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Electrochemical Oxidation of 5-Hydroxytryptamine
and 5-Hydroxyindoleacetic Acid at Ultrasmall
Gold Ring Electrodes

by

Y. Y. Lau, D. K. Y. Wong, G. Luo, A. G. Ewing

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Department of Chemistry
Penn State University
University Park, PA 16802

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ELECTROCHEMICAL OXIDATION OF 5-HYDROXYTRYPTAMINE
AND 5-HYDROXYINDOLEACETIC ACID AT
ULTRASMALL GOLD RING ELECTRODES

Yau Yi Lau, Danny K.Y. Wong, Guoan Luo and Andrew G. Ewing*

Department of Chemistry
152 Davey Laboratory
Penn State University
University Park, PA 16802

Received _____

*To whom correspondence should be addressed.

ABSTRACT

Ultrasmall gold ring electrodes have been constructed by electrodeposition of gold onto carbon ring electrodes. Electrodes thus formed show similar adsorption/desorption peaks for oxygen in H_2SO_4 solution compared to those obtained at conventional-sized electrodes. These electrodes have been used in conjunction with linear scan voltammetry and subsequent cleaning pulses to obtain repeatable quantitative results for the electrochemical oxidation of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid. This technique involves the use of a linearly scanned applied potential, followed by a large positive potential to clean the electrode oxidatively and negative potential for electrode activation. The result of the cleaning pulses is that the oxidation products of 5-hydroxytryptamine are cleaned off the electrode and reproducible voltammograms are obtained.

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INTRODUCTION

5-Hydroxytryptamine (5-HT) is a neurotransmitter present in high concentrations in mammalian hypothalamus, midbrain and brain stem, while 5-hydroxyindoleacetic acid (5-HIAA) is its major metabolite [1]. 5-HT is stored in synaptic vesicles in nerve endings and has been implicated in such illnesses as insomnia, hyperthermia, and schizophrenia as well as sensitivity to pain, control of blood pressure and regulation of sleep mechanisms [1]. Methods to directly monitor the concentration of 5-HT in neurons and relate this information to its neuronal activities would be extremely useful in the eventual understanding of 5-HT as a neurotransmitter. Although 5-HT is easily oxidized at physiological pH, it has been difficult to carry out quantitative studies of the electrochemical oxidation of 5-HT. The voltammetric signal is observed to decay rapidly during repeated voltammograms. The oxidation products of 5-HT have been reported to form an insulating film on the electrode surface [2,3], thus hindering the diffusion of 5-HT to the electrode and causing a deterioration of the voltammetric signal. Baur et al. [4] have recently demonstrated the use of fast scan cyclic voltammetry (200 V s^{-1}) at carbon fiber electrodes to obtain reproducible voltammetry of 5-HT for at least fourteen successive scans. The oxidation products formed during the forward scan in this method are rapidly converted back to reactant on the reverse scan, not allowing sufficient time for passivation.

Johnson and coworkers [5-14] have developed a pulsed amperometric detection technique for HPLC that minimizes electrode fouling while maintaining the reactivity of gold and platinum electrodes in experiments involving aliphatic solutes. In this technique, a sequence of three potential pulses is used to obtain each current point. After an oxidation detection potential pulse, a positive potential oxidatively cleans the electrode surface, followed by a negative potential which dissolves the passivating oxide layer formed during cleaning and restores the native reactivity of the clean metal surface. Successive electrochemical pretreatment steps prior to each current measurement result in continuous amperometric monitoring of several species ordinarily not determined by voltammetry. Pulse amperometric detection has been incorporated in liquid chromatography as a useful post-column detection technique for the determination of carbohydrates, amino acids and sulphur compounds [5, 9, 11, 15]. A method involving pulsed activation of platinum or gold electrodes combined with sweeping the potential to obtain voltammetric information for both qualitative and quantitative analysis has not been demonstrated to date.

In this paper, procedures to construct and briefly characterize ultrasmall gold ring electrodes are described. These electrodes are then used in conjunction with integrated pulse linear scan voltammetry to minimize electrode passivation during electrochemical oxidation of 5-HT and 5-HIAA.

EXPERIMENTAL

Chemicals. Dopamine (3,4-dihydroxyphenethylamine), 5-HT and 5-HIAA, (Sigma Chemical Co., St. Louis, MO), were used without further purification. All solutions were prepared with doubly distilled water (Corning Mega-Pure MP-3A). Citrate/phosphate buffer was used to prepare fresh dopamine, 5-HT and 5-HIAA solutions immediately before voltammetric measurements. 1.0×10^{-3} M AuCl_3 was prepared by dissolving gold powder (20 mesh, 99.9%, Aldrich Chemical Company, Inc., Milwaukee, WI) in aqua regia. All solutions were purged with nitrogen for 20 min prior to voltammetry. After deaeration, a blanket of nitrogen was kept over the solutions.

Electrodes and Apparatus. Ultrasmall carbon ring electrodes were constructed and voltammetrically tested as described previously [16-18]. Carbon ring electrodes were initially tested in 1.0×10^{-4} M dopamine in pH 7.4 citrate/phosphate buffer. Only electrodes which showed a well-defined sigmoidal response in the cyclic voltammograms for oxidation of dopamine with very little capacitive charging current were used. These electrodes were coated with gold (1.0×10^{-3} M AuCl_3 in 0.50 M H_2SO_4) by reduction at -0.8 V vs SSCE.

Cyclic voltammetry and integrated pulse linear scan voltammetry were carried out with an EI-400 potentiostat (Ensmann Instrumentation, Bloomington, IN). Potential waveforms for integrated pulse linear scan voltammetry and data acquisition were

carried out with an IBM personal computer and a commercial interface (Labmaster, Scientific Solutions, Solon, OH). Electrochemical experiments employed a cell consisting of a 30 mL glass vial, filled to 25 mL, with holes drilled in a plastic cap to facilitate a three-electrode system. A sodium chloride saturated calomel electrode (SSCE) was used as the reference electrode and a platinum wire as the auxiliary electrode. All experiments were carried out in a copper mesh Faraday cage at room temperature.

RESULTS AND DISCUSSION

Characterization of gold electrodes. The deposition procedure used for coating gold onto carbon ring electrodes corresponds to a successive irreversible reduction of Au^{3+} to Au^0 resulting in a gold film thickness that is dependent on deposition time. Figure 1 shows the background cyclic voltammogram obtained for a 2 μm gold plated carbon ring electrode in 0.50 M H_2SO_4 , after a deposition time of 15 s. The observed anodic and cathodic peaks correspond to adsorption and desorption of oxygen on the gold electrode surface. These features of the voltammogram are similar to those obtained at larger diameter ultrathin gold ring electrodes [19] and conventional sized gold disk electrodes [20].

Figure 1

Figure 2 shows a cyclic voltammogram obtained with a 2 μm

gold-coated ring electrode in 1.0×10^{-4} M dopamine solution. These voltammograms are consistently sigmoidal indicating that the gold deposition procedure does not result in a damaged or leaky electrode. The combined observation of the classical oxygen waves in H_2SO_4 and the sigmoidal voltammetry in neutral dopamine solutions indicates that we have successfully deposited gold onto the carbon ring electrode.

Figure 2

The small structural dimension of the electrodes employed here precludes morphological examination by conventional microscopy. It is also difficult to determine the thickness of the carbon film. This thickness could potentially be different even for electrodes of similar total structural diameters. Hence, electrodeposition has been carried out potentiostatically for specific durations in this work, rather than galvanostatically. We have found it sufficient to deposit gold, for durations ranging from 15 s to 1 min, onto carbon ring electrodes of 1-5 μm structural diameter. Cyclic voltammograms obtained at these electrodes then show the characteristic adsorption/desorption peaks of oxygen in sulphuric acid with a sigmoidal voltammogram with little charging current obtained for the oxidation of dopamine in pH 7.4 citrate phosphate buffer.

Integrated Pulse Linear Scan Voltammetry. The waveform for integrated pulse linear scan voltammetry is shown in Figure 3. In this technique, voltammograms are recorded as the potential is swept linearly from an initial potential (E_i) to a final potential (E_f). The potential is then stepped to a large anodic potential (E_a) for oxidative cleaning of the electrode surface and subsequently to a cathodic potential (E_c) for reactivation by dissolution of surface oxide. Voltammograms obtained at ultrasmall gold ring electrodes using this waveform show well-defined sigmoidal responses for the oxidation of dopamine. Integrated pulse linear scan voltammetry can provide voltammetric information which is not usually obtained with pulsed amperometric detection alone. The pulses after each scan maintain the reactivity of the electrode surface and thus provide more sensitive and reproducible measurements which cannot be achieved by voltammetry alone for the oxidation of 5-HT and 5-HIAA.

Figure 3

Voltammetry of Hydroxyindoles. Voltammetric studies of 5-HT have been hindered by the adsorption of oxidation products to form a passivating layer on the electrode surface. Figure 4 shows the 1st, 10th and 20th successive conventional linear scan voltammograms for the oxidation of 20 μ M 5-HT and 20 μ M 5-HIAA in pH 7.4 citrate/phosphate buffer at gold ring electrodes. As expected, the oxidation currents for both 5-HT and 5-HIAA decay

rapidly with repeated scans due to the formation of an insulating film on the electrode surface.

Figure 4

Figure 5 shows the 1st, 10th and 20th successive integrated pulse linear scan voltammograms for the oxidation of 5-HT and 5-HIAA at gold ring electrodes under the same conditions used in Figure 4. A well-defined sigmoidal wave with a relatively flat baseline is obtained for all the 5-HT voltammograms. In contrast, voltammograms for 5-HIAA appear to be less Nerstian and do not achieve a limiting plateau in the potential range used. In both cases, however, there is minimal deterioration of oxidation current for the twenty successive scans in Figure 5. Further, for oxidation of either 5-HT and 5-HIAA, after two hundred repeated voltammograms the current is diminished by only 25% and 5%, respectively. These observations reflect a relatively effective cleaning and reactivity restoration of the gold electrode surface by E_a and E_c in integrated pulse linear scan voltammetry. Plots of oxidation current at +0.50 V vs concentration for 5-HT and 5-HIAA, concentrations from 10 to 100 μM and 10 to 200 μM , respectively, are linear (slope = 1.6 $\mu\text{A}/\text{M}$ and 3.3 $\mu\text{A}/\text{M}$; correlation coefficients are 0.9972 and 0.9955 for 5-HT and 5-HIAA, respectively). At lower concentrations the small amount of oxidation products of 5-HT and 5-HIAA formed on the electrode can be easily removed by the pulses. However, as the concentrations of 5-HT get higher than 100 μM , peak

shaped voltammograms are obtained and calibration plots become nonlinear. Although peak shaped voltammograms are not obtained as the concentration of 5-HIAA get higher, a deviation from linearity is observed at concentrations higher than 200 μM . The peak shaped voltammograms obtained at higher concentrations appear to result from formation of an insulating film resulting from an increase in adsorption of oxidation products of 5-HT. Apparently, the cleaning and activation pulse scheme used is not sufficient to completely offset passivation of the electrode surface at these concentrations. Optimuzation of the cleaning pulse length and potential might result in an ability to carry out repeated voltammetry in more concentrated solutions; however, we have not been successful in our attempts to date.

Figure 5

CONCLUSIONS

A simple procedure to construct ultrasmall gold ring electrodes has been reported. Voltammograms of 5-HT and 5-HIAA have been examined at these gold ring microelectrodes. A technique involving cleaning pulses integrated between linear scan voltammetry scans has been used to minimize electrode fouling during oxidation of these substances. This procedure allows 20 repeated voltammograms to be carried out in 5-HT and 5-HIAA without any significant fouling of the electrode and reduces electrode

fouling to only 25% and 5% of signal lost after 200 voltammograms in 5-HT and 5-HIAA, respectively. This is an important step in developing schemes to dynamically monitor the neurotransmitter, 5-HT, and metabolite, 5-HIAA, in microenvironments.

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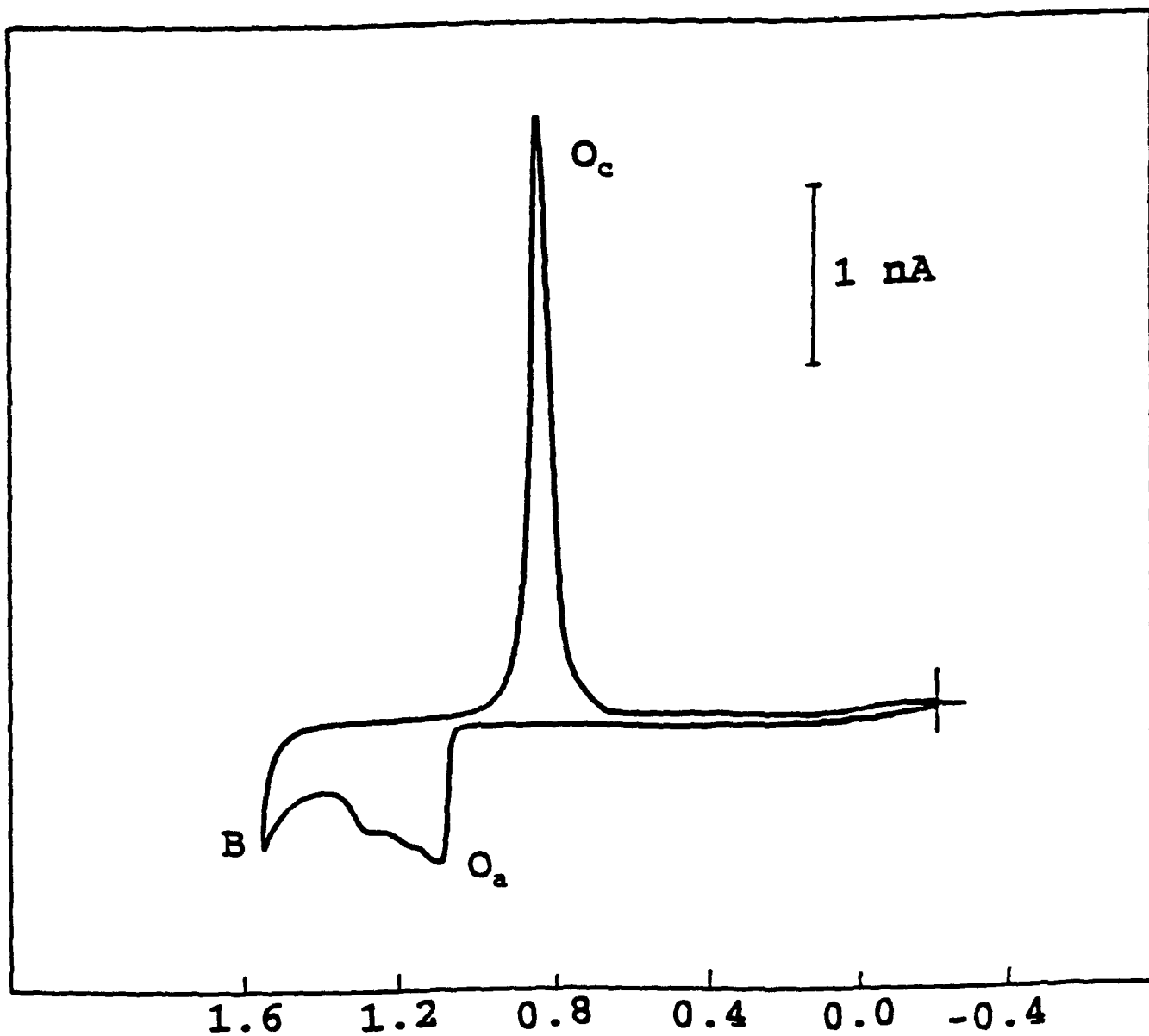
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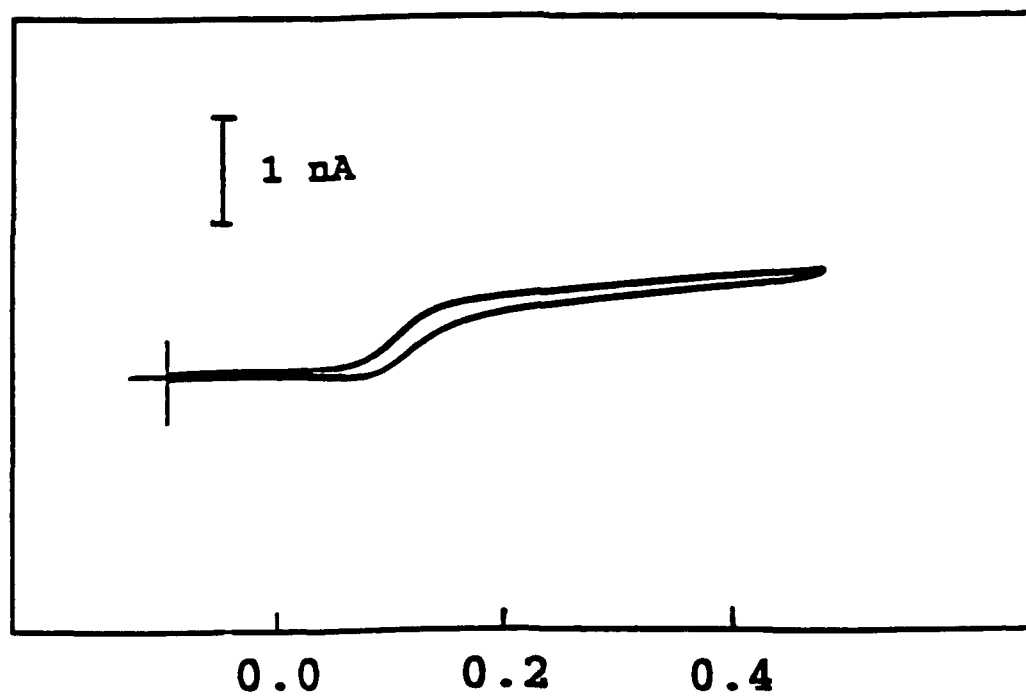
FIGURE CAPTIONS

- Figure 1 Cyclic voltammetry in 0.50 M H_2SO_4 at a 2 μm tip diameter gold-coated ring electrode. Gold was deposited from AuCl_3 for 15 s. Scan rate = 200 mV s^{-1} . Peak predictions: O_c , ion of reduction of oxide layer; O_a , formation of adsorbed oxygen or gold oxide; B, bulk evolution of oxygen. Oxidation current is plotted down and to the left.
- Figure 2 Cyclic voltammogram of 1.0×10^{-4} M dopamine in pH 7.4 citrate/phosphate buffer at a 2 μm total tip diameter gold-coated ring electrode. Scan rate = 100 mV s^{-1} . Oxidation current is plotted up and to the right.
- Figure 3 Schematic diagram illustrating the waveform used for integrated pulse voltammetry. E_a denotes an oxidative cleaning potential held for a time of t_a , E_c an electrode reactivation potential held for t_c , while the detection voltage is ramped from the initial voltage E_i to a final voltage E_f .
- Figure 4 Repeated linear scan voltammograms obtained at gold-coated carbon ring electrodes. Voltammograms shown are the 1st (solid line), 10th (open circles) and 20th (solid dots) scans obtained. (A) 20 μM 5-HT at a 2 μm diameter electrode, (B) 20 μM 5-HIAA at a 4 μm diameter electrode. Scan rate = 200 mV s^{-1} .

Figure 5 Repeated integrated pulse linear scan voltammograms obtained at gold-coated carbon ring electrodes. Voltammograms are the 1st (solid line), 10th (open circles) and 20th (solid dots) scans obtained. (A) 20 μM 5-HT at a 2 μm diameter electrode, (B) 20 μM 5-HIAA at a 4 μm diameter electrode. Scan rate = 200 mV s^{-1} . $E_a = +1.2 \text{ V}$, $E_c = -0.3 \text{ V}$, $t_a = t_c = 100 \text{ ms}$.



E vs SSCE (V)



E vs SSCE (V)

