**Imaging catalytic surfaces by multiplexed capillary electrophoresis with absorption
detection**

by

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ABSTRACT

A new technique for *in situ* imaging and screening heterogeneous catalysts by using multiplexed capillary electrophoresis with absorption detection was developed. By bundling the inlets of a large number of capillaries, an imaging probe can be created that can be used to sample products formed directly from a catalytic surface with high spatial resolution. In this work, we used surfaces made of platinum, iron or gold wires as model catalytic surfaces for imaging. Various shapes were recorded including squares and triangles. Model catalytic surfaces consisting of both iron and platinum wires in the shape of a cross were also imaged successfully. Each of the two wires produced a different electrochemical product that was separated by capillary electrophoresis. Based on the collected data we were able to distinguish the products from each wire in the reconstructed image.

CHAPTER 1. INTRODUCTION

Capillary Electrophoresis

Electrophoresis, as a separation technique, was first introduced in 1937 by Tiselius ¹ who demonstrated the electrophoretic separation of proteins. Virtanen ² provided one of the earliest demonstrations of the capillary electrophoresis using small diameter silk fibers for the determination of 100 pg of RNA contained within a single cell. Since the early 1980's, after Jorgensen and his colleagues laid down the foundation for running electrophoresis in a narrow bore ($< 100\ \mu\text{m}$) fused silica tube to separate charged species, ^{3, 4} capillary electrophoresis has begun to achieve scientific recognition and has been undergoing an explosive progress. Capillary electrophoresis offers some exciting features: 1) very small amount of sample is required for each analysis (a few nL), 2) extremely fast and highly efficient separations of both ionic species and neutral compounds can be performed, and 3) the capillary tube can be used to carry microreactions, after which separation and quantification are executed. A wide spectrum of samples can be analyzed by this technique. Small ions, as well as neutral molecules and large biological molecules can be separated and analyzed with different modes of capillary electrophoresis.

The separation mechanism is dependent on the electroosmotic flow and the electrophoretic migration of ions in the applied electric field. ⁵ The total velocity of each molecule is the vector sum of the electrophoretic velocity and the velocity imparted by the electroosmotic flow. All molecules that have a net velocity toward the detector will eventually be detected.

Today, capillary electrophoresis has become a collective name for a family of separation techniques that use capillaries as the separation channels and an electric field as the separation driving force. Depending on the analytes, several modes of capillary electrophoresis have been developed to perform quick and efficient analyses. The most common modes are open tubular or capillary zone electrophoresis (CZE),⁴ micellar electrokinetic capillary chromatography (MEKC),^{6,7} capillary gel electrophoresis (CGE), and capillary electrochromatography (CEC).⁸

To date, CZE has been the most popular capillary electrophoresis mode. The analytes in the capillary migrate under a high electric field and are separated based upon their differences in their mass to charge ratio. In CZE, separated analytes migrate as separate non-continuous zones. CZE has been used to separate a wide range of simple charged organic molecules, inorganic ions, and peptides.

Molecules and ions with similar mass to charge ratio, such as DNA and large peptides, have very similar electrophoretic mobilities in free buffer solution and are impossible to effectively separate by CZE. These analytes can be effectively resolved by CGE via a different separation mechanism.^{9, 10} In this technique, the capillary is filled with a polymer gel, which forms a sieving matrix that has characteristic pore sizes. Different size molecules penetrate the gel at different speeds and are separated. Various polymers, such as linear polyacrylamide and its derivatives,¹¹ poly(ethylene oxide),¹² polyvinyl alcohol,¹³ and polyvinylpyrrolidone,¹⁴ have been utilized for the analysis of nucleic acids and proteins.

MEKC was first introduced by Terabe^{7, 15} in 1984. Unlike CZE, MEKC allows for separation of both charged and uncharged molecules. In this mode of operation,

usually a certain surfactant is added to the buffer solution at a concentration above its critical micelle concentration, so that micelles are formed as a pseudo phase. To resolve neutral compounds, micelles are required to be charged and thus have an electrophoretic mobility. Neutral analytes are separated based on the differences in their partitioning between the solution phase and the micellar phase. Sodium dodecyl sulfate is the most popular surfactant used¹⁶ although a number of other surfactants have been studied.¹⁷ By choosing chiral micelles, several works^{6, 18, 19} demonstrated that MEKC can be used to separate chiral compounds such as D,L-amino acids and chiral drug molecules.

Capillary electrochromatography is a separation technique that is a hybrid of capillary HPLC and capillary electrophoresis. In CEC, solvent is driven through capillary tubes by electroosmotic flow generated by the applied electric field across the capillary instead of the hydraulic flow induced by pressure HPLC. The capillaries are packed with conventional HPLC stationary phase. The analytes are separated based on their different partition ratios between the electrolyte mobile phase and the packed stationary phase. With this technique, both neutral and ionic analytes can be separated.

Optical Detection in Capillary Electrophoresis

Detection of extremely small amounts of materials is always challenging for any detection method. The often nanoliter range of analytes used in capillary electrophoresis requires highly sensitive and fast response detectors, which do not disturb the electric field for running capillary electrophoresis. A number of detection methods have been shown to be effective and sensitive for capillary electrophoresis analysis. Absorbance and fluorescence detection will be discussed briefly in this section.

UV/visible Absorbance Detection. UV/visible absorbance detectors are the most commonly used detectors for capillary electrophoresis. As with HPLC, the primary reason for the popularity of the absorbance detection schemes is that the majority of compounds analyzed absorb somewhere in the UV or visible region. In general, light from a lamp is focused on the capillary. Light collected from the capillary is passed through a monochromator or a filter is used to isolate the specific wavelength for the absorbance measurement. The transmitted intensity is detected with a photodetector and digitized by a computer that is also used to convert the transmitted intensity to an absorbance reading. This conversion is achieved using the Beer - Lambert law:

$$A = \log \frac{I_o}{I} = \epsilon bc$$

where A is the measured absorbance, I_o is the intensity of the incident radiation upon the sample, I is the intensity of radiation emergent from the sample, ϵ is the molar extinction coefficient of the sample at the selected wavelength, b is the optical path-length, and c is the molar concentration of the sample. As observed from the Beer - Lambert law, for a certain analyte (ϵ) and a certain concentration (c), the measured absorbance (A) is directly proportional to the optical path-length (b). For capillary electrophoresis, the optical path is restricted to the internal diameter of the capillaries used that is generally in the range of 20-100 μm . Increasing the path-length is the most popular choice for improving the concentration limit of detection (LOD). Several different methods have been employed to address this problem such as using rectangular capillaries,²⁰ bending capillaries to form Z-shaped flow cells,²¹ using axial or whole column illumination,^{22, 23} and forming

“bubble cells” which have been extensively studied by Xue and Yeung.²⁴ The LOD can also be improved by using a double beam optical approach in order to compensate for fluctuations in the light source.²⁵

While direct UV/visible absorbance detection is applicable to an abundance of analytes, it is not truly universal. Compounds whose structures do not include π bonds, such as small inorganic ions and carbohydrates, usually have very small molar extinction coefficients even at low wavelengths. An alternative detection method is to use indirect absorbance detection. In this technique, an ionic additive of high molar extinction coefficient is introduced into the buffer. In the absence of analyte, the continually eluting buffer generates a high background absorbance level. The presence of ionic analyte in the capillary leads to the displacement of the buffer additive ions due to the constant electric field. As a result, elution of the analyte results in a decrease in the absorbance of the buffer which is proportional to the amount of analyte present.

Laser Induced Fluorescence Detection. Because fluorescence detection can provide the highest sensitivity for capillary electrophoresis it is more popular than absorption detection, particularly for the determination of biologically interesting molecules that exhibit native fluorescence or can be labeled with fluorescent dyes. Compared to absorption detection, fluorescence detection typically results in sensitivity gains of 1-3 orders of magnitude, depending on the light source, its intensity and stability, analyte fluorescence efficiency and background interference as well as detector configuration.²⁶ The large difference in LOD between absorption and fluorescence measurements results from the way the signal is monitored. In absorption, the small signal from the transmitted intensity is monitored in the presence of a relatively high

background signal. In general, it is not easy to measure a very small signal change on a high background, since a small fluctuation on the background can conceal the small signal. On the other hand, fluorescence measurements can be performed with a negligible background signal, so that even very small signals can be recognized easily. With the high power laser beam used to excite fluorescence, the short optical path-length of the capillaries is less problematic for achieving low LOD, even though the fluorescence intensity is also dependent on the path-length.

While laser induced fluorescence provides superior sensitivities for fluorescence dyes and properly labeled molecules, its application is mostly limited to large biomolecules such as nucleic acids and proteins, for which fluorescence labeling chemistry is well developed. Unfortunately most small organic and inorganic molecules do not possess strong enough native fluorescence for sensitive detection. Also, development of derivatization chemistry for these smaller molecules is labor intensive since no universal solution is available.

High-throughput Analysis using Multiplexed Capillary Electrophoresis

Combinatorial chemistry has advanced to a stage that it is revolutionizing the discovery of new drugs,²⁷ novel materials, and efficient catalysts.²⁸ It aims to substantially increasing the throughput of discovery by scanning and testing vast number of possibilities in a parallel fashion. Combinatorial chemists have developed and continue developing novel methods for synthesizing large numbers of compounds simultaneously. Characterization and screening of all the compounds produced by these parallel synthesis methods imposes new pressure on analytical chemistry for the development of high-

throughput analysis technologies. In the past several years, a number of research groups have been involved in developing multiplexed capillary electrophoresis systems with several hundreds of capillaries, thus greatly increasing system analysis throughput. Until recently, this work was driven almost exclusively by efforts to accommodate capillary gel electrophoresis with laser induced fluorescence detection for high-throughput DNA sequencing.²⁹ More recently multiplexed capillary electrophoresis has been used for high-throughput PCR analysis,³⁰ genotyping,³¹ and mutation detection.³²

The major difference between the various multiplexed capillary electrophoresis systems with laser induced fluorescence detection lies in the detection scheme. Generally, the laser induced fluorescence modes fall into two categories: scanning^{33, 34} and imaging.^{35, 36} For the scanning detection mode, a single detector is used and signal from each capillary is detected sequentially. In an imaging system all capillaries are illuminated and detected simultaneously.

More recently a multiplexed capillary electrophoresis system using absorption detection was reported.³⁷ This technique allows high-throughput separations of analytes that are not fluorescent while at the same time providing a more simplified experimental setup. Various applications have been reported using this technique such as screening enzyme activities,³⁸ screening of homogeneous catalysts,³⁹ and peptide mapping.⁴⁰ A variation of this system is used here and will be discussed in more detail in Chapter 2.

Catalytic Surface Imaging

Combinatorial chemistry has had a tremendous impact in recent years in areas ranging from drug discovery in the biotechnology and pharmaceutical industries to the

discovery of new solid state materials^{41, 42}. Preparation of solid-state heterogeneous catalysts has been reported using combinatorial methods such as thin-film deposition,^{41, 42, 43} and liquid dosing.⁴⁵ Such techniques can create catalytic materials in a high-throughput fashion, which in turn increases the demand for high-throughput screening. Recently, techniques such as IR thermography,^{46, 47} microprobe sampling mass spectrometry,⁴⁸ laser-induced resonance-enhanced multiphoton ionization,⁴⁹ laser-induced fluorescence imaging,^{50, 51} and fluorescence indicators⁵² have been reported for screening catalysts.

The optical methods listed here allow for high-speed acquisition of data on the activities of individual catalysts in a library without the need to take samples for off-line analysis. However, these methods also present some significant shortcomings. For example, IR thermography does not provide any information on selectivity or specific reaction products. Fluorescence techniques require the products to be fluorescent or that a product-specific fluorescent indicator is available. The most successful screening method has been microprobe sampling mass spectrometry because of its maturity and its ability to analyze complex gaseous mixtures. For the techniques listed above, characterization of multiple products from a single catalyst would be difficult. Furthermore, only laser-induced fluorescence imaging is suited for screening multiple catalysts at the same time. All other techniques rely on serial operation.

An *in situ* imaging technique for heterogeneous catalytic reactions in liquids is reported here. The technique is based on a multiplexed capillary electrophoresis system with absorption detection previously described.³⁷ The system allows for the simultaneous screening of multiple catalysts or multiple positions on the same catalytic surface. To

create the imaging probe, a large number of capillaries in the array are grouped together to sample the liquid immediately adjacent to a surface where the generation of UV/visible absorbing products occurs. The main advantage of this technique is the ability to utilize the separation power of capillary electrophoresis for distinguishing among compounds produced at the same location on the surface. Electrochemical reactions occurring on metal wires (platinum, gold and iron) in various shapes were used to demonstrate the imaging and separation performance of this system.

CHAPTER 2. EXPERIMENTAL

Materials

Potassium iodide, sodium phosphate and 1,10-phenanthroline (Phen) were obtained from Fisher Scientific (Fair Lawn, NJ). Potassium ferrocyanide was obtained from Aldrich (Milwaukee, WI). The gold (25 μm and 50 μm diameter) and platinum (50.8 μm diameter) wires were obtained from Alfa Aesar (Ward Hill, MA). The iron wire (65 μm diameter) was obtained from Goodfellow (Berwyn, PA).

Capillary Array Electrophoresis System

The experimental setup used is similar to the one previously described.³⁷ A photograph of the experimental setup is shown in Figure 2.1. Briefly, a total of 102 fused-silica capillaries (Polymicro Technologies Inc., Phoenix, AZ) with 75 μm I.D. and 150 μm O.D., 60 cm effective length and 90 cm total length, were packed side by side at the detection window and clamped together in a plastic mount after a window was created on each capillary. A tungsten lamp powered by a 12 V car battery was used as a light source. The light was passed through an interference filter to select the desired absorption wavelength and then expanded using a pair of cylindrical lenses in order to uniformly illuminate the detection window. The transmitted light was then focused on a photodiode array (PDA) (model C5964-SPL01, Hamamatsu, Japan) by using a quartz camera lens (Nikon; f.l. = 105 mm, $f = 4.5$). The PDA incorporated a linear photodiode array chip with 1024 diodes and driver/amplifier/temperature control electronics. Each diode was 25 μm in width and 2500 μm in height. The linear diode array was cooled thermoelectrically

to 0 °C. The PDA was interfaced to a computer via a National Instruments E series data acquisition board with 16-bit resolution. Data were collected using an in-house program written using LabView 6.0 (National Instruments, Austin, TX). Figure 2.2 shows an image of the data collected from the PDA. A high-voltage power supply (Spellman, Hauppauge, NY) was used to drive the electrophoresis. In all experiments gravity injection and flow was implemented by raising the inlet vial 20 cm above the outlet vial. Experiments with platinum and iron electrodes were performed at 456 nm while experiments with gold electrodes were performed at 420 nm.

Imaging Probe Fabrication

At the inlet the capillaries were bundled together either in PEEK (Upchurch Scientific, Oak Harbor, WA) or heat shrink tubing. Care was taken so that the inlets of the capillaries were at the same level in order to form a flat imaging tip. Epoxy glue was applied between the capillaries in the PEEK tubing so that their position would be fixed. The capillaries in the heat-shrink tubing were held in place by shrinking the tubing around them after assembly. The resulting imaging probe (Figure 2.3) had a roughly circular shape approximately 1.1 mm in diameter. The position of each capillary was determined by pushing water through the aligned outlet end of each capillary while observing the imaging tip under a microscope. Figure 2.4 shows an image of the imaging probe tip with the position of each capillary labeled.

Electrode Fabrication

The electrodes used as model imaging surfaces were constructed by using metal

wires attached with epoxy glue to a microscope slide. A platinum and an iron wire were used to form a cross-shaped electrode. Gold wire was used to make cross, square, triangle, and linear electrodes. Two of the electrodes used are shown in Figure 2.5.

Experimental Procedure

Using gold electrodes. The capillary array was first flushed with deionized water for cleanup and then filled with 5 mM potassium ferrocyanide in 0.1 M phosphate buffer, pH 9.2. The same solution also served as the reaction mixture. The electrode was placed in a petri dish filled with the reaction mixture. Using a three-dimensional stage the imaging probe was positioned about 10-20 μm over the electrode. The gold electrode was imaged by the oxidation of the ferrocyanide to ferricyanide (Reaction 1), which was achieved by applying 0.85 V vs. Ag/AgCl to the gold electrode.

The duration of the oxidation reaction was also the injection time. For the linear gold electrodes a single 7-s injection was performed. For the cross, square, and triangle gold electrodes two 3-s injections followed by an injection of ferricyanide into all capillaries were performed with 3 min between injections. Since only one compound was produced at the electrode no separation was necessary. The injected peak was allowed to simply flow through the detection window.

Using iron/platinum electrodes. The capillary array was first flushed with deionized water and then filled with 50 mM potassium iodide and 0.25 mM 1,10-

phenanthroline. The same solution was also employed as the reaction mixture for the two electrochemical reactions as well as the running buffer. The electrode was placed in a petri dish filled with the reaction mixture, and the imaging probe was placed over the electrode using a three-dimensional stage. At the iron electrode we have the oxidation of the iron wire to Fe^{2+} and Fe^{3+} . The two ions complex with the phenanthroline present in the solution which results in a colored complex. At the platinum electrode we have the generation of triiodide anion (Reactions 5-7) as previously described.⁵⁴ When 1.50 V, from a AA battery, was applied to the two electrodes the following reactions occurred:

Iron electrode:

Platinum electrode:

As in the imaging of the gold electrodes the duration of the electrochemical reaction served as the injection time. First a 3-s injection of an iron-phenanthroline solution to all capillaries was performed followed by a simultaneous 4-s injection from the iron electrode and a 10-s injection from the platinum electrode 5.0 min later. The high

voltage (+2.0 kV) was turned on 2.5 min after the last injection. This provides sufficient time to remove the cross electrode surface from the solution. During electrophoresis a large amount of unidentified material was produced at the high-voltage electrode. To avoid clogging the imaging probe, the high-voltage electrode was placed in a micro bio-spin chromatography column (Bio-Rad, Hercules, CA) that acted as a filter. The gel in the column was replaced with the running buffer and the column was immersed into the same petri dish as the imaging probe. Prior to electrophoresis the electrode surface that was to be imaged was removed from the petri dish.

Figure 2.1. Photograph of the experimental setup.

Figure 2.2. Image of the 102 capillaries on the photodiode array. Each peak represents a capillary.

Figure 2.3. Two photographs of the imaging probe made using heat shrink tubing.

Figure 2-4. Figure of the tip of the imaging probe. Each circle represents the position of a capillary. The number in each circle is the capillary represented at that position.

Figure 2.5. (A) Picture of the cross Iron/Platinum electrode. (B) Close-up picture of the square cross electrode. The black circle represents the approximate position of the imaging probe over the electrode.

CHAPTER 3. RESULTS AND DISCUSSION

Sampling

In order to achieve reliable sampling, a continuous gravity flow was employed. As products were generated at the electrode they were immediately injected into the capillary (or capillaries) right above the electrode. Differences in liquid flow in adjacent capillaries could cause some mixing of the products.

Imaging Resolution

The maximum resolution obtainable is defined by the outside diameter of each capillary in addition to the spacing between capillaries. In our experiments the outside diameter of each capillary was about 165 μm (including the cladding around each capillary). We estimate the resolution of the imaging probe to be about 170-200 μm depending on the location. According to a recent report,⁵⁵ fused-silica capillaries can be pulled to an outer diameter of about 1 μm with an inner diameter of a few hundred nanometers. Utilizing such techniques in the imaging probe construction in the future, it should be feasible to eventually achieve spatial resolution in the order of a few micrometers.

Imaging Probe Positioning

The position of the imaging probe in relation to the surface to be imaged is very important. The surface and the probe need to be close to each other in addition to being perpendicular to each other. Failure to meet either of these requirements could result in

unclear images due to lateral diffusion of the products prior to injection into the capillaries. Additional difficulties were encountered in positioning the imaging probe over the square and triangle test electrodes since the surface size is very similar to the size of the imaging probe.

Gold Electrode Imaging

Four different shapes of electrodes were imaged in our experiments. The reconstructed images are shown in Figures 3.1 through 3.4. In all cases, the size and orientation of the reconstructed image correspond to the size and orientation of the electrodes with respect to the imaging capillaries. In Figures 3.2, 3.3 and 3.4 the ratio of the ferricyanide peak area divided by the area of the control peak is plotted. In Figure 3.1 the ferricyanide peak area divided by the migration time is plotted. This calculation was done to correct for any differences in injection efficiency between capillaries. In all cases, the darker the circle, the more sample was injected. The variation in the amount of material injected in each capillary could be attributed to variations in activity along the electrode, mixing of the material as it was produced at the electrode, and imperfect positioning of the probe with respect to the electrode.

Iron/Platinum Electrode Imaging

A cross electrode, made of a platinum and an iron wire, was chosen for this experiment. A different reaction occurs at each wire with two different products that can be separated by capillary electrophoresis. The reconstructed images from two different experiments are shown in Figures 3.5 and 3.6. Based on the position of each peak in the

collected electropherograms (Figure 3.7B), the identity of each compound was determined. The first peak is the control peak. Since the control and the product at the iron electrode are the same species and since they were injected 5 min apart, they were separated by 5 min in the electropherograms. The triiodide anion produced at the platinum electrode has a migration time different from the control that is greater than 5 min. The electropherogram in Figure 3.7B-3 comes from a capillary at the junction of the platinum and iron wires (Figure 3.7A). It clearly demonstrates the ability to detect two different compounds produced at the same location of the catalytic surface. Based on the migration times, identification of the catalyst is possible (platinum or iron). In both experiments presented, the orientation of the reconstructed image corresponds to the orientation of the electrode with respect to the imaging probe. As with the gold electrode images, the variation in the amount of material injected could be attributed to variations in electrode activity, sample mixing, and probe positioning.

Conclusions

In this work, we have successfully demonstrated a new technique for *in situ* heterogeneous catalyst screening and imaging in liquids. This complements previous studies in our group on *in situ* imaging of gas-phase products at a solid surface.^{51, 52} The technique has the ability to simultaneously screen multiple catalysts present at the surface, e.g. a combinatorial array, or be used to image catalytic surfaces for local variations in activity. By using capillary electrophoresis, multiple products from a single catalyst can be identified and quantified. The catalyst can then be characterized and evaluated based on the products formed. As a continuous sampling probe, the time

resolution is in the order of peak widths in electrophoresis, or a few s. Detection of any UV/visible absorbing product is feasible by simply changing the filter and the light source. For example, at 214 nm (Zn lamp), almost all organic functional groups absorb. Universal detection is then possible provided that the solvent does not absorb at that wavelength. Finer imaging should be possible if capillaries with smaller I.D. are used or if the capillaries are drawn down in diameter after assembly. Although 102 capillaries were used here, many more can be employed by scaling up since the present PDA already possesses 1024 elements. To perform spatial correlation between the location of a particular capillary at the probe end and at the detection window, automated injection of an absorbing species into each capillary can be performed while checking for absorption at the PDA elements. This last operation could be implemented following a binary tree, i.e., involving different halves of the capillary array at a time. In that case, a 1024-member (2^{10}) imaging probe can be correlated in only 10 steps.

Figure 3.1 Reconstructed image of a gold linear electrode. Each circle represents a capillary in the imaging probe. The darker the circle the more material was injected.

Figure 3.2. Reconstructed image of a gold cross electrode. Each circle represents a capillary in the imaging probe. The darker the circle the more material was injected.

Figure 3.3. Reconstructed image of a gold square electrode. Each circle represents a capillary in the imaging probe. The darker the circle the more material was injected.

Figure 3.4. Reconstructed image of a gold triangle electrode. Each circle represents a capillary in the imaging probe. The darker the circle the more material was injected.

Figure 3.5. Reconstructed image of a iron/platinum electrode. The ratio of the sample peak area to the control peak area was plotted. Each circle represents a capillary in the imaging probe. The darker the circle the more material was injected.

Figure 3.6. Reconstructed image of a iron/platinum electrode. The ratio of the sample peak area to the control peak area was plotted. Each circle represents a capillary in the imaging probe. The darker the circle the more material was injected. More details about this figure are presented in Figure 3.7.

Figure 3.7. Reconstructed image of a iron/platinum electrode. The ratio of the sample peak area to the control peak area was plotted. Each circle represents a capillary in the imaging probe. The darker the circle the more material was injected. Selected electropherograms from A are presented in B. The peak at 7 min is the iron-phenanthroline control injected into all capillaries prior to the electrolytic reaction. The peak at 12 min is the iron-phenanthroline complex found only in the capillaries positioned over the iron wire. The peak at 14 min in the triiodide anion found only in capillaries positioned over the platinum wire. The electropherogram in B-3 corresponds to the capillary at the junction of the two types of electrodes. There, both compounds were detected and separated.

APPENDIX. SUPPLEMENTAL DATA

The following figures are the electropherograms collected for the imaging experiments presented here.

Figure A.1. Electropherograms of the data presented in Figure 3.1.

Figure A.2. Selected electropherograms of the data presented in Figure 3.2. The first two peaks are the two consecutive injections from the gold cross electrode. The third peak is the ferricyanide control. All injections were performed in 3 min intervals.

Figure A.3. Selected electropherograms of the data presented in Figure 3.3. The first two peaks are the two consecutive injections from the gold square electrode. The third peak is the ferricyanide control. All injections were performed in 3 min intervals.

Figure A.4. Selected electropherograms of the data presented in Figure 3.4. The first two peaks are the two consecutive injections from the gold triangle electrode. The third peak is the ferricyanide control. All injections were performed in 3 min intervals.

Figure A.5. Electropherograms of the data presented in Figure 3.5. The first peak is the iron-phenanthroline control peak. The second peak is the iron-phenanthroline complex produced at the iron electrode and the third peak is the triiodide anion produced at the platinum electrode.

Figure A.6. Electropherograms of the data presented in Figure 3.6. The first peak is the iron-phenanthroline control peak. The second peak is the iron-phenanthroline complex produced at the iron electrode and the third peak is the triiodide anion produced at the platinum electrode.

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