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Detection of electrochemiluminescence from floating metal platelets in suspension

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We present a generation of electrochemiluminescence (ECL) signal, based on square shaped gold electrodes with a size of 50 μm positioned inside a fused silica capillary. The ECL was generated using electric pulses with duration in the range from 100 ms to 5 s and an electrical field strength from 300 V cm^{-1} to 500 V cm^{-1}. We have demonstrated that the electrochemical reaction with detectable optical output can be produced using freely moving and thus disposable electrodes.

Introduction

Microfluidics represents a promising technology for the development of high throughput and low cost systems for analytical and clinical applications,[1] lowering the required sample amount and shortening the time for the analysis. The small sample volume makes the detection of the reaction product more challenging and popular electrochemical methods are often replaced with the more sensitive fluorescence-based methods. Compared to the fluorescence techniques, chemiluminescence[2–5] effectively has no background signal, making it an attractive alternative. It is also characterized by the relatively simple instrumentation and, unlike the fluorescence methods, does not require an external light source.

Electrochemiluminescence (ECL) is chemiluminescence which can be generated during specific redox reactions at electrode surfaces. ECL represents a valuable combination that takes advantage of both optical and electrochemical detection techniques. The labelling of the analyte molecules with ECL active compounds has been documented in the sensitive detection of biomolecules.[6–9] Redox reactions can be generated on any metal material immersed in the solution conducting electric current. The electric field in the solution is distorted by the metal, leading to the potential difference at the metal/solution interface and formation of both anodic and cathodic overpotentials on the opposite ends of the metal.[10,11] This reaction system has been previously described as a bipolar electrode arrangement, since the electrode behaves as the anode and cathode at the same time.[12–14]

On the macroscale, the metal electrodes can be created from wires, foils and sheets. In microdevices, electrodes are mainly prepared using thin film technology such as sputtering or evaporation. The shape of the electrodes can be defined by the photolithographic process and then by etching the metal. If these processes are conducted on the sacrificial layer, metal patterning and the removal of the sacrificial layer will result in free platelets. This process is typically denoted as top-down preparation[15] and has potential for use in analytical applications and microfluidics.[16–19]

In this study, we use similar techniques based on the combination of the vacuum metal sputtering deposition and photolithography to prepare well-defined microscale metal platelets and explore their use as disposable electrodes for ECL detection in microfluidics. To our knowledge, this is the first time that ECL signal has been observed on a microscale freely moving electrode, gold platelet.

Experimental

Fabrication of gold platelets

The platelets were fabricated from lithographically patterned thin film metal deposited on a sacrificial layer. The fabrication was carried out on 50 mm × 75 mm glass substrates (Corning, Ted Pella, Redding, USA). The substrate was first cleaned with H_2SO_4/H_2O_2 (piranha) solution. Next, it was covered by a spincoating technique for 30 s at 3000 rpm with MaN-420 negative photoresist (Micro resist technology GmbH, Berlin, Germany) diluted with acetone in ratio 1 : 3. Photoresist was cured on a hotplate for 60 min at a temperature of 100 °C. This material was only used as the sacrificial layer. Gold layer with a thickness of 0.3 μm was deposited by magnetron sputtering. Subsequently, it was covered with Positiv 20 positive photoresist (CRC Industries Europe N. V., Zele, Belgium) by spincoating for 15 s at 400 rpm. The substrate was cured on a hotplate for 60 min at 70 °C temperature. Using a tabletop

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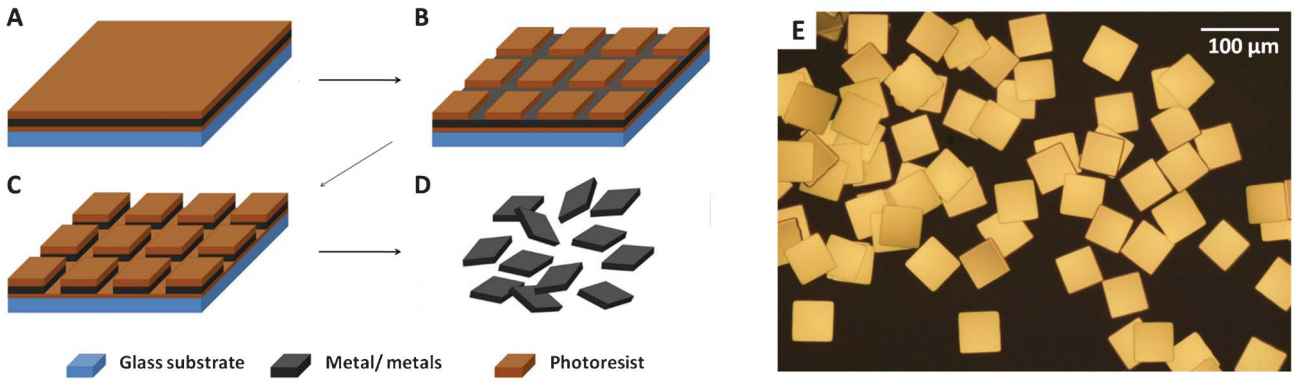

Fig. 1 Fabrication process. (A) Sacrificial photoresist layer was spin coated on the glass substrate followed by metal sputtering and second photoresist layer deposition on the top. (B) Exposition and development of the top photoresist layer. (C) Exposed metal sections were etched away. (D) Release of platelets by acetone. (E) Resulting gold platelets, square shaped and size of 50 μm, thickness of 0.3 μm.

laser pattern generator (μPG 101, Heidelberg Instruments Mikrotechnik GmbH, Heidelberg, Germany), a pattern containing 50 μm squares separated from each other by 10 μm was transferred to the photoresist layer. After the exposure, the top photoresist was developed by 7 g dm^{-3} NaOH solution and the gold layer was etched using conventional KI/I_2 etch solution. The bottom sacrificial layer was then removed using acetone. During this step, the platelets were separated from the glass substrate. Centrifuged platelets were transferred into an aqueous buffer solution ready for the experimentation. The scheme of the fabrication process with the resulting suspension of the gold platelets can be seen in Fig. 1. While several metals, including silver, copper and nickel were tested for fabrication of the electrodes, all but gold exhibited strong electrode corrosion. Hence only gold platelets were used in this study.

Electrochemiluminescence reaction on the gold platelets

ECL measurements were performed in a fused silica capillary with a transparent (acrylic) outer coating (Polymicro Technologies, Phoenix, AZ, USA). The capillary had a length of 8 cm, an inner diameter of 100 μm and an outer diameter of 400 μm. The capillary was mounted on a laboratory-assembled holder, enabling the fine movement of the capillary in the *x* and *y* directions. Once firmly mounted, the capillary was filled with the solution of the ECL reactants and platelets. Both ends of the capillary were immersed in 0.5 ml polypropylene vials, serving as reservoirs for the ECL reactants. Platinum wires immersed into the reservoirs served as driving electrodes.

ECL light emission from one particle was collected by a microscope objective 40× and 0.65 N.A. (Oriel, Stratford, CT). The flat surface of gold platelets had excellent optical properties. Here we used the reflectivity of the platelets to locate them within the capillary and positioning the capillary in front of the microscope objective prior to the ECL experiment. Since the ECL signal generated on such a small scale is not easy to observe visually, we first illuminated the platelets with a laser beam and rotated the capillary with the platelet inside to maximize the amplitude of reflected laser light in the direction of the PMT. Once the amplitude of the light was maximized, the laser was turned off and the photomultiplier (PMT) tube R 647-01 (Hamamatsu, Japan) was used for recording of the ECL signal.

The detected amplitude of ECL signal was recorded *via* an A/D converter interface and chromatographic integrator CSW 1.6 (Apex, Prague, Czech Republic) with a sampling frequency of 400 Hz. The ECL reaction was performed with different voltages and pulse durations. An electronically controlled voltage power supply with highest output voltage up to 6 kV provided square wave pulses, adjustable in 1 ms increments from 10 ms to 999 ms. The measurement setup is schematically shown in Fig. 2. The whole system was enclosed in a black box and covered with black fabric during the measurements, thus protecting the set-up from ambient light.

ECL reaction of the tris(2,2′-bipyridyl) dichlororuthenium(II) hexahydrate $[Ru(bpy)_3]^{2+}$ with co-reactant 2-(dibutylamino)ethanol DBAE was selected as an indicator of the

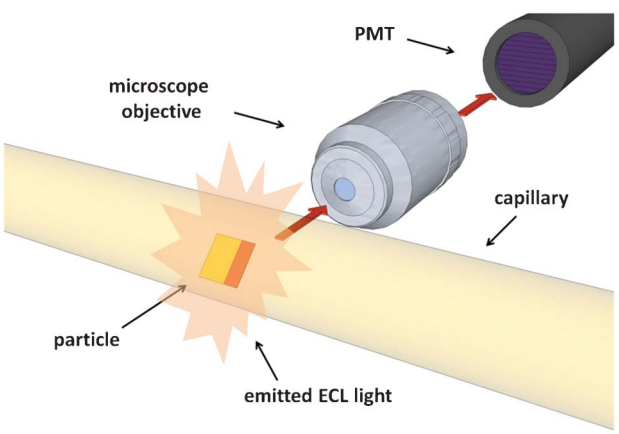

Fig. 2 Schematic diagram of the detection system. The particle was positioned inside the transparent capillary filled with the ECL reactants. Both ends of the capillary were immersed into reservoirs that were connected to a high voltage power supply. The application of electrical voltage across the capillary resulted in the ECL reaction accompanied by light emission that was collected by the PMT.

electrochemical reaction. We decided to use DBAE for its higher selectivity and wider dynamic range, compared to the predominantly used tripropylamine (TPA). Additionally, DBAE is less toxic and volatile than TPA and does not require high buffer concentrations.[20] The reaction mechanism of the $[Ru(bpy)_3]^{2+}$/DBAE system has been already described earlier.[21] Since the metal platelet has lower electrical resistance than the surrounding electrolyte solution, most of the current will be transported by the platelet. This electric current will polarize the metal platelet and create an overpotential at each platelet side. When this overpotential reaches the redox potential of the $[Ru(bpy)_3]^{2+}$/DBAE system the reactants are electrooxidized on the platelet electrode surface and the reaction is accompanied by ECL light emission.

All measurements were performed using a solution containing 5 mM DBAE and 50 μM $[Ru(bpy)_3]^{2+}$ in 13 mM TRIS–phosphate buffer (pH 7.8). Both ends of the capillary with the platelets were immersed in the electrode reservoirs. Application of the voltage will create potential drop along the buffer solution with ECL reactants inside the capillary. Consecutively, each of the metal platelets present in the capillary will be polarized. When increasing the applied voltage the overpotentials on the platelet electrode will increase accordingly. Once sufficient overpotentials are reached, the ECL emission occurs and further increases with increasing electric field. The magnitude of the overpotentials depends on the length of the platelet and the potential gradient along the capillary; as described previously.[22]

Results and discussion

The first experiment was done using a DC power supply but the electrolyte inside the capillary with inner diameter of 100 μm turned out to be conductive and corresponding amplitude of Joule heat lead to the boiling of the electrolyte. We then used a pulsed voltage source. It eliminated the problem of capillary overheating as the duration between two pulses allowed the electrolyte to cool down.

Fig. 3 shows light emissions generated at the surface of gold platelets as a result of the ECL reaction between $[Ru(bpy)_3]^{2+}$ and DBAE. A series of pulses with increasing durations were recorded at different voltages applied at the capillary. The first pulse length was 0.1 s and every 5 s another pulse was applied with 0.1 s longer duration (0.1 s–0.9 s) (Fig. 3A–B).

There was no detectable light emission when there was no platelet inside of the capillary or when the platelet was outside of the objective focus. We tested different voltages, starting at 2.4 kV and the minimum voltage at which we could detect the ECL emission was 3.5 kV, applied on the capillary for at least 0.5 s (Fig. 3A). This corresponds to the electric field strength of 437.5 V cm^{-1} in the capillary containing the 50 μm square platelet. As mentioned before, generation of the ECL light emission depends on the overpotentials at the ends of the gold platelet which depends on both the platelet length and the potential gradient along the capillary. Since the size of the platelet is meant to be fixed for future analytical applications, we studied the effect of increasing the applied voltage to 3.75 kV (Fig. 3B). This allowed us to detect higher signals compared to the signal collected at 3.5 kV, which is in agreement with

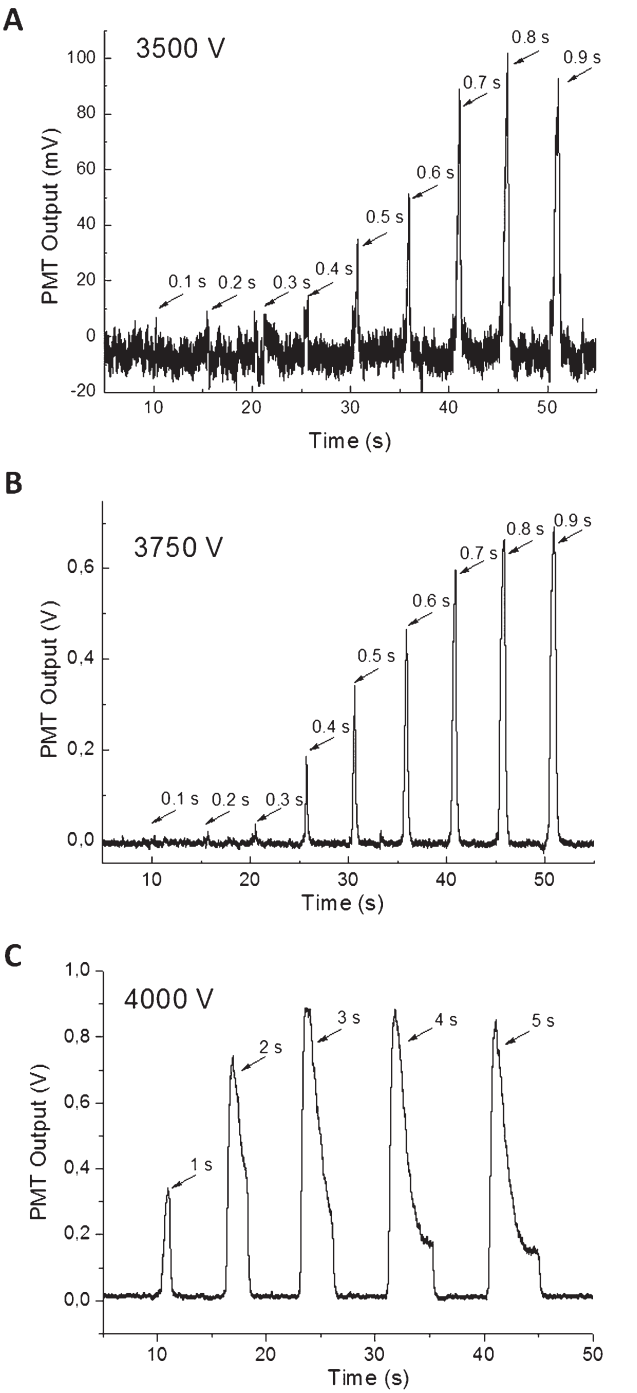

Fig. 3 The dependence of the ECL emission on the applied voltage and time. Comparison of the light emission signals from a gold platelet with size of 50 μm × 50 μm and thickness of 0.3 μm. Different high voltage pulses were applied between two driving electrodes (A: 3.5 kV; B: 3.75 kV; C: 4 kV) in increasing time periods, ranging from 0.1 s to 5 s (as indicated at the peaks) and light emission signals were collected. ECL active solution was 5 mM DBAE and 50 μM $[Ru(bpy)_3]^{2+}$ in the 13 mM TRIS–phosphate buffer (pH 7.8).

theoretically predicted behaviour. Increasing the applied voltage at the capillary ends results in increased overpotentials on the platelet. When combining an applied voltage of 4 kV with pulse duration starting at 1 s and every 5 s increased by another 1 s (1 s–5 s), the light emission reached a maximum with the signal decreasing during the following pulse (Fig. 3C). Since both of the components, $[Ru(bpy)_3]^{2+}$ and DBAE are transported to the electrode mainly by diffusion, the supply of reagents for the ECL reaction is diffusion limited. In the first set of experiments (Fig. 3A–B) with maximum duration of the pulses 0.9 s, the reaction components were not exhausted and were supplied in sufficient amounts by diffusion, resulting in peak heights increasing with pulse voltage and time until a plateau was reached. In the second set of experiments with pulse duration ranging from 1 s to 5 s, the reagents in close proximity to the platelet were consumed, leading to the signal decrease by the end of the pulses. The signal decreased until equilibrium was reached where all of the components brought to the electrode surface (diffusion, residual flow) were immediately consumed by the ECL reaction. Further increase of the pulse duration was not possible due to the Joule heating causing bubble formation and disruption of the electric current.

Conclusions

We have shown that metal platelets can be positioned inside an electrophoretic channel with the applied voltages corresponding to electric field strengths typical in electrokinetic separations. The ECL signal was detected with electric field strength amplitude of above 350 V cm^{-1} using square electrodes of 50 μm. It corresponds to 1.75 V voltage drop along the platelets which is in line with results published earlier. We assume that critical ECL field strength in the fluid is dependent on platelet size. Bigger platelets should require lower amplitudes of critical electric fields.

The main advantage presented by freely moving metal platelets is its economical value, as opposed to fixed metal electrodes that cannot be reused. The preparation of fixed metal electrodes has to be integrated with the fabrication of the microfluidic device. Once the electrodes are damaged or contaminated, the entire device has to be disposed. Floating platelets are prepared separately from the particular microfluidic device, thus making them disposable. The platelets are brought into the appropriate locations within the microfluidic device by external forces. If damaged by electrochemical processes, the platelets can be easily replaced with fresh ones, while keeping the original microfluidic device in place. This makes the entire process much more economical as the microfluidic device can be recycled for further experimentation.

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