Electrochemistry

Simultaneous Detection of Single Attoliter Droplet Collisions by Electrochemical and Electrogenerated Chemiluminescent Responses**

Jeffrey E. Dick, Christophe Renault, Byung-Kwon Kim, and Allen J. Bard

Abstract: We provide evidence of single attoliter oil droplet collisions at the surface of an ultra-microelectrode (UME) by the observation of simultaneous electrochemical current transients (i–t curves) and electrogenerated chemiluminescent (ECL) transients in an oil/water emulsion. An emulsion system based on droplets of toluene and tri-n-propylamine (2:1 v/v) emulsified with an ionic liquid and suspended in an aqueous continuous phase was formed by ultrasonification. When an ECL luminophore, such as rubrene, is added to the emulsion droplet, stochastic events can be tracked by observing both the current blips from oxidation at the electrode surface and the ECL blips from the follow-up ECL reaction, which produces light. This report provides a means of studying fundamental aspects of electrochemistry using the attoliter oil droplet and offers complementary analytical techniques for analyzing discrete collision events, size distribution of emulsion systems, and individual droplet electroactivity.

The electrochemistry of discrete events at an electrode surface is important due to the widespread interest in understanding stochastic phenomena as opposed to techniques that involve measurements over ensemble quantities. This research can provide important information on the characteristics of single micro- and nanoparticles, hard or soft, including size distribution and electroactivity. Moreover, understanding discrete events might even have analytical implications and uncover phenomena not found in ensemble measurements. The electrochemistry of single particle collisions has been investigated using various types of particles, such as metal particles (gold,[1] platinum,[2] silver,[3] nickel,[4] copper[5]), oxide particles (iridium oxide,[6] titanium oxide,[7] cerium oxide,[8] silicon oxide[9]), and hard organic particles (polystyrene,[10] indigo,[11] aggregates of fullerene[12]). Recently, we have observed collision events involving soft particles, such as toluene droplets suspended in water, on an ultra-microelectrode (UME).[13] In that study, we suggested the concept of the emulsion oil droplet as an attoliter

electrochemical reactor for the electrochemistry of hydrophobic molecules (such as ferrocene) in an aqueous continuous phase. Thus, the emulsion droplet reactor (EDR) serves as an attoliter electrolysis cell.

Optical techniques, including electrogenerated chemiluminescence (ECL) and fluorescence microscopy, also allow imaging of individual particles[14] and can further be coupled to an electrochemical cell for simultaneous detection using electrochemistry and spectroscopy. For example, we demonstrated previously that 25 nm diameter poly(9,9-dioctylfluorene-*co*-benzothiadiazole) nanoparticles immobilized on an indium tin oxide (ITO) electrode can be individually imaged by ECL.[15] We also previously demonstrated the ECL detection of platinum nanoparticles.[16] By using fluorescence detection, the collisions of polystyrene microbeads labeled with a fluorescent dye could be used to monitor discrete events between the microbeads and an UME by electrochemistry and fluorescence microscopy simultaneously.[17] Simultaneous detection is important because it provides more evidence when distinguishing a collision event from background noise.

Here, we describe a novel method for the simultaneous observation of single attoliter oil droplet collisions on a 50 μm Au working electrode with both electrochemical and ECL detection. When an oil droplet diffuses to the electrode surface, the constituents—an ECL luminophore and a coreactant—are oxidized, and light is generated. Thus, the detection of current through amperometry and the detection of light through the follow-up ECL reaction allow an observable correlation between electrochemistry and ECL. This is the first report of single nano- and micro-sized oil droplets colliding with an electrode and producing electrochemical and ECL signals concurrently.

In the current study, ECL is produced using rubrene as the luminophore and tri-*n*-propylamine (TPrA) as the co-reactant. An emulsion system based on toluene and TPrA (2:1 v/v), emulsified with an ionic liquid, is suspended in water by ultrasonication. The highly hydrophobic rubrene is dissolved only in the toluene phase (ca. 15 mM). The TPrA is mainly contained in the toluene phase (ca. 1.8 M), leaving a small concentration of TPrA (ca. 5 mM) dissolved in the aqueous continuous phase.[18] The emulsion containing rubrene and TPrA is introduced into a spectroelectrochemical cell, where an UME faces a photomultiplier tube (PMT; a schematic diagram of the electrochemical reaction and experimental configuration is shown in Figure S1 in the Supporting Information).

Previously we showed that rubrene and TPrA can be oxidized in toluene droplets at a relatively low potential

[*] J. E. Dick,[+] Dr. C. Renault,[+] Dr. B. K. Kim, Prof. Dr. A. J. Bard
Center for Electrochemistry, Department of Chemistry
The University of Texas at Austin, Austin, TX 78712 (USA)
E-mail: ajbard@cm.utexas.edu

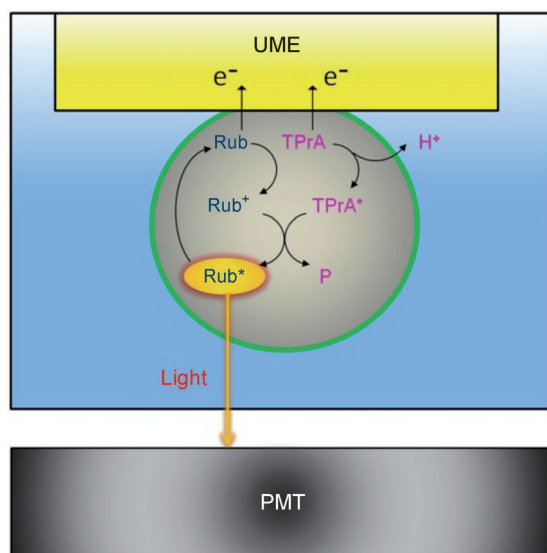[+] These authors contributed equally to this work.

Figure 1. Schematic representation of the mechanism to produce ECL from rubrene (Rub) and TPrA oxidation in a single emulsion droplet on an UME. The light blue and gray colors correspond to the water and toluene phases, respectively. The green line represents the ionic liquid. This figure is not drawn to scale.

(0.82 V versus Ag/AgCl) and produce a strong ECL signal.[18] Therefore, the collision of an individual toluene droplet on the UME was expected to produce discrete current and ECL events. The proposed model for the oxidation of electroactive species in the oil emulsion droplet and the subsequent light emission is shown in Figure 1. First, rubrene and TPrA are oxidized at the electrode. Note that the electron transfer can occur either at the electrode/oil or electrode/oil/water interface.[19] This is currently under investigation for our system. The oxidized TPrA then loses a proton, which balances the charge upon oxidation to yield a strong reducing agent (TPrA·). This latter species reduces the rubrene radical cation to its neutral excited state through an energetic radical annihilation that produces light.[20] At least two electrons are collected at the electrode for every photon emitted in the emulsion droplet.

Figure 2 shows the current (black line) obtained with a 50 µm diameter Au UME biased at 1.1 V versus Ag/AgCl and dipped into a 40 pM emulsion solution in 5 mM pH 7 phosphate buffer (PB). The ECL (red line) signal was simultaneously monitored with the PMT facing the electrode (see Figure S1 in the Supporting Information for details about the experimental setup). Figure 2A shows a clear correlation between the current and ECL spikes. These concomitant current and ECL spikes are attributed to the collision of single oil droplets. Control experiments where the electrode is biased at a potential (0.75 V versus Ag/AgCl) where only TPrA is oxidized, produce current spikes, but no light is observed. This observation supports the mechanism presented in Figure 1, where the oxidation of both rubrene and TPrA is necessary to produce significant ECL. Control experiments where the droplet is loaded with only TPrA also produces current spikes without any generation of light. In the absence of both rubrene and TPrA, neither current nor
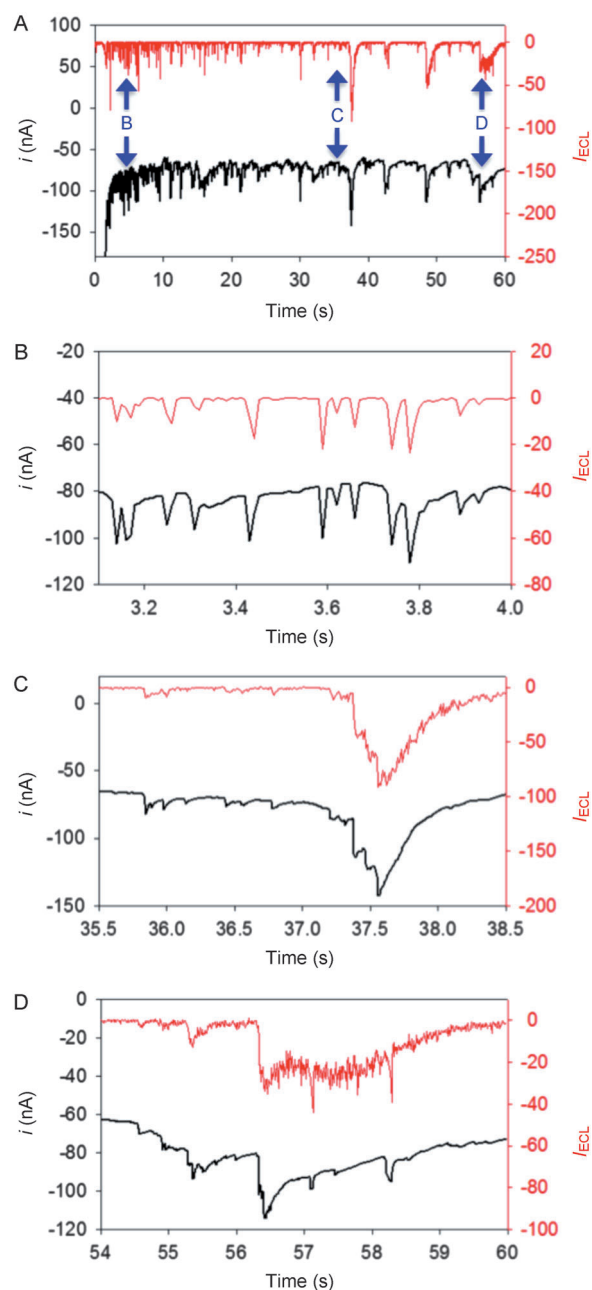

Figure 2. A) ECL intensity (red line) and amperometric *i–t* curve (black line) of a 40 pM emulsion containing 15 mM rubrene and 1.8 M TPrA on a 50 µm Au UME in 5 mM PB (pH 7). The UME is biased at 1.1 V versus Ag/AgCl. B–D) are higher resolution portions of Figure 2A, indicated with the blue arrows.

light spikes are observed. Figure 2A also shows a large current background on the amperometric *i–t* curve, while there is no background on the ECL trace. The background faradaic oxidation of TPrA (see the steady-state current of ca. −60 nA in Figure 2A) dissolved in the aqueous continuous phase does not produce significant ECL.

The relationship between the ECL and current blips is emphasized in Figure 2B–D. As shown in Figure 2B, the detected time, relative intensity, and shape of each current peak (black trace) correlate well with the ECL peaks (red

trace). This observation is in agreement with the mechanism proposed in Figure 1, where the quantity of photons generated by ECL is proportional to the current density at the electrode.[21] A few peaks may show multiple collisions within a short time period, as shown in Figure 2C (36.4 to 38.5 s) and 2D (56.3 to 58.3 s), where the amperometric i–t curve shows convoluted peaks that lead to a broader current signal. In general, the shape of the current signal matches well with the ECL trace, which suggests that each of the events effectively corresponds to single droplet collisions.

The diameters of the emulsion droplets, measured by dynamic light scattering (DLS), are plotted in Figure 3 (black trace). This ensemble-type measurement shows a bimodal distribution of droplet sizes centering on diameters of roughly
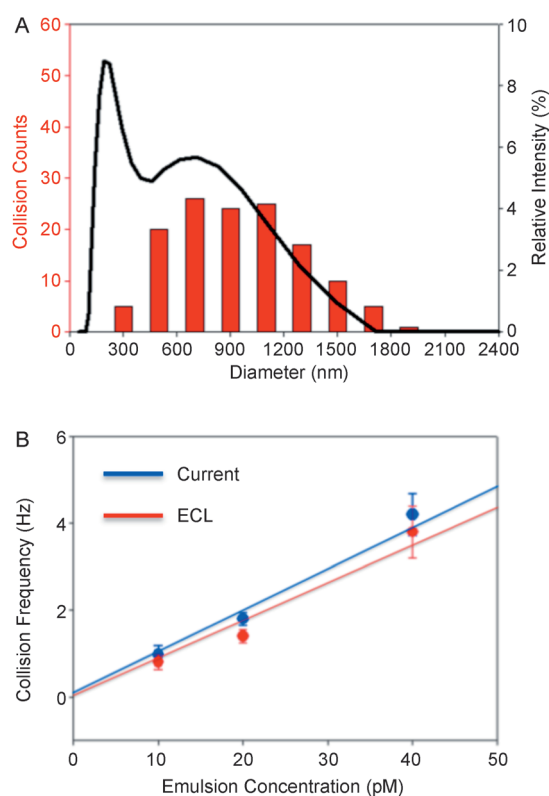

Figure 3. A) Comparison of the emulsion diameter calculated from Equation (1) (red bars) and DLS data (black solid line). B) Plots of collision frequency as a function of the droplet concentration for current and ECL.

250 nm and 730 nm. These diameters correspond to droplet volumes of approximately 8 aL and 20 fL, respectively. The size of the droplets can also be estimated from the electrochemical data and the EDR model, assuming total consumption of all the rubrene and TPrA molecules in a collision. The total amount of charge (Q) contained in a droplet is obtained by the integration of single current blips in the i–t curve shown in Figure 2. Equation (1)[13] is then used to calculate the droplet diameter (d_{drop}):

$$d_{drop} = 2\left(\frac{3Q}{4\pi F(n_{Rub}C_{Rub} + n_{TPrA}C_{TPrA})}\right)^{1/3} \qquad (1)$$

where F is the Faraday constant, n is the number of electrons (TPrA is oxidized in an overall two-electron reaction), and C is the concentration of redox species (Rub = rubrene). The droplet is assumed to be spherical, and the electrolysis of rubrene and TPrA is considered complete. The diameter of single droplets, calculated over more than 150 events using Equation (1), is plotted in Figure 3A (red bars). Only one size distribution with an average diameter of 945 nm is observed. This plot matches pretty well with the higher distribution of the DLS data. Under our experimental conditions, the minimum size of a droplet that can be detected by electrochemistry and ECL is about 300 nm. The ECL signal produced by 300 nm diameter droplets arises from about 1.3×10^5 molecules of rubrene in a 14 aL droplet.

The collision frequency for both the current measurement and the ECL measurement is shown in Figure 3B as a function of the droplet concentration. The similar values of frequency obtained from the current and ECL measurements reflect the good correlation between the two techniques. A rough estimation of the collision frequency (f^{dif}) based on diffusion was calculated with Equation (2), assuming every collision is detected for droplets above 300 nm:

$$f^{dif} = 4D_{drop}C_{drop}r_{elec}N_A \qquad (2)$$

where D_{drop} is the diffusion coefficient, calculated by the Stokes–Einstein relation, of a 945 nm diameter droplet (5×10^{-9} cm^{2}s^{-1}), C_{drop} is the concentration of droplets, r_{elec} is the radius of the electrode, and N_A is Avagadro's number (see the Supporting Information for details about the values of D_{drop} and C_{drop}). The calculated frequency is about 1.2 Hz for a 40 pM concentration of droplets on a 50 μm diameter Au UME. The actual current blip frequency for the 40 pM concentration (4.2 ± 0.49 Hz) is four times larger than the calculated frequency. This discrepancy could arise from uncertainty in the value of C_{drop} or migration effects (the zeta potential of the droplet is −14 mV). The frequency was calculated by counting blips (in the current and ECL) that were at least three times the background noise.

In summation, we have provided evidence for a clear correlation between current blips and ECL blips when tracking stochastic collisions of attoliter oil droplets loaded with electroactive species. When a droplet collides with the electrode, its constituents are oxidized at the applied potential, which generates a current blip. If the constituents within the droplet are an ECL luminophore and a co-reactant, the follow-up reactions produce light in accord with reported ECL mechanisms,[21] thereby allowing the simultaneous observation of nano- and micro-sized droplet collisions using electrochemistry and ECL. Although the amperometric i–t curve presents a large background current, we showed that the ECL signal is essentially background-free and particularly suitable for sensing applications. Thus, this novel technique allows two different, simultaneous measurements to look at individual emulsion droplets, calculate their size, and also analyze their contents. This research should have wide implications in studying fundamental aspects of electrochemistry as well as providing a basis upon which analytical sensor

techniques can be built using single attoliter droplet electrochemistry and ECL.

Experimental Section

The emulsion preparation is based on a previously reported method.[13,18] In brief, deionized Milli-Q water (5 mL, > 18 MΩ cm, MilliQ Reagent Water System, Millipore, Billerica, MA) was added to a vial containing 15 mm rubrene (100 µL, Aldrich, St. Louis, MO) in toluene (> 99.9 %, Sigma Aldrich, St. Louis, MO). Then, the ionic liquid trihexyltetradecylphosphonium bis(trifluoromethylsulfonyl)-amide (30 µL, > 95 %, Aldrich, St. Louis, MO) and TPrA (50 µL, TCI co. LTD, Tokyo, Japan) were successively injected in the vial. The contents of the vial were sonicated with a horn ultrasonicator (Q500 ultrasonic processor with a microtip probe, Qsonica, Newtown, CT) to form a finely dispersed emulsion with a milky orange color. The droplets size distribution was measured by dynamic light scattering (Zetasizer Nano ZS, Malvern, Westborough, MA) less than 1 h after the sonication of the emulsion. The experimental setup is shown in Figure S1 in the Supporting Information. Briefly, a 1 cm path length plastic cuvette (Fisher Scientific, Waltham, MA) was used to fabricate the spectroelectrochemical cell. The UME was inserted in the cuvette through a hole drilled in one face of the cuvette. The distance between the surface of the UME and the opposite cuvette wall is about 1–2 mm. The cell was filled with a 5 mm phosphate buffer pH 7 solution (2 mL, KH_2PO_4 and K_2HPO_4 were purchased from Sigma Aldrich, St. Louis, MO) and then an appropriate volume of the stock solution emulsion (at 110 pm) was added to achieve the desired final concentration in the cell. A platinum wire (counter electrode) and Ag/AgCl 1m KCl reference electrode (CH Instrument, Austin, TX) were used. The PMT (Hamamatsu R4220, Tokyo, Japan) was powered at −700 V using a Kepco DC power supply (New York, NY). The signal of the PMT was read with an electrometer (Keithley 6517, Solon, OH) and then digitalized using an analog to digital converter (ADC 164, Eco Chemie Autolab, Utrecht, Netherlands) integrated with the potentiostat (Eco Chemie Autolab PGSTAT100, Utrecht, Netherlands). The potentionstat simultaneously read the current and signal from the electrometer.

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