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The Development and Characterization of Dual Electrodes for Use with Scanning Electrochemical Microscopy

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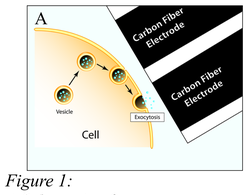
Abstract

Scanning Electrochemical Microscopy (SECM) utilizes electrode probes that can be positioned just abutting cells and can detect the presence of small quantities of chemicals known as neurotransmitters. In order to detect neurotransmitter release from a cell, the electrode must be positioned very close to the cell surface to detect the change in current that accompanies the oxidation/reduction of the neurotransmitters released. The peaks in current were analyzed in order to detect differences in release mechanisms, such as slow or fast-release. Furthermore, dual electrodes were made and characterized in order to monitor two different locations on a cell simultaneously. They can also be modified to observe two different electrochemical processes, such as amperometry and potentiometry.

Introduction

Carbon-fiber electrodes are fabricated and then characterized using cyclic voltammetry. Cyclic voltammetry is useful to characterize dual electrodes since it makes sure that both carbon fibers have the same response to the same voltage conditions applied, and reveals the size of the electrochemical surface. After characterization, Scanning Electrochemical Microscopy (SECM) is used to monitor neurotransmitter release from PC12 cells. PC12 cells are excellent model neurons since they can synthesize, store, and release a variety of neurotransmitters (such as dopamine or norepinephrine) upon KCl stimulation. The probe of the SECM is able to make multiple simultaneous electrochemical measurements. This allows for careful positioning of the electrode and detection of neurotransmitter release.

Dual electrodes are useful since they can monitor two different locations on one cell or monitor two different processes simultaneously. For example, one carbon fiber electrode can be modified to record pH measurements, while the other can remain unmodified to measure the current.



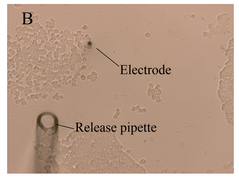


Figure 1: A.Schematic of neurotransmitter release detected by a dual electrode. B. Photograph of PC12 cells.

Experimental

Dual electrodes were constructed with two 7 µm diameter carbon fibers (Thornel T650, Cytec Industries) and beveled at 90^0 on a diamond polishing wheel (model BV-10, Sutter Instrument Company).[2] Cyclic Voltammetry was used to characterize the electrodes. An Ag/AgCl reference electrode and a platinum wire counter electrode were used. The potential of the working electrode was cycled from −0.5 V to +0.2 V, at a sweep rate of 0.1 V/s. A solution of ruthenium hexaamine trichloride, iridium oxide (IrO_2), and hydrosulfuric acid were used to test the electrodes. After characterization, amperometric measurements were made. Voltammetric measurements were conducted with a bipotentiostat in the 3-electrode mode (EI400, Cypress Systems), and potentials were reported against an Ag/AgCl reference electrode. Experiments were conducted in Hanks buffer at pH 7.2. Current and impedance measurements were monitored simultaneously during dopamine release, which was initiated by a 100 mM KCl stimulus.

Figure 2: Multicomponent Imaging setup.

Figure 3: Dual carbon fiber electrode with each fiber about 7µm in diameter. .

Results and Discussion

Amperometry can be used to detect neurotransmitter release from PC12 cells.

A single carbon fiber electrode can be used to detect and monitor neurotransmitter release from PC12 cells. The electrode is kept at a constant oxidizing potential, which oxidizes the neurotransmitter being released. The tall and sharp shape of the current peaks indicate that vesicular release is occurring. Figure 4 shows vesicular release as detected by a single carbon fiber electrode.

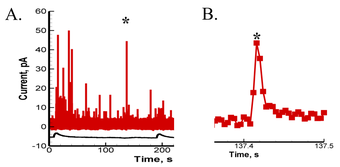


Figure 4: A. Vesicular release from a PC12 cell. B. Zoomed in release peak.

Cyclic Voltammetry can be used to characterize electrodes.

The dual electrodes are first tested in ruthenium hexaamine trichloride to make sure that each electrode measures the same change in current as the potential sweeps from -0.5 V to +0.2 V (Figure 5). Then, iridium oxide is deposited onto the carbon fiber as shown in Figure 6.

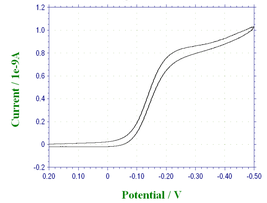


Figure 5: Electrode test in Ruthenium hexaamine trichloride.

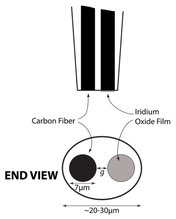


Figure 6: The deposition of Iridium Oxide on a carbon fiber electrode.

Dual electrodes are able to monitor neurotransmitter release at two different locations on the same PC12 cell.

Dual electrodes are useful since they can monitor neurotransmitter release at two different locations on the same cell simultaneously. Figure 7 shows two different patterns of vesicular releases on the same PC12 cell detected by two different carbon fiber electrodes.

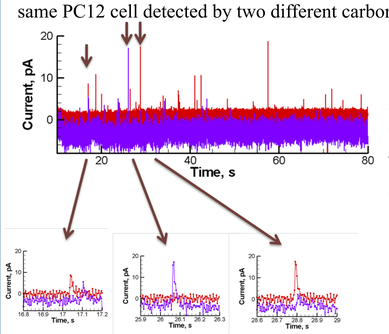
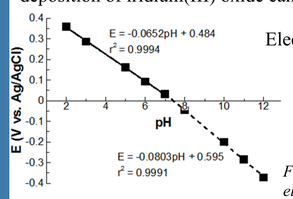


Figure 7: Vesicular release by a PC12 cell. The red lines represent the signals from one electrode and the purple represent the signals from the other electrode.

Modified dual electrodes can monitor pH and current changes simultaneously.

An unmodified electrode can monitor the change in current, while a modified electrode can monitor the pH change during neurotransmitter release. This is useful because two different electrochemical processes can be monitored simultaneously. Also, the pH change from the deposition of iridium(III) oxide can be detected by the pH electrode.



Electrodeposited Hydrous Iridium Oxide

$$2IrO_2 + 2H^+ + 2e^- \rightleftarrows Ir_2O_3 + H_2O$$

Figure 8: The calibration of the electrode's response to pH changes.

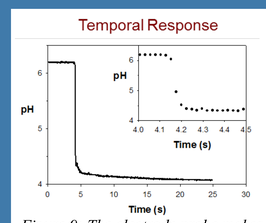


Figure 9: The electrode probe makes a rapid response to a pH change.

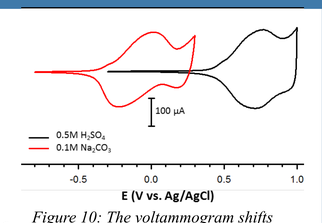


Figure 10: The voltammogram shifts based on the pH.

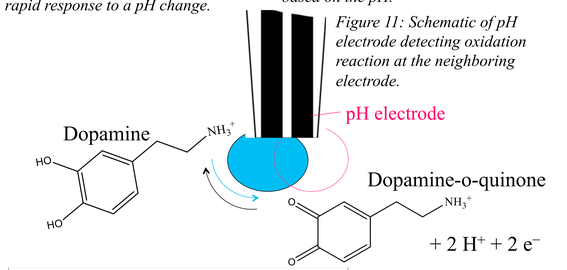


Figure 11: Schematic of pH electrode detecting oxidation reaction at the neighboring electrode.

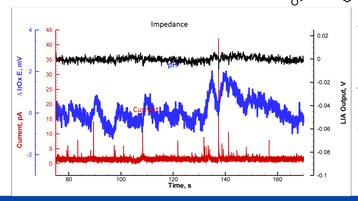


Figure 12: Graph depicting vesicular release from an undifferentiated PC12 cell. A decrease in pH is due to the oxidation reaction of dopamine at the neighboring electrode.

Conclusion

Amperomertry can be used to detect neurotransmitter release from the cells. Dual electrodes can be characterized for accuracy using cyclic voltammetry. Dual electrodes are useful because they can monitor two different locations on one cell or two different processes simultaneously. In the future, fast scan cyclic voltammetry can be used to distinguish between different neurotransmitters since each neurotransmitter has a distinct voltammogram.

References

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2. Kelly R.S. and Wightman R.M. "Beveled carbon fiber ultramicroelectrodes." *Analytica Chimica Acta.* (1986): 187:79-87.
3. J.E. Baur and T.W. Spaine, *J. Electroanal. Chem.* 443 (1998): 208-216.

Acknowledgments

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Dr. John Baur and Dr. Melinda Baur