.6 V vs. time trace obtained for three consecutive 10 nA ejections at times indicated by the horizontal bars. Lower left: Color representation of the iontophoretic ejections shown, with applied voltage at the carbon fiber plotted vs. time and the current measured in response to dopamine ejection plotted in false color. Right: Cyclic voltammogram obtained at the time indicated by the white dashed line in the color plot.

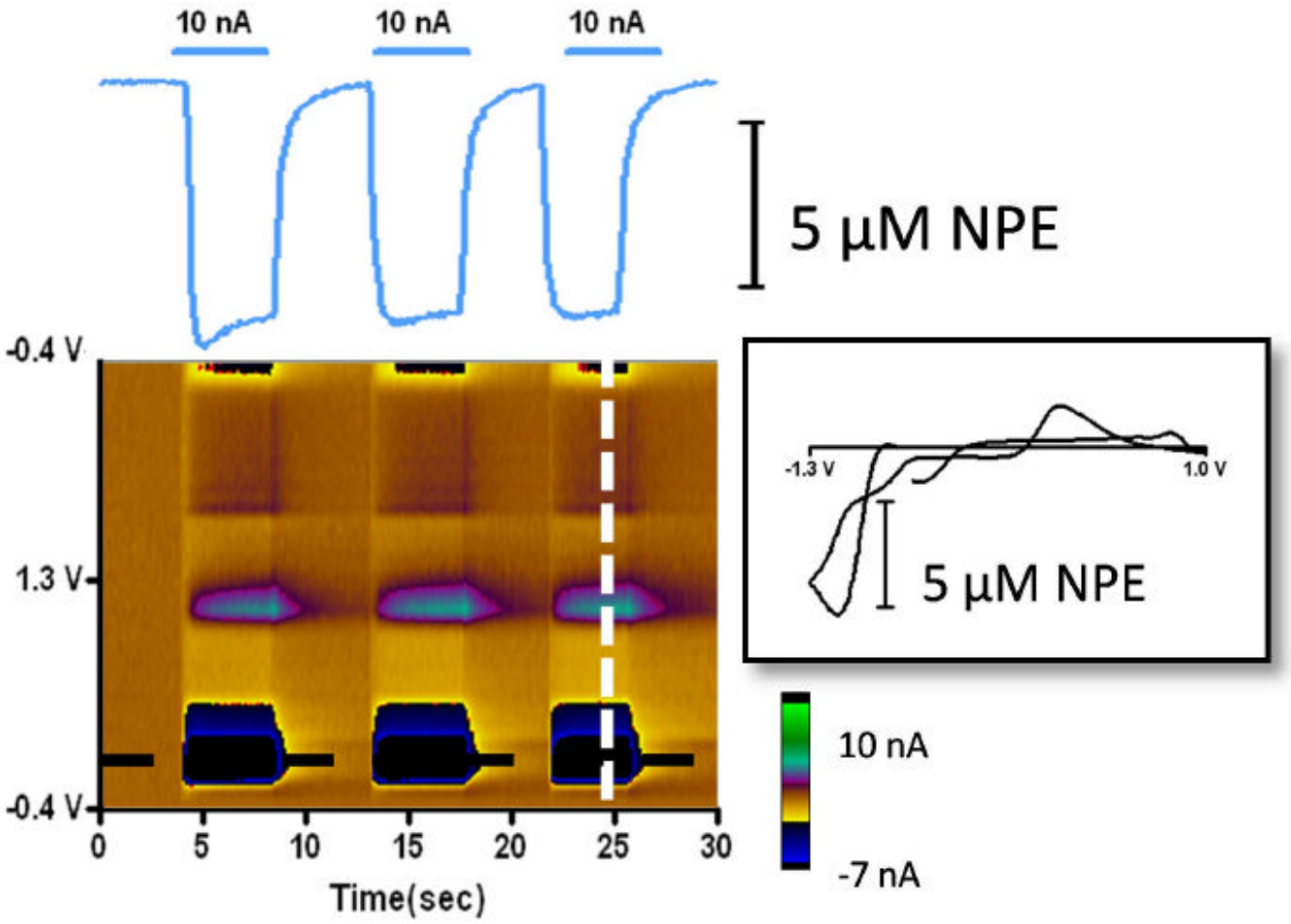

Figure 3.

Analysis of consecutive iontophoretic ejections of a neutral marker compound, NPE. Carbon-fiber iontophoresis probes were used to eject a solution of NPE at pH 5.8 into a solution of TRIS buffer at pH 7.4. Upper left: The solid line represents [NPE] at -1.2 V (light blue solid line) measured vs. time trace obtained for three consecutive 10 nA ejections at times indicated by the horizontal bars. Lower left: Color representation of the iontophoretic ejections shown, with applied voltage at the carbon fiber plotted vs. time and the current measured in response to NPE ejection plotted in false color. Right: Cyclic voltammogram obtained at the time indicated by the white dashed line in the color plot.

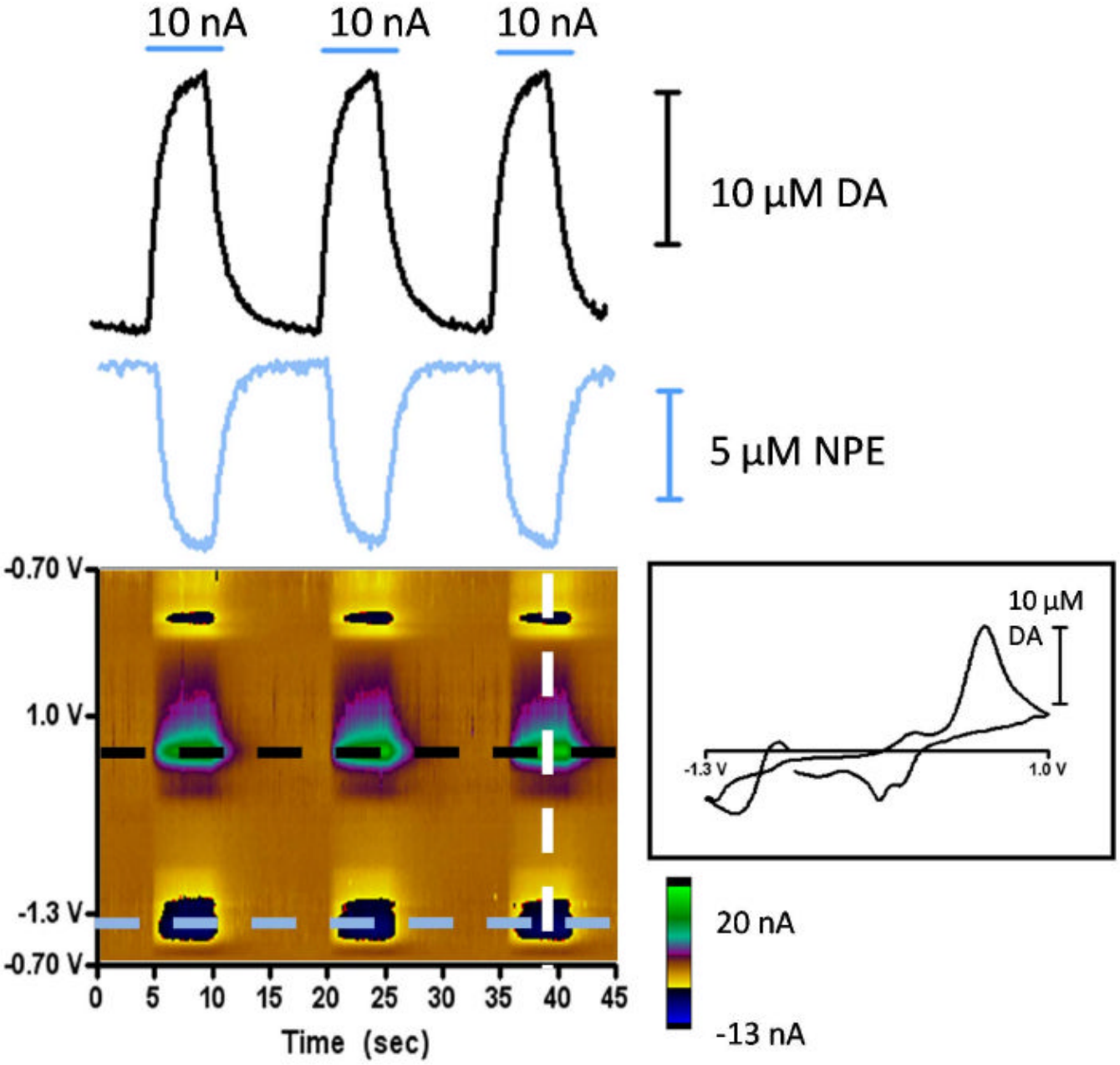

Figure 4.

Analysis of consecutive iontophoretic ejections of dopamine and NPE. Carbon-fiber iontophoresis probes were used to eject a mixture of dopamine and NPE at pH 5.8 into a solution of TRIS buffer at pH 7.4. Upper left: The solid lines represent [DA] at 0.6 V (black) and [NPE] at -1.2 V (light blue) measured vs. time trace obtained for three consecutive 10 nA ejections at times indicated by the horizontal bars. Lower left: Color representation of the iontophoretic ejections shown, with applied voltage at the carbon fiber plotted vs. time and the current measured in response to dopamine and NPE ejection plotted in false color. Right: Cyclic voltammogram obtained at the time indicated by the white dashed line in the color plot.

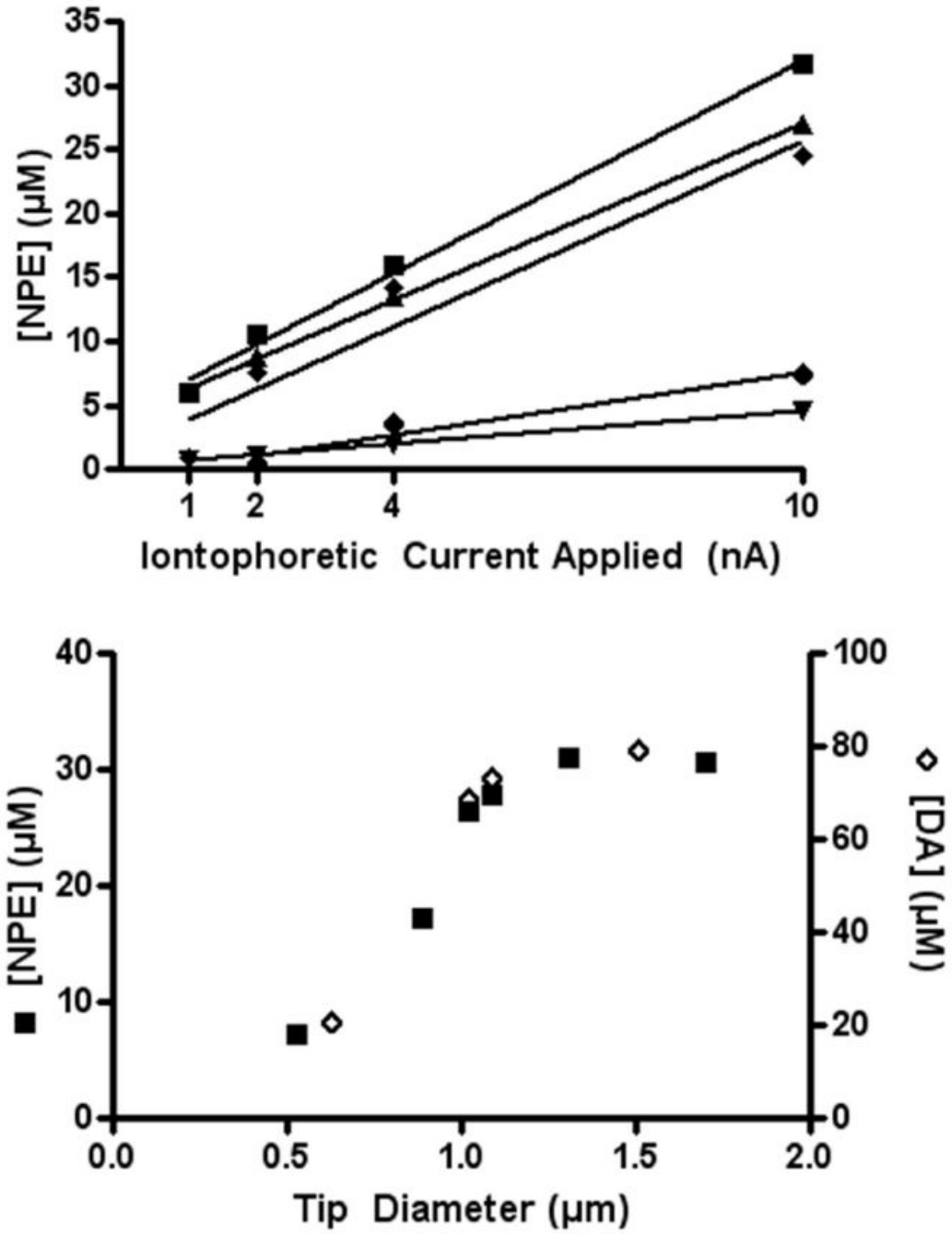

Figure 5.

Variability of iontophoretic ejections for different barrels. Upper: 5 different probes were used to eject a mixture of dopamine and NPE at currents ranging from 1 -10 nA into TRIS buffer. Each line represents the linear dependence of applied current to the amount of NPE ejected, as well as the difference in ejection efficiency for each barrel. (Dopamine showed an identical relationship, although not shown here). Lower: Different probes were used to eject dopamine or NPE into TRIS buffer using a 10 nA ejection current. After the ejections, SEM images of the probes were obtained to estimate the tip diameter of the iontophoresis probes. Plotted is the relationship between the amount of NPE (solid squares) and dopamine (empty diamonds) ejected at 10 nA and the diameter of tip.

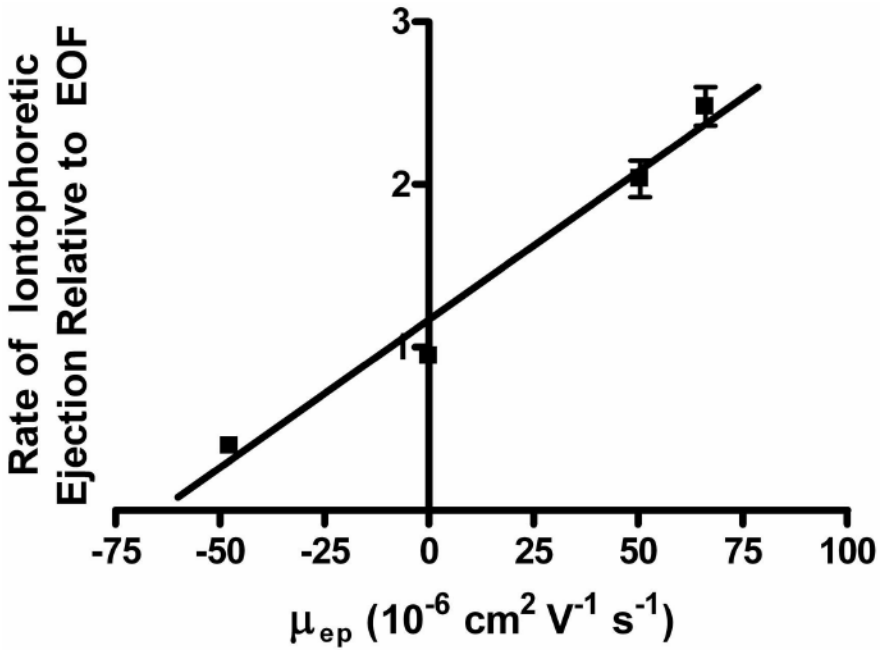

Figure 6.

Correlation between iontophoretic and electrophoretic mobility. Iontophoretic mobility was determined as a ratio of the amount ejected by iontophoretic and electroosmotic forces to the amount ejected by electroosmotic forces only. This was done by ejecting a mixture of a charged compound (DA, NE, or UA) with a neutral marker, NPE. The amount of the charged compound ejected corresponded to the amount ejected by iontophoretic and electroosmotic forces, and the amount of NPE ejected was taken as the rate of EOF. Electrophoretic mobilities were obtained for each of these compounds using Equation 5 and plotted against the rate of transport relative to EOF given in Table 1.

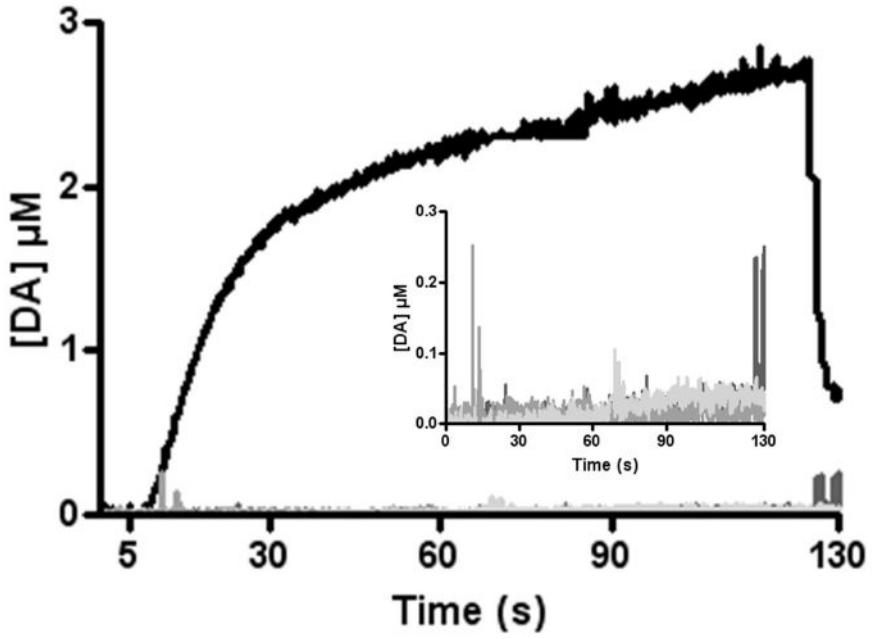

Figure 7.

Effects of retention current on iontophoretic ejections. Carbon-fiber iontophoresis probes were used to ejected dopamine cations after the application of retaining currents ranging from -10 nA to 0 nA. Each retaining current was applied for 6 minutes prior to the application of a higher ejection current for 6 minutes. The solid lines (amplified in the inset) represent the amount of dopamine ejected after the application of -10 nA retaining current followed by -5 nA (dark gray) ejection current, -5 nA retaining current followed by -3 nA ejection current (gray), and -3 nA retaining current followed by 0 nA (light gray). The black solid line shows the amount of dopamine ejected after holding the barrel at 0 nA for 6 minutes and then applying +3 nA for ejection current.

Table 1

Rate of iontophoretic delivery of charged compounds relative to EOF as measured by FSCV.

Compound Ejected by Iontophoresis	Concentration Ejected Relative to NPE
Dopamine (pH 4.0)	3.56 ± 0.27
Dopamine (pH 5.8)	2.57 ± 0.30
Norepinephrine (pH 5.8)	2.04 ± 0.27
Hydroquinone (HQ) (pH 5.8)	1.01 ± 0.02
Acetaminophen (AP) (pH 5.8)	0.95 ± 0.06
Uric Acid (pH 5.8)	0.49 ± 0.18

Carbon-fiber iontophoresis probes were used to deliver a mixture consisting of a charged compound (10 mM) and the EOF marker, NPE (10 mM), into 1X TRIS buffer (pH 7.4). The values given are the average of at least 6 different barrels for each of the compounds tested. For each barrel, 25 consecutive ejections were averaged at ejection currents ranging from 1 nA to 100 nA as needed to deliver concentrations of NPE in the 5-50 μM range.