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A Two-Channel Microfluidic Sensor That Uses Anodic Electrogenerated Chemiluminescence as a Photonic Reporter of Cathodic Redox Reactions

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This paper describes a new approach for sensing electrochemically active substrates in microfluidic systems. This two-electrode sensor relies on electrochemical detection at one electrode and electrogenerated chemiluminescent (ECL) reporting at the other. Each microfabricated indium tin oxide electrode is located in a separate microfluidic channel, but the channels are connected downstream of the electrodes to maintain a complete electrical circuit. Because of laminar flow, there is no bulk mixing of the fluids in the detecting and reporting channels. This approach allows the ECL reaction to be physically and chemically decoupled from the sensing channel of the device, which greatly expands the number of analytes that can be detected. However, because the cathode and anode are connected, electron-transfer processes occurring at the sensing electrode are electrically coupled to the ECL reaction. Charge balance permits the ECL light output to be quantitatively correlated to electrochemical reductions at the cathode. The system is used to detect $Fe(CN)_6^{3-}$, $Ru(NH_3)_6^{3+}$, and benzyl viologen and report their presence via $Ru(bpy)_3^{2+}$ (bpy = bipyridine) luminescence. Each different redox target initiates ECL at a unique potential bias related to its standard redox potential. The influence of the concentrations of $Ru(bpy)_3^{2+}$ and the target analytes is discussed.

Here, we report a dual-channel microfluidic sensor that detects target analytes electrochemically and reports their presence via electrogenerated chemiluminescence (ECL). We reported an analogous single-channel device recently and demonstrated that it had the following two desirable features.[1] First, the electrochemical sensing reaction is chemically decoupled from the ECL reporting reaction under most conditions. This differs in an important way from previously reported ECL detection schemes, because in this new approach the target need not directly influence the ECL reactants.[2−8] Accordingly, the number of targets that can be detected is greatly expanded. Second, the electrical connection between the cathode (detection electrode) and anode (reporting electrode) necessitates charge balance, which ensures a direct correlation between the ECL intensity and the concentration of the target. A limitation of the previously reported single-channel system is that under certain conditions the detection and reporting reactions interfere with one another because of their close spatial proximity, which can lead to quenching of the ECL reaction and a corresponding loss of reporting fidelity. The new two-channel system reported here ensures that these reactions are fully chemically decoupled under all circumstances. As shown in Scheme 1, this is accomplished by physically separating the anode and cathode reactions into different channels of a microfluidic device. Importantly, this strategy also permits use of solvents and other solution components that are incompatible with the ECL reporting reaction.

Since their introduction in 1990,[9] the advantages of analytical microsystems, including high performance, design flexibility, reagent economy, miniaturization, and automation, have been well documented.[10,11] Such microsystems have been coupled with a variety of detectors, ranging from laser-induced fluorescence[12] (LIF) to mass spectrometry (MS).[13] These methods offer exquisite sensitivity and a high density of information content; however, both LIF and MS require bulky, off-chip support instrumentation that compromises the benefits of miniaturization and portability. New high-sensitivity detection methods that are lightweight, require little real estate on the chip, and that have minimal power requirements are essential for the development of compact, portable microsystems. Electrochemical methods meet these requirements with just one exception: their sensitivity is not competitive with LIF or MS.[14−20] The method reported here

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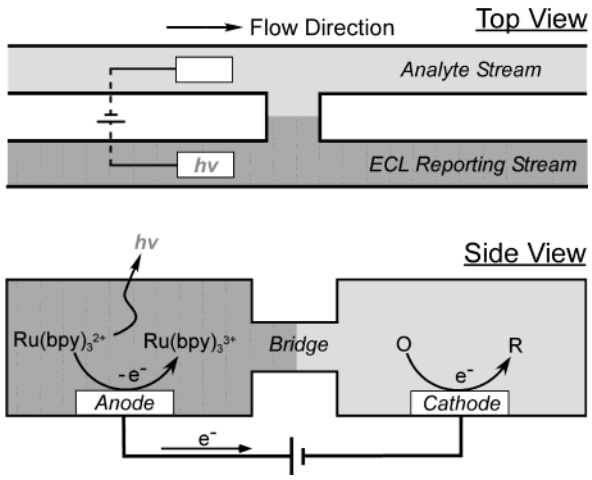
Scheme 1



addresses this issue by coupling electrochemical detection methods, along with all of their desirable properties, to a simple, low-background photonic reporting system that has minimal power requirements.

In this work, we used the well-established anodic ECL reaction that involves oxidation of $Ru(bpy)_3^{2+}$ (bpy = bipyridine) and tripropylamine (TPA) to report the presence of analytes that can undergo reduction. The two-channel microfluidic system permits electrical contact between the ECL reporting stream and the target-containing stream, while at the same time eliminating mixing of the two streams. In our previously reported single-channel system, the maximum detection concentration for a model target, benzyl viologen (BV^{2+}), was 100 μM. Above this concentration, the anode (reporting) and cathode (sensing) reactions overlapped in space, resulting in luminescence quenching. The two-channel system reported here eliminates diffusion-layer overlap and of the electrode reactions, and therefore, concentrations of BV^{2+} up to 10 mM can be detected with high fidelity.

EXPERIMENTAL SECTION

Chemicals. Hexaammineruthenium chloride ($Ru(NH_3)_6Cl_3$, 99%) and $Ru(bpy)_3Cl_2 \cdot 6H_2O$ (bpy = 2,2′-bipyridine) (minimum 98%) were purchased from Strem Chemicals (Newburyport, MA). TPA (99+%) and benzyl viologen dichloride (97%) were obtained from the Aldrich Chemical Co. (Milwaukee, WI). Potassium ferricyanide ($K_3Fe(CN)_6$) was obtained from Fisher Scientific (Fair Lawn, NJ). Deionized 18 MΩ·cm water (Milli-Q reagent water system, Millipore, Bedford, MA) was used to prepare all aqueous solutions. All chemicals were used without additional purification.

Device Fabrication. Methods used to fabricate the indium-doped tin oxide (ITO) microelectrodes and the microfluidic devices have been reported previously.[1] Briefly, ITO-coated (thickness, ~200 nm; resistance, ~10 Ω/square) aluminosilicate glass slides (Delta Technologies, Ltd., Stillwater, MN) were first cleaned. A positive photoresist layer (~15 μm thick, AZ P4620, Clariant Co., Somerville, NJ) was then spin-coated onto the ITO-coated glass slide. Next, the photoresist layer was patterned by

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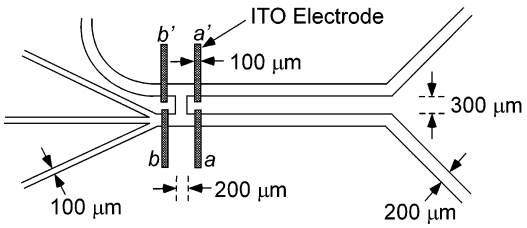
Scheme 2



illumination with a 365-nm light (EFOS Lite, UV spot lamp, Mississauga, ON, Canada) through a mask (photographic film) designed using a computer graphics program. These patterns were then transferred to the substrate by developing the photoresist and etching the ITO surface with an aqueous acid solution (5% HNO_3 and 20% HCl). The remaining photoresist was removed with acetone.

Microfluidic devices were fabricated by a published method using poly(dimethylsiloxane) (PDMS, Dow Corning Sylgard Silicone Elastomer-184, Krayden, Inc.) molds.[21] The layout and dimensions of the device are shown in Scheme 2. PDMS molds were aligned over the patterned ITO-coated glass slides with the aid of an *x*,*y*,*z*-micropositioner (462 series, Newport Co., Irvine, CA) and a motion controller (model 861, Newport Co.) under a microscope having a 10× lens (Optiphot, Nikon). A syringe pump (PHD 2000, Harvard Apparatus, Holliston, MA) was used to deliver fluids to the channels. The potential biases applied between the two ITO microelectrodes were generated by a dc power supply (potential range, 0−25 V, model E3620A, Hewlett-Packard). Electrical contacts were made to the ITO electrodes using Ag paste (Epo-tek, Epoxy Technology, Billerica, MA).

Electrochemical Measurements. Conventional three-electrode experiments were carried out using a Pine AFRDE4 bipotentiostat (Grove City, PA) and a Kipp and Zonen XYY′ chart recorder (Bohemia, NY). Clean ITO slides (Delta Technologies, Ltd., Stillwater, MN) were used as working electrodes with an exposed area of ~0.45 cm^2. In all cases, the counter electrode was a Pt mesh and the reference was Ag/AgCl (3 M NaCl) (Bioanalytical Systems, West Lafayette, IN).

ECL Measurements. ECL spectra were acquired using a liquid nitrogen-cooled, CCD-based (LN/CCD, Roper Scientific, Tucson, AZ) spectrometer (SpectraPro-300i, Acton Research Co., Acton, MA) under darkroom conditions. This spectrometer was connected to the side port of an optical/fluorescence-inverted microscope (Eclipse TE300, Nikon). Prior to making luminescence measurements, the light path was aligned to ensure maximum sensitivity. The ECL acquisition time was typically 5 s with a slit width of 1 mm.

RESULTS AND DISCUSSION

Scheme 2 is an illustration of the essential components of the microfluidic device used in this study. The microfluidic channels were fabricated from PDMS using standard methods that are described in the Experimental Section. The channels were sealed by irreversibly attaching the PDMS mold to a glass plate. The device consists of two main channels having a width and height

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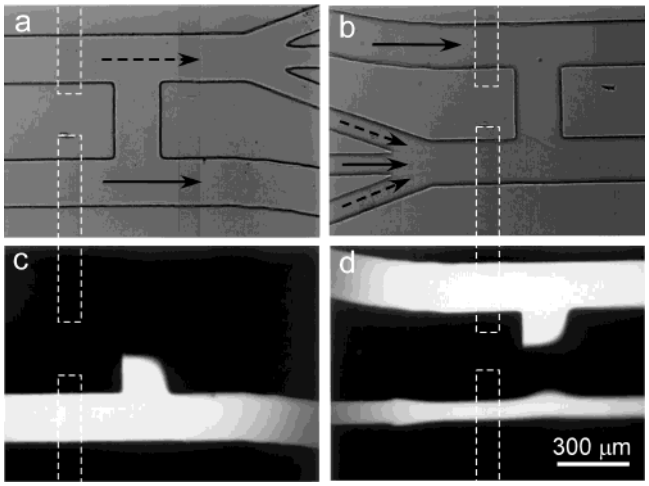

Figure 1. Demonstration of the flow characteristics of the device illustrated in Scheme 2. (a) and (b) are optical micrographs of the same device. The dashed white lines highlight the shape and positions of the microfabricated ITO electrodes. The arrows indicate the direction of flow. Specifically, the solid arrows indicate flow of solutions containing 2 mM fluorescein in an aqueous, pH 6.9 phosphate buffer solution, and the dashed arrows indicate flow of buffer only. (c) and (d) are fluorescence micrographs corresponding to (a) and (b). The flow rate in the two channels in (c) was 1 μL/min. In (b), the flow rate in the upper channel was 1.5 μL/min and the flow rate in the three small subchannels was 0.5 μL/min. These fluorescence micrographs indicate well-behaved laminar flow even though the two main channels are in hydrostatic contact via the crossover channel. Note that no fluorescence is observed above background in the buffer-only flow stream.

of 200 and 26 μm, respectively. A short cross channel connects the two main channels. This cross channel has the important function of providing electrical and pressure contact between the fluids in the two main channels. When the fluids in the two channels flow at the same velocity, each stream will tend to remain in its own channel because the shear force generated at the two ends of the junction cancel. Fluid could be pumped through the main channels in either direction. The lower channel has three input/output subchannels on the left side for increased design flexibility. Two pairs of 100-μm-wide ITO electrodes were microfabricated opposite one another on the glass plate affixed to the bottom of the PDMS mold. Each electrode had an exposed area of 2×10^4 μm^2.

To confirm that mixing of fluids does not occur at the liquid junction between the two main channels of this microfluidic H-cell, a fluorescent dye, 2 mM fluorescein in an aqueous, pH 6.9, 0.1 M phosphate buffer, was pumped through one channel at 1 μL/min and buffer only was pumped through the other at the same flow rate. Figure 1a is an optical micrograph of the device illustrated in Scheme 2. One pair of electrodes is highlighted by dashed white lines. Figure 1c is a fluorescence micrograph corresponding to the optical micrograph in Figure 1a. In this experiment, buffer solution (indicated by a dashed arrow) was pumped through the top channel and the fluorescent dye (solid arrow) was pumped through the lower channel. The direction of flow is indicated by the arrows in Figure 1a (with reference to Scheme 2, the flow direction is from right to left). The important result is that there is no detectable mixing of the solutions in the cross channel.

The optical micrograph in Figure 1b represents the situation when the flow direction is reversed (left to right in Scheme 2). Here, the dye is pumped through the upper main channel and through the center subchannel leading into the lower main channel. Buffer is pumped through the two outer subchannels of the lower main channel. Here again there is no detectable crossover of fluid from one main channel to the other (Figure 1d).[22] We did occasionally observe crossover of one or the other stream, presumably resulting from slight differences in flow velocity, but it always occurred downstream of the crossover channel. Therefore, even in this case, the fluids in the two channels are separated in the vicinity of the first pair of electrodes.

To illustrate the electrochemical sensing function of this two-channel device, we chose three target redox compounds: $K_3Fe(CN)_6$, $Ru(NH_3)_6Cl_3$, and BV^{2+}. Figure 2 presents cyclic voltammograms for these three compounds obtained using a conventional three-electrode cell. In all cases, the target was present at a concentration of 5 mM, the electrolyte was aqueous 0.1 M Na_2SO_4, the working electrode was ITO (area, 0.45 cm^2), and the counter electrode was a platinum mesh. The formal potential for each redox reaction (eqs 1−3) was calculated by averaging the

$$Fe(CN)_6^{3-} + e^- = Fe(CN)_6^{4-} \quad (1)$$

$$Ru(NH_3)_6^{3+} + e^- = Ru(NH_3)_6^{2+} \quad (2)$$

$$BV^{2+} + e^- = BV^{\bullet +} \quad (3)$$

potentials of the peak anodic and cathodic currents (indicated by the dashed lines in Figure 2): (a) $K_3Fe(CN)_6$, 0.21 V; (b) $Ru(NH_3)_6Cl_3$, −0.22 V; and (c) BV^{2+}, −0.57 V. Voltammogram d was obtained under the same conditions as the others, but in the absence of an intentionally added redox-active target. The cyclic voltammetry of the components necessary for the ECL reaction (voltammogram e: 5 mM $Ru(bpy)_3^{2+}$ plus 25 mM TPA) is consistent with previous reports.[23] The electrochemical response of the targets and the ECL reporter reaction all occur within the polarizable limit of the electrolyte solution (voltammogram d). The potential differences between the onset of current for the ECL process and the estimated formal potential for each of the three redox targets (ΔE_{onset}) is shown at the bottom of Figure 2.

The voltammetry shown in Figure 2 was correlated to the appearance of ECL. With reference to Scheme 2, these experiments were performed as follows. The ECL solution (1 mM $Ru(bpy)_3^{2+}$ and 25 mM TPA in 0.1 M aqueous phosphate buffer) was pumped from right to left in the upper main channel, and each of the three redox targets (1 mM in 0.1 M aqueous Na_2SO_4 electrolyte solution) were sequentially pumped in the same direction through the lower channel. The flow rate of the solution in both channels was 1 μL/min. Potential biases of varying magnitude were then applied between ITO electrodes a and a′ (Scheme 2), and at each potential, an ECL spectrum was obtained at the anode.

Figure 3 shows the normalized ECL intensity recorded at λ_{max} = 610 nm for reduction of each of the three redox targets at many different applied biases (E_{app}). We estimated the onset of ECL as the bias required to observe a signal-to-noise ratio of 2 (that is, when the ECL signal was twice the background of the detector).

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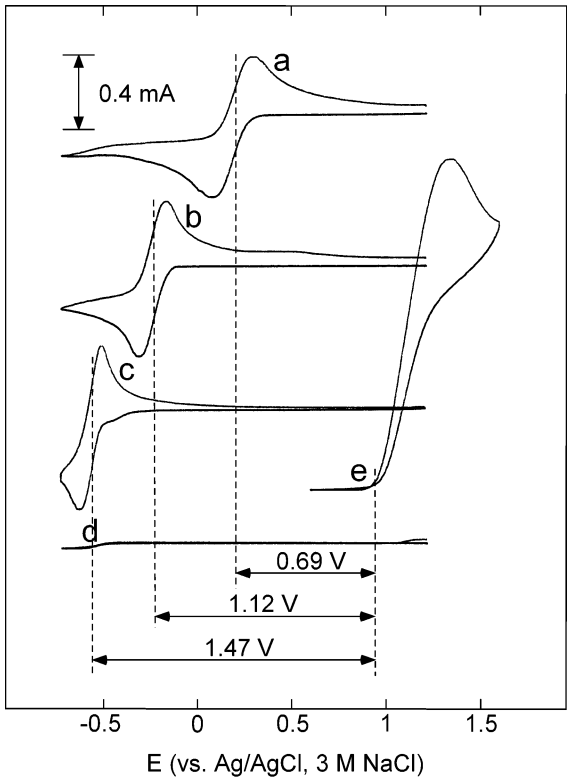

Figure 2. Cyclic voltammograms obtained using a conventional three-electrode cell. In (a)−(d), aqueous 0.1 M Na_2SO_4 was used as the supporting electrolyte, and in (e) 0.1 M aqueous phosphate buffer (pH 6.9) was used. (a) 5.00 mM $K_3Fe(CN)_6$; (b) 5.00 mM $Ru(NH_3)_6Cl_3$; (c) 5.00 mM BV^{2+}; (d) 0.1 M Na_2SO_4 electrolyte solution only; (e) 5 mM $Ru(bpy)_3Cl_2$ and 25 mM TPA. The ITO working electrode had an area of 0.45 cm^2, and the scan rate was 100 mV/s. The dashed lines are estimates of the formal potentials of the three reduction reactions and the onset potential for the ECL reaction.

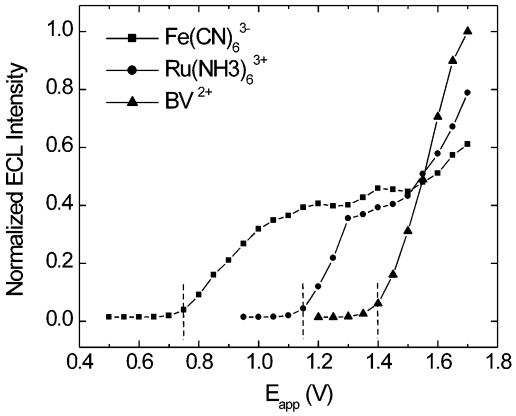

Figure 3. Normalized ECL intensity at $\lambda_{max} = 610$ nm as a function of applied potential bias (E_{app}). The redox targets $K_3Fe(CN)_6$, $Ru(NH_3)_6Cl_3$, and BV^{2+} were present at a concentration of 1.00 mM in aqueous 0.1 M Na_2SO_4. The ECL reporting stream contained 1 mM $Ru(bpy)_3Cl_2$ and 25 mM TPA in 0.1 M phosphate buffer (pH 6.9). With reference to Scheme 2, the flow direction was from right to left and the ECL spectra were collected from the ITO anode (electrode a′). The dashed lines indicate the onset potential for the three reduction reactions, defined as the applied potential necessary to establish an ECL signal-to-noise ratio of 2. The flow rate was 1 μL/min.

These onset biases were found to be 0.75, 1.15, and 1.40 V, for $Fe(CN)_6^{3-}$, $Ru(NH_3)_6^{3+}$, and BV^{2+}, respectively. These values differ by no more than 10% from the potential differences calculated from the voltammetry shown in Figure 2. We conclude that any redox target in the analyte stream can be detected if $E_{app} > \Delta E_{onset}$. Moreover, by precisely controlling E_{app}, one or more reductions having relatively close formal potentials can be detected by ECL. Finally, this experiment also demonstrates that this two-channel strategy can be used to sense and report the presence of targets that do not participate in the ECL chemistry. It is obvious that even targets that are incompatible with the ECL components can be detected by using this approach.

Because the microfluidic device is operated under flowing conditions, reactants (e.g., ECL reagents and target analytes) are continuously replenished at the electrode surface. This means that a steady-state ECL signal should be observed for a particular set of experimental conditions (e.g., target concentration, flow rate, electrode area, etc.). The stability of the ECL signal was evaluated by making 36 consecutive measurements over a time period of 3 min. The ECL signaling channel contained 5 mM $Ru(bpy)_3^{2+}$ and 25 mM TPA in 0.1 M aqueous phosphate buffer adjusted to pH 6.9, and the target analyte present in the other channel was 5.0 mM BV^{2+} in a 0.1 mM Na_2SO_4 aqueous solution. The applied potential bias (E_{app}) between the two electrodes was 1.5 V, and the flow rate of the two streams was 1 μL/min. The relative standard deviation (RSD) of the ECL data for all 36 experiments was found to be less than 1%, indicating that this sensor is quite robust.

To investigate the relationship between the detection and reporting functions of the two-channel microfluidic system, we designed and executed the following two experiments. In the first experiment, the concentration of $Ru(bpy)_3^{2+}$ was held constant at 1.00 mM in the reporting channel and the concentration of BV^{2+} in the detection channel was varied between 0.25 and 5.00 mM. In the second experiment, the BV^{2+} concentration was held constant at 1.00 mM and the concentration of $Ru(bpy)_3^{2+}$ was varied between 0.25 and 5.00 mM. The results are summarized in Figure 4. The obvious trend is that higher concentrations of either the reporter reagents or the target analyte lead to higher ECL intensities, even though E_{app} and the flow rate were constant at 1.5 V and 1 μL/min, respectively. This result provides additional evidence that the anode and cathode processes are correlated.

Interestingly, the ECL versus concentration data in Figure 4 do not attain a limiting value spanning the range of ECL reagent-to-target analyte ratios of 1:5−5:1. This is a consequence of convective−diffusive transport. Finally, even though the ECL response does not vary linearly as a function of analyte concentration, reliable quantification can still be achieved through careful calibration.

Thus far, we have discussed investigations of single-target detection. However, from a technological perspective, solutions containing multiple targets are more important. Experiments designed to understand multianalyte detection were carried out using the device shown in Scheme 2, but in this case, the flow direction was from left to right, which made it possible to pump up to three different targets through each of the three small subchannels leading into the lower main channel. The ECL components were pumped into the upper channel and the flow direction was also from left to right. In the first experiment, 1.00 mM BV^{2+} was pumped into the upper subchannel and electrolyte

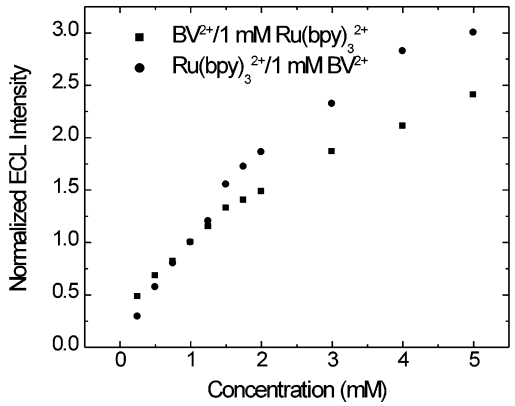

Figure 4. ECL intensity at λ_{max} = 610 nm as a function of the concentration of $Ru(bpy)_3^{2+}$ in the reporter channel and BV^{2+} in the detection channel (Scheme 2). The data are normalized at a $Ru(bpy)_3^{2+}$ or BV^{2+} concentration of 1.00 mM. The data indicated by filled squares were obtained using a fixed concentration of 1.00 mM $Ru(bpy)_3Cl_2$ (together with 25 mM TPA in aqueous 0.1 M phosphate buffer, pH 6.9) and varying the concentration of BV^{2+} from 0.25 to 5.00 mM (in aqueous 0.1 M Na_2SO_4). The data indicated by filled circles were obtained using a fixed concentration of 1.00 mM BV^{2+} (in aqueous 0.1 M Na_2SO_4) and varying the concentration of $Ru(bpy)_3Cl_2$ from 0.25 to 5.00 mM (together with 25 mM TPA in 0.1 M phosphate buffer, pH 6.9).

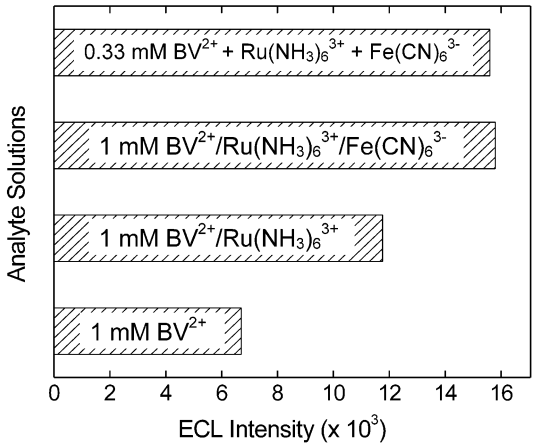

Figure 5. Normalized ECL intensities correlated to different combinations of redox-active targets in the detection channel. In all cases, the reporter channel contained 1 mM $Ru(bpy)_3Cl_2$ and 25 mM TPA in 0.1 M phosphate buffer (pH 6.9). Data for the bottom three bars were collected for flow from left to right (Scheme 2). The detection channel contained the indicated redox target(s) in separate laminae, which were pumped through the three small subchannels as described in the text. Data for the top bar were obtained by mixing the three redox targets in the same solution and pumping from right to left (Scheme 2). The flow rate in the main channels was 1.5 μL/min, and in each of the subchannels it was 0.5 μL/min.

solution only (0.1 M aqueous Na_2SO_4) was pumped through the other two subchannels. The bias applied between electrodes b and b′ was 1.55 V. The measured ECL intensity is shown as the bottom bar in Figure 5. Next, the electrolyte-only solution in the middle subchannel was replaced with 1.0 mM $Ru(NH_3)_6^{3+}$, and the ECL signal was collected under the same conditions used for the BV^{2+}-only experiment. Finally, the remaining (bottom) electrolyte-only stream was replaced with 1.0 mM $Fe(CN)_6^{3-}$, and this led to a further increase in the measured ECL intensity.

When the three targets are simultaneously pumped through each of the three subchannels, each target only has access to about one-third of the total electrode area because of laminar flow. Accordingly, the ECL response from this experiment will reflect the total current originating from each of the three target-containing laminae. Moreover, if all three targets are present at the same concentration, undergo fully thermodynamically reversible electron-transfer reactions, and have the same mass-transfer characteristics, then each should account for one-third of the total current. Figure 5 shows that the actually percentages, relative to the ECL intensity of the experiment in which all three redox targets are present, are BV^{2+}, 43%; $Ru(NH_3)_6^{3+}$, 32%; and $Fe(CN)_6^{3-}$, 25%. These differences are probably due to the different heterogeneous electron-transfer rate constants for each redox target,[24] different overpotentials for each, and slightly different diffusion coefficients and flow rates.

In another experiment, a mixture of the three redox targets was pumped from right to left (Scheme 2) through the lower main channel. The concentration of each target was 0.33 mM. The ECL reagents, present at the same concentrations as in the previously described experiment, were pumped through the upper main channel. Electrodes a and a′ were used for this experiment. As shown by the top bar in Figure 5, the ECL responses from the mixed and separated streams are nearly identical. This simple experiment indicates that the concentrations of different targets can be determined by taking advantage of the laminar flow characteristics of this device.

SUMMARY AND CONCLUSIONS

We have described a new method to detect redox-active targets electrochemically and report their presence photonically via electrogenerated chemiluminescence. We showed previously that this approach does not require the target to directly interact with the reactants required for ECL.[1] This greatly expands the number of analytes that can be reported by ECL.

In this new report, we have taken the additional step of physically separating the ECL reporting reaction from electrochemical detection events. This is accomplished by placing electrodes in each of two separate channels, which are connected by a stagnant plug of solution in a crossover channel. This approach further expands the number of analytes that can be detected, because even those that might interfere with the ECL cocktail are accessible. Moreover, this two-channel approach opens up the possibility of using completely different solution conditions (for example, different solvents or electrolytes) in the detection and reporting channels.

We described two additional interesting new findings. First, it was possible to take advantage of the useful laminar flow characteristics of microfluidics to detect three different analytes, each confined to their own separate laminae. This might be important if different solvents were required for each target or if precipitation would occur if two of the targets were in the same bulk-phase solution. Second, the measurements reported here were all obtained under continuous-flow conditions, which opens up the possibility of real-time detection. Coupling this general strategy with microfluidics also has the general advantage of low power requirements and portability.

The results reported here and in our previous paper[1] clearly indicate that this new ECL-based analysis approach can be used

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for quantitative analysis of redox-active targets with high reliability and stability. At present, target selectivity is accessible only via the different redox potentials of the target analytes. However, there are a number of means for improving selectivity. For example, there are many examples of using chemically modified electrodes for this purpose. Moreover, the fraction of the detection column leading up to the electrode could be modified in such a way that specific analytes could be preconcentrated or separated prior to detection. Work toward the improvement and extension of the analytical capability of this microfluidic sensor is currently underway in our laboratory.

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