# AN OVERVIEW OF CARBON FIBER ELECTRODES USED IN NEUROCHEMICAL MONITORING

by

Melissa J. Buckshire

B. S. Chemistry, B. S. Physical Science, Concordia University-Nebraska 2006

Submitted to the Graduate Faculty of

Arts and Sciences in partial fulfillment

of the requirements for the degree of

Master of Science, Chemistry

University of Pittsburgh

2008

UNIVERSITY OF PITTSBURGH

FACULTY OF ARTS AND SCIENCES

This thesis was presented

by

Melissa J. Buckshire

It was defended on

June 13, 2008

and approved by

Shigeru Amemiya, Ph.D., Associate Professor

Stephane Petoud, Ph.D., Associate Professor

Thesis Director: Adrian C. Michael, Ph.D., Associate Professor

**AN OVERVIEW OF CARBON FIBER ELECTRODES USED IN NEUROCHEMICAL MONITORING**

Melissa J. Buckshire, M.S.

University of Pittsburgh, 2008

Neurochemistry has always been a topic that many scientists are interested in researching because the brain is such a fascinating and complex organ. Electrochemical methods have proven to be a successful tool for scientists to use for their brain-researching endeavors. Many types of probes and analytical devices have been invented and used in conjunction with electrochemical methods over the past several decades to investigate the inner workings of the brain. In particular, the carbon fiber electrode has become a popular device among scientists due to its favorable qualities.

The carbon fiber electrode has several unique characteristics to give it an advantage over other techniques. Carbon fiber electrodes have the ability to monitor in a subsecond time frame and record in real time. Because they are so small, carbon fiber electrodes are also able to sample very small environments, such as a single cell or vesicular volumes, where other devices cannot because they are too big. Evidence has shown that carbon fiber electrodes appear to cause less disruptive tissue damage when implanted into a brain than other devices, for instance a microdialysis probe. On top of that, carbon fiber electrodes are also excellent devices for those seeking greater sensitivity and selectivity by making electrode modifications tailored for the analyte of interest. In addition, carbon fiber electrodes provide a wider range of detectable species, again by simply making slight modifications.

One can clearly see that the future for neurochemical monitoring lies heavily in the hands of the carbon fiber electrode. Its advantages over other devices make it superior in many aspects. Researchers will no doubt continue to use the carbon fiber electrode and keep improving it to make it suitable for countless more experiments.

## TABLE OF CONTENTS

## LIST OF FIGURES

# 1.0 INTRODUCTION

Some advances in medical and clinical care have resulted from innovatively new ways of taking measurements of important biological components. Of particular interest in this paper, is the use of carbon fiber electrodes to make such measurements, one tool that is being used more and more to measure neurochemicals in the brain. The brain is an extremely complex entity that no one knows everything about; however, neuroscientists have taken giant leaps to discovering the intricacies of neurological function using such devices as the carbon fiber electrode (CFE). Since its invention, many other scientists have come along to make improvements in the CFE design to enhance sensitivity and selectivity.

The success of the CFE in neurochemical monitoring is due largely to the electrochemistry principles that are working within the milieu of the brain's environment to generate a measurable signal from a charge-transfer process at the electrode interface. The signal is measured from a change in current or potential, and is proportional to the concentration of the substances within the environment immediately surrounding the electrode (by theory or by calibration). The neuronal environment consists of numerous electrochemically detectable targets of interest to neurochemists, such as glucose, lactate, ascorbate, urate, hydrogen peroxide, oxygen, nitric oxide, simple inorganic ions, catecholamine and indolamine neurotransmitters and their metabolites, and pH. Hence the reason that CFE have been so useful and helpful in giving

scientists a better look at the inner workings of the brain and will continue to do so for many future generations.

# 2.0 CHEMICAL SENSORS AND BIOSENSORS

## 2.1 DEFINITION OF A SENSOR

According to the book Chemical Sensors For I*n Vivo* Monitoring edited by Anthony P. F. Turner, a chemical sensor is "a compact analytical device containing a chemically-sensitive element either integrated with or in intimate contact with a transducer and capable of generating a continuous signal proportional to a specific chemical or group of chemicals (Turner 1993)." Also, according to Turner, a biosensor is a "type of chemical sensor in which the chemically sensitive element is biologically derived (Turner 1993)." A carbon fiber electrode is therefore a chemical biosensor. In the laboratory, CFE's are implanted into the brain, usually of a rat, and connected to a potentiometer or amperometer to record any signals. Once in the brain, the carbon fiber surface is the interface where the charge transfer occurs. The substance of interest becomes either oxidized or reduced near the interface due to either the negative or positive potential (i.e. charge) of the electrode and a transfer of electrons occurs.

Other chemical biosensors besides the CFE used in neurochemical monitoring include the microdialysis probe and electrodes of other materials such as platinum, gold, diamond, and carbon paste to name a few. These biosensors have contributed much to the field of neuroscience. They each have unique capabilities which are most suitable for different situations of neurochemical measurements. Of course, one of the reasons for developing such biosensors

comes from a practical and useful need for them in the medical, or clinical, field. Scientists and doctors are always looking for new and improved ways to monitor patients.

## 2.2 CLINICAL USE FOR SENSORS

In general, the sooner and more precisely a doctor and/or nurses know critical information about a patient, the sooner they can administer proper care and possibly save a life. Knowing information about the patient in real time, as opposed to taking a sample and sending it to a lab to wait for results, can significantly change the outcome of an emergency situation. Biosensors have this capability of monitoring a signal continuously, allowing for real-time analysis.

One good example of a biosensor, which was invented in the 1980's, to help improve medical care, is the blood glucose tester, used by people who have diabetes and must constantly test the level of glucose in their blood because their own bodies lack that ability. One of the more recent versions of a blood glucose tester can take a measurement in a matter of seconds. A person can use, for example, the OneTouch® UltraMini® Blood Glucose Meter by first inserting a test strip into the device. Then only a speck of blood (about 0.5 μL) from the forearm or palm needs to be placed on the test strip. Once this is done, a digital read-out of the blood glucose level is displayed on the screen about 5 seconds later.

Another type of a biosensor which has much potential but has yet to be commercialized, detects the level of lactate in the blood. For a patient suffering from shock due to congestive heart failure, pulmonary edema, hemorrhage, myocardial infarction or septicemia, monitoring the level of lactate is of great importance (Heller et al., 1997). This is because the shock, due to associated anaerobic metabolism, may cause lactic acidosis on a life-threatening scale. Blood

lactate concentrations that surpass 7-8 mM are indicative of inevitable death, thus the advancing increase and decrease in the amount of lactate in the blood is a foreshadowing of the probability of survival. With that being said, if real-time monitoring of lactate levels could be accomplished *in vivo*, then the more quickly hospital personnel can administer proper care upon the detection of an abnormal range in lactate levels and the more likely the patient may survive. These are just two of the many examples that show that biosensors have become and will become very helpful in clinical use and healthcare.

# 3.0 CARBON FIBER ELECTRODES

## 3.1 BACKGROUND

Carbon fiber electrodes were first invented in the late 1970's by François Gonon and colleagues (Ponchon et al. 1979, Gonon 1980). The basic manufacturing of their CFE is as follows: the carbon fiber is threaded through a pulled glass tube with a tapered tip, secured in place with a polyester resin and graphite powder mixture forced down into the tip, a contact wire pushed down into the resin and dried 24 hours or more. The carbon fiber is cut to a length of 0.5 mm prior to use. Gonon et al. took a significant step forward in the fields of neuroscience and electrochemical methods by giving chemists and neuroscientists a new way of probing the inner workings of a brain. In their first experiment, Gonon and colleagues measured oxidation currents of dopamine (DA) using normal pulse polarography and cyclic voltammetry in vitro and *in vivo*. They found that the carbon fiber electrode had a detection limit better than that of the carbon paste electrode. They also determined that, depending on the electrochemical method applied, compounds with similar oxidation and reduction peaks can be better differentiated. For instance, the determination of dopamine and norepinephrine is difficult using normal pulse polarography, however, they are better differentiated using cyclic voltammetry.

Once the CFE was invented, Gonon and colleagues began to discover the great potential of the CFE's abilities to qualitatively and quantitatively detect species by developing an

electrochemical pretreatment process done to the CFE before implantation (Gonon, 1981). This pretreatment helped to resolve oxidation currents measured in the rat brain into definite peaks scanned across the potential axis. This study was only one of the many studies done by several contributors to develop and enhance CFE's in the use of selectively measuring electrochemical species in vitro and *in vivo*.

Before the use or invention of the carbon fiber electrode, Ralph Adams implanted his carbon paste microelectrodes into a rat brain in the 1970's. This was a major breakthrough for neuroscientists in that time. Adams is the scientist generally credited with being the one who first measured the *in vivo* concentration of catecholamine neurotransmitters and their metabolites in the extracellular space (Adams 1976). It should be mentioned, however, that he was not really the first to make *in vivo* measurements, and Adams actually gives credit in his paper for the one who did. Leland Clark carried out voltammetry experiments in brain tissue back in the mid-1960's using is invention, the Clark oxygen electrode. Adams preliminary studies also showed early on that neurotransmitters were merely a percentage of species present in the brain able to oxidize and reduce at the electrode surface, hence the need for guidelines/ rules of detection and identification of *in vivo* species. In the 1980's, Mark Wightman and colleagues determined such guidelines, which are now well known as the "Five Golden Rules." Scientists wanting to monitor a certain compound *in vivo* should use these rules as a checklist for choosing the best biosensor, selectivity and sensitivity parameters, and detection system to optimize the resolution of the response signal in whatever pre-defined environment and signal-inducing criterion the scientist has planned. These golden rules are discussed in a book on voltammetry in the neurosciences (Justice 1987) and the journal Trends in Analytical Chemistry (Philips and Wightman 2003).

## 3.2 SIZE AND SPEED: THE BENEFITS

Microelectrodes come in all shapes and sizes. For instance, there are the disk, needle, elliptical, double barrel, and band electrodes. These all have their own set of characteristics and serve their own unique purpose for a specific set of experiments. However, for the use in neurochemical monitoring, the two main kinds of shapes used for carbon fiber electrodes are the needle and disk electrodes. These shapes are what will be assumed when referring to CFE's throughout this paper.

CFE's have relatively small dimensions in comparison to other biosensors used *in vivo*. Typical dimensions of the carbon fiber tip range from 7-20 μm in diameter and 400 - 800 μm in length. The order of magnitude of the area of a CFE is in the $10^{-4}$ $cm^2$ range. It might have seemed like too great of a challenge for something so small to be selective and sensitive enough in an environment stocked with a vast amount of electrochemically detectable species to give a discernable response. Fortunately, there are several benefits due to the CFE's small size.

One major benefit to the small size of the CFE is real-time subsecond recording capability. Since the electrode is directly implanted into the brain, any signal recorded has only a time delay of the length of time it takes the analyte (e.g. dopamine) to diffuse across a gap approximately 3-4 μm to the electrode, which is on the order of milliseconds. For example, naturally occurring dopamine concentration increases have been detected by voltammetric methods within a millisecond time scale (Robinson et al., 2003; Cheer et al., 2004). In the case of microdialysis, the same transient increases would never be detected because the required sampling time takes nearly 100 times longer than the time it takes for a transient to occur. Other things, such as *in vivo* diffusion of neurotransmitters and kinetics of drug injections (Jones et al., 1995; Greco and Garris, 2003), can only be resolved with these fast real-time resolutions.

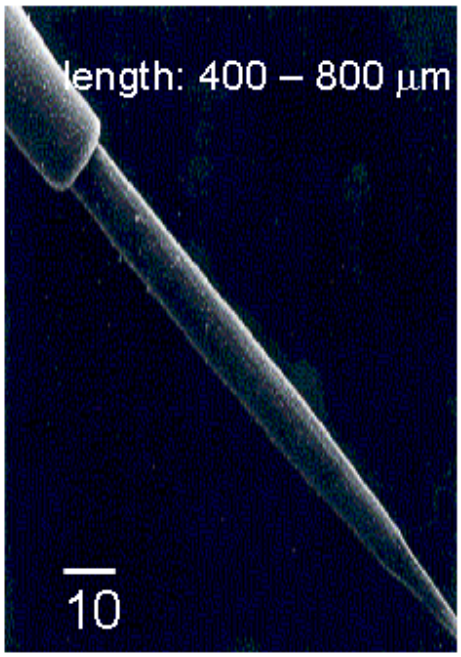


**Figure 1. Image of a carbon fiber electrode.**

In addition, CFE's can be used to measure cellular and subcellular events easily. These kinds of investigations are unrealistic and incapable for larger measuring devices. Monitoring neurochemicals released from single cells have been investigated using microelectrodes in conjunction with many electrochemical techniques (Leszczyszyn et al., 1991; Wightman et al., 1991; Boudko et al., 2001; Kumar et al., 2001). Also, quantal size and vesicular volume have been investigated (Kozminski et al., 1998; Pothos et al., 2000; Colliver et al., 2000). This is something not possible for larger devices because they typically cannot sample single cells, but rather whole cells, thus negating the goal of the spatial resolution obtained from the use of microelectrodes. Microdialysis probes are an example of devices that are too large to quickly and accurately sample the contents of vesicles, despite their superb sensitivity and selectivity.

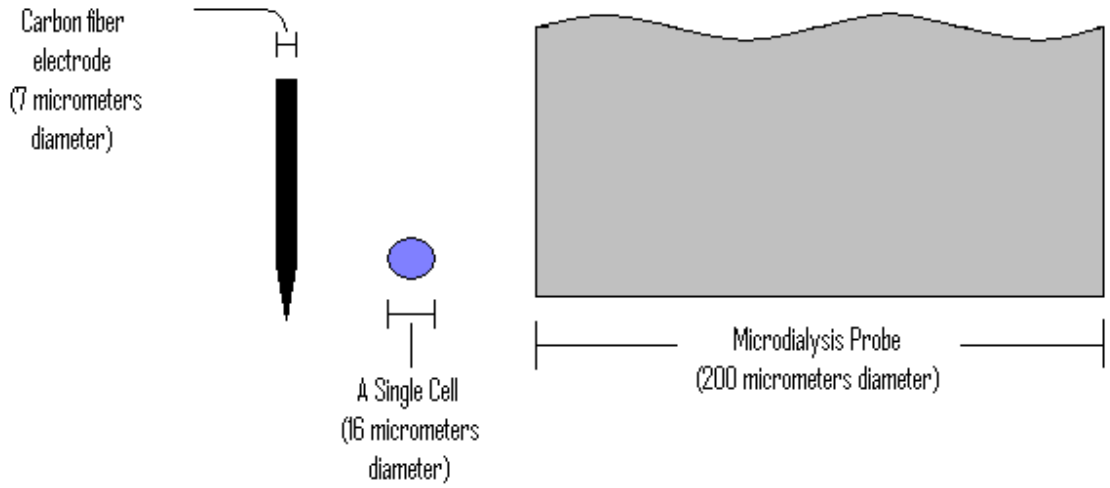


**Figure 2. A comparison of the relative sizes of a CFE and a microdialysis probe next to a single cell.**

Another advantage of the small size of carbon fiber electrodes used in neurochemical monitoring lies in the observation that, when implanted in the brain, it has less of an impact in detrimental damage to the surrounding tissue near the implantation site than much larger devices. Microscopy studies have observed evidence of damage surrounding the implantation site of a microdialysis probe extending at least 1 mm (Clapp-Lilly et al., 1999; Zhou et al., 2001). The studies observed a hole surrounded by broken blood vessels and dead cells. This evidence of damage to the tissue in the brain upon probe implantation suggests there is a disruption to normal physiology and also suggests a reason that microdialysis probes may be too large. More recent evidence of damage suggests disruption of blood flow and possibly compromising the intactness of the blood-brain barrier near the implantation site of a microdialysis probe seen by Mitala, C.M. and Michael, A. C. (unpublished data). Other studies further support the notion that

microelectrodes (e.g. CFE) cause minimal damage. Light microscopy studies cannot follow the tiny tracts of the brain tissue's ultrastructure surrounding an implanted microelectrode (Peters et al., 2004). There was no visible damage except for an occasional neuron with darkened cytoplasm, suggesting little negative impact microelectrodes have on surrounding tissue structure. Another study using microelectrodes and cyclic voltammetry to monitor the activity of dopamine within the range of 220 μm or less of an implanted microdialysis probe revealed a gradient of disrupted dopamine release and uptake (Borland et al., 2005). This study not only supports the idea that larger probes have a negative affect on the structure and function of its surrounding tissue, but also supports the idea that the much smaller microelectrodes are advantageous for studying functionally intact brain tissue as well as small environments, such as single cells and vesicles. Therefore, because of its many advantages based on size and speed, the carbon fiber electrode has become an essential analytical tool for the studying of neurochemical monitoring.

## 3.3 SELECTIVITY

### 3.3.1 Detection of Electroactive Species

Another appealing aspect of the CFE is the potential for it to detect a wide range of species in the brain. Although it is well-known that dopamine is easily detected using CFE's, there are other possibilities. Focusing first on electroactive species, CFE have been largely used to monitor neurotransmitters, catecholamines and their metabolites, but also pH, ascorbic acid, oxygen, and hydrogen peroxide, to name a few. These species can become either oxidized or reduced on the

electrode surface. This transfer of the electrons is what produces the electrode's signal and thus detection. Examples of more frequently studied species include dopamine, serotonin, and norepinephrine and their metabolites. *In vivo*, or in physiological media, these species become oxidized and release electrons as shown in Figure 3, and have been studied with microelectrodes and voltammetry (Daws et al., 1998; Avshalumov et al., 2003). The reason for the ability of the CFE to selectively detect different species is that one species tends to have its own unique potential where it becomes oxidized and reduced. By focusing the scanned potentials to a specific range, a person can be more confident in their assessment of the identification of the detected and measured signal.

Electrochemical methods are sometimes used to detect things other than neurotransmitters and their metabolites. This would include the measurement of things such as small molecules and biologically significant ions. Ion selective electrodes are quite often used *in vitro*; however, they can be miniaturized for *in vivo* use for various applications, such as the pH electrode (Boudko et al., 2001). Another electrode that has the ability to apply to *in vivo* selection is the Clark oxygen sensor. It is a small-molecule electrode rather than an ion-selective one. Oxygen is reduced as shown in Figure 4 to produce a current measurable at the electrode surface. This oxygen sensor is composed of a membrane-coated platinum wire electrode. Carbon fiber cylinder electrodes, however, have also been used in place of platinum, to detect $O_2$ (Kennedy et al., 1992). Another small molecule of interest to neuroscientists that can be detected *in vivo* is nitric oxide (NO, Fig 4). Sensors for nitric oxide closely resemble that of the oxygen sensors and have been constructed to measure and study NO in many different kinds of environments (Kumar et al., 2001; Zhang et al., 2000). Yet another small molecule relevant to *in vivo* monitoring is hydrogen peroxide. ($H_2O_2$, Fig 4). This easily detected molecule is reduced on

the surface of carbon fiber and platinum electrodes. It also poses a problem because often it becomes oxidized in the same window of potential as several other electroactive species. Thus, in order to detect and measure $H_2O_2$, a more selective sensor or electrode is necessary. Fortunately, there are naturally occurring enzymes that are selective for peroxide and can be coupled with electrodes to make a sensor specifically for $H_2O_2$ (Kulagina and Michael, 2003).

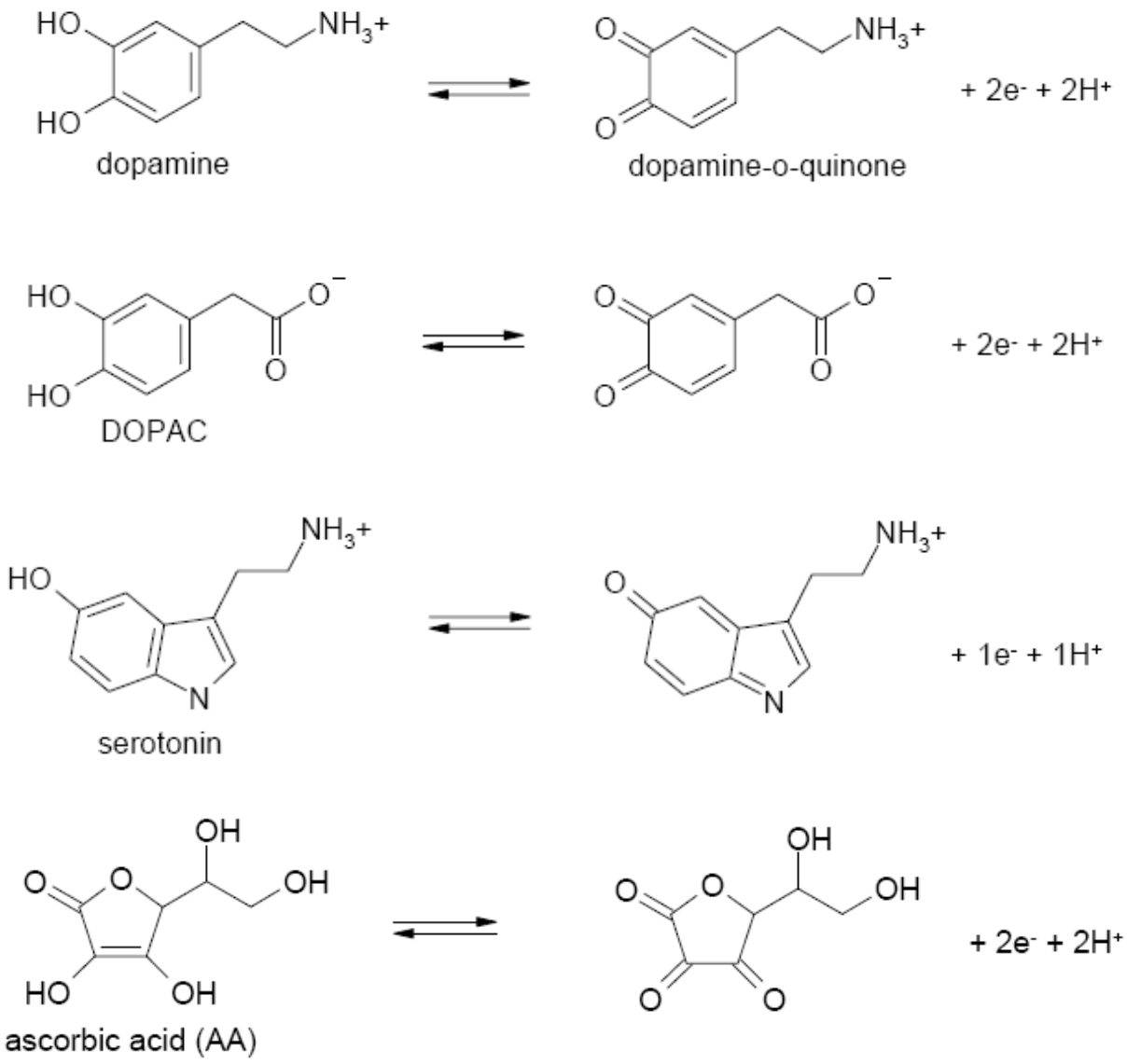


**Figure 3. Oxidation reactions of electroactive species.**

$$NO\cdot \rightarrow NO^{+} + 1e^{-}$$

**Nitric oxide**

$$O_2 + 4H^{+} + 4e^{-} \rightarrow 2H_2O$$

**Oxygen**

$$H_2O_2 + 2H^{+} + 2e^{-} \rightarrow 2H_2O$$

**Hydrogen peroxide**

**Figure 4. Additional electroactive species found in the brain.**

Ascorbic acid is another extremely relevant neurochemical in the brain. It is actually one of the most prevalent molecules in the brain, with concentration levels reaching 200-400 μM. Ascorbic acid plays a definite role in behavior and neurotransmitter activity, and it has been studied extensively (Rebec and Pierce, 1994; Rebec and Wang, 2001). Ascorbic acid, however, is also another molecule that becomes oxidized at potentials near those of other neurochemicals, specifically dopamine and norepinephrine. This makes detection of dopamine and norepinephrine difficult due to the overpowering signal from ascorbic acid. Luckily, this interfering of ascorbic acid can be controlled by increasing the scan rate of the applied potential. Catecholamines diffuse away from the electrode much more slowly than the oxidized ascorbic acid. An increased scan rate means the diffusion of the oxidized ascorbic acid away from the electrode surface also increases. This greatly decreases the likelihood of the species reducing back to ascorbic acid, and thus decreases the interference of the voltammetric signaling from the catecholamines.

This interference problem has also given rise to improvements on selectivity with the addition of coatings on the electrode surface. These coatings sometimes serve as a barrier between the detection of wanted and unwanted species of interest. Electrode coatings include polyelectrolytes (Nafion), electrically conducting polymers (polypyrrole), and electrodeposited polymers (1,2 diaminobenzene). Coatings with a thin film of polyelectrolytes, Nafion for example, are a simple way to increase electrode selectivity to certain analytes. Nafion-coated electrodes are ion-selective, thus the anionic composition of Nafion films promotes the selection of only cations to pass through (Gerhardt et al., 1984).

### 3.3.2 Detection of Nonelectroactive Species

This discovery, that by putting coatings on an electrode before implantation greatly improved the selectivity of the sensor, opened the doors to the prospect that one can detect species that are inherently not electroactive. It was actually Leland Clark's idea in the 1960's to coat an electrode with the glucose enzyme for greater selectivity (Clark and Lyons, 1962). Others quickly caught on to his idea and it spread rapidly. Now some scientists use polyelectrolyte films, as well as many different conducting and non-conducting polymers, to capture or immobilize enzymes onto the electrode surface. This creates a sensor that is species-selective, depending on what enzyme is immobilized (Bartlett and Cooper, 1993).

These enzyme-based microsensors, some of which are made from carbon fiber electrodes, are used to detect analytes that are of interest to neuroscientists but are non-electroactive, such as choline, glutamate, glucose and lactate. A different electrode is constructed for a different analyte, each specialized with its own coatings and additives that will selectively produce electrochemical signals. The most commonly used enzymes are the oxidase enzymes,

particularly for the detection of choline, glutamate, glucose and lactate. These enzymes act as the go-between for the analyte and electrochemical signal. Typically, the analyte reduces the enzyme on the surface of the electrode, then the enzyme gets oxidized in a reaction with a co-substrate (Bartlett and Cooper, 1993). The reaction scheme typically goes as follows as seen in Figure 5:

$$\text{substrate(RED)} + \text{enzyme(OX)} \rightarrow \text{product(OX)} + \text{enzyme(RED)}$$

$$\text{enzyme(RED)} + \text{co-substrate(OX)} \rightarrow \text{enzyme(OX)} + \text{co-product(RED)}$$

**Figure 5. Reaction scheme for enzyme-based electrodes.**

Primarily, the co-substrate in this scenario is oxygen ($O_2$), which generates hydrogen peroxide ($H_2O_2$) as the final co-product. Hydrogen peroxide can be electrochemically oxidized and detected on the electrode surface, as mentioned previously. The rate of production of $H_2O_2$ would ideally be directly related to the concentration of the substrate detected by the enzymes. As an example, the oxidation of the substrate glutamate and subsequent oxidation of $H_2O_2$ would be:

$$\text{Glutamate} + \text{GluOx(OX)} \rightarrow \alpha\text{-ketoglutarate} + NH_3 + \text{GluOx(RED)}$$

$$\text{GluOx(RED)} + O_2 \rightarrow \text{GluOx(OX)} + H_2O_2$$

$$\text{At electrode surface: } H_2O_2 \rightarrow 2H^+ + O_2 + 2e^-$$

The idea behind immobilizing the enzymes in a thin film to the surface of the electrode in this kind of sensor is to minimize the distance $H_2O_2$ must travel to the electrode surface to be oxidized. The thin films also help to limit the diffusion of peroxide or the excessive reaction of the peroxide, both of which would result in a lower sensor sensitivity. The minimizing of the film's thickness also decreases the response time of the electrode because of the decreasing distance the substrate itself must travel into the film to react with its enzyme (Heller, 1992). For

all of these reasons, thin film coatings have become a big breakthrough in the detection of neurochemical species in the brain and continue to be used and improved.

Another type of electrochemical sensor that uses enzymes for species detection does not require that the enzyme be immobilized on the electrode surface. This other type of sensor utilizes what is called crosslinked redox gels composed of a poly(vinylpyridine) complexed to $Os(bpy)_2Cl$ that carries the signal to the electrode with moving enzymes (Gregg and Heller, 1990; Heller, 1992; Kulagina and Michael, 2003). The redox polymer shapes the three-dimensional gel which contains the mobile enzymes, but at the same time allows for fast in and out diffusion of both substrates and products. Electrons transferred during the enzyme's reduction are quickly carried through the polymer's redox centers to the surface of the electrode where they are detected through changes in current response. Different non-electroactive species can thus be detected and measured utilizing these types of electrochemical sensors by changing the functional enzyme within the gel, from glucose oxidase to lactate oxidase for example. Other sub-types of enzyme-based electrodes use more than one enzyme to transfer electrons, such as a glutamate detector utilizing glutamate oxidase, which reacts with glutamate and produces $H_2O_2$, and horseradish peroxidase, which reduces the $H_2O_2$ and oxidizes the osmium redox polymer (Kulagina et al., 1999). This chain of events leads to the final generation of current response changes to detect glutamate.

The addition of special coatings on carbon fiber electrodes has greatly increased the range of detectable analytes in the brain and thus has expanded the research in the neurosciences. These further insights into the inner workings of the brain have been and will continue to be of great interest and value for scientists and patients alike.

It should, however, be mentioned here that, like most other things, carbon fiber electrodes in conjunction with electrochemical methods do have their drawbacks. One disadvantage is that measuring basal concentrations for any species in the brain is simply unachievable. It is rather best suited for measuring the changes in concentration that occur. Another disadvantage of the CFE is its sensitivity to pH changes (Runnels et al., 1999). Usually, pH is not the desired measurement in an experiment, and therefore any changes in its value during an experiment can bring about misconstrued or misinterpreted data. Also, a word of caution for some species that are not yet well-studied, positive identification of the analyte responsible for current changes may be more difficult. This is simply due to the facts that it is impractical to be able to control every single possible experimental variable and that there is a plethora of species that are electrochemically detectable.

Future directions for the carbon microelectrode are likely related to its size. How small can one make a functional electrode? Some scientists are trying to find out and carbon nanotubes are the point of interest. Carbon nanotubes have unique electronic properties, as well as mechanical, chemical and geometrical properties, with the potential for applications in bioelectrochemistry (Campbell et al., 1999). Others are incorporating carbon nanotubes into the construction of carbon fiber nanoelectrodes to improve stability, reproducibility, sensitivity, and spatial resolution (Chen et al., 2003; Valcarcel et al., 2007).

# 4.0 PRINCIPLES OF ELECTROCHEMISTRY

## 4.1 FUNDAMENTALS

An electrochemical process is one in which a chemical reaction or change occurs in a system caused by a passing of electrons (an electrical current), which is basically a production of electrical energy by a chemical reaction. In the neurosciences, these chemical reactions occur on the electrode surface, and these reactions can be either oxidation or reduction. Oxidation is the transfer of electrons away from the analyte, whereas reduction is the transfer of electrons to the analyte. At an electrode surface, these reactions occur when a potential is applied and reaches the specific potential where the analyte becomes either oxidized or reduced. This potential is known as the formal potential ($E^0$). When the formal potential has been reached, the transfer of electrons must be rapid in order to record electrochemical measurements. If it is not, the information used to identify and quantify the analytes will be obscured and too difficult to interpret. The rate of which this transfer of electrons occurs from analyte to electrode is called the current. The current, i, can be calculated using the equation:

$$i = \frac{\partial Q}{\partial t} = \frac{nF\partial N}{\partial t} \qquad \text{(Eqn. 1)}$$

where n is the number of electrons transferred per molecule of analyte, F is Faraday's constant, Q is charge, and N is the moles of analyte. This equation shows that the current is regulated by the number of moles of the analyte, i.e. the concentration, at the surface of the electrode. In order

for the analyte to undergo a chemical reaction, it must be in direct contact with the electrode surface. The main mode of transportation of the analyte from the bulk solution to the electrode surface is by diffusion, or random molecular motion. The rate at which the analyte diffuses correlates to the tendency of the analyte to experience continuous random motion and is known as the flux (J). Flux depends upon the diffusion coefficient (D) for the analyte of interest as well as the concentration gradient that builds up due to the reduction or oxidation reactions occurring at the electrode surface. The correlation between flux and the concentration gradient is described by Fick's first law:

$$J = -D \frac{\partial C}{\partial x} \qquad \text{(Eqn. 2)}$$

where C is the concentration and x is the distance. Now the quantity of the current response can be defined as the flux of the analyte to or from the electrode surface. The current that is produced from the reduction or oxidation of the analyte at the electrode surface is called the faradaic current. The current relies upon the magnitude of the applied potential of the electrode, which has a major role in formulating the rate of electron transfer and the consequent electrolysis of the analyte. The quantity of current can be obtained by multiplying by the area of the electrode and a factor for the number of coulombs per mole of reactant:

$$i = n\mathrm{FAD}\left.\frac{\partial C}{\partial x}\right|_{x=0} \qquad \text{(Eqn. 3)}$$

where A is the surface area of the electrode. The subscript x = 0 in Equation 3 states that the concentration at the electrode surface is zero, which creates the gradient and causes molecules to move.

## 4.2 CHRONOAMPEROMETRY

To put the fundamentals of electrochemistry in action requires the consideration of an electrochemical method. Chronoamperometry is the simplest method used to monitor the current of an analyte produced at the electrode surface. Chronoamperometry begins with the electrode held at an initial "resting" potential, where reduction or oxidation of the analyte does not occur. Then the potential is swiftly stepped up to a higher potential, higher than the formal potential of the analyte, initiating the oxidation reaction. This step potential diagram is found in Figure 6A. This step-wise change in potential is what makes chronoamperometry a so called potential pulse method. Once the potential has been stepped up, any analyte in contact with the electrode surface immediately becomes oxidized. Therefore, the concentration of the analyte at the electrode surface becomes zero, creating the gradient between the electrode and bulk solution, driving the diffusion of the analyte toward the electrode. The current monitored during this potential step experiment is proportional to the concentration of the analyte, and over time, the concentration of the solution next to the electrode will become depleted. Thus, under diffusion controlled conditions, chronoamperometry produces a distinct current-time relationship, as demonstrated in the Cottrell equation:

$$i(t) = \frac{nFA\sqrt{D_o}C_o}{\sqrt{\pi \cdot t}} \qquad \text{(Eqn. 4)}$$

where $D_o$ is the diffusion coefficient, $C_o$ is the bulk concentration of the analyte and t is time. The concentration profile for the analyte being investigated using chronoamperometry is shown in Figure 6C. The current versus time graph for the same chronoamperometry experiment is diagrammed in Figure 6B.

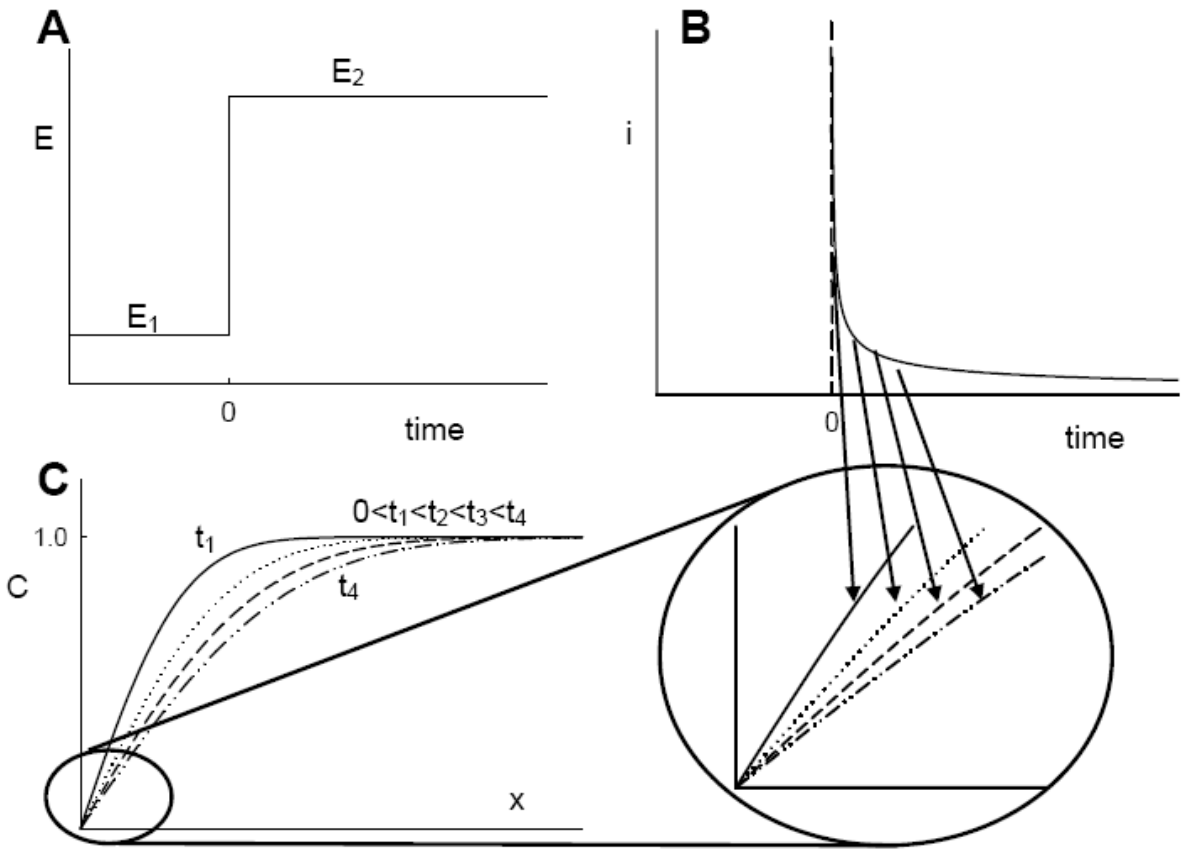


**Figure 6 (Bard & Faulkner, 2000). (A) Diagram of the step potential used in chronoamperometry. (B) Current vs. time plot for a chronoamperometric experiment. (C) Concentration profile for times after the commencement of a chronoamperometric experiment. Circled inset: A decrease in the current response during an experiment relates to a decrease in the slope of the concentration, which is characterized by the Cottrell equation (Equation 4).**

## 4.3 VOLTAMMETRY

Another potential pulse method is an extension of the chronoamperometric technique, called normal pulse voltammetry (NPV). In this method, the electrode is held at some base potential ($E_b$), at which electrolysis does not occur. Then the potential is stepped up to a higher, oxidizing

potential (E) for a very short period of time until the potential is dropped back down to the base potential. After a designated time interval, the potential is again stepped up from $E_b$ to a higher E potential, this potential being higher than the first step. Again, after a short period of time, the potential is dropped back to the base potential and the cycle keeps repeating, with each successive step higher than the previous step. The potential profile for NPV is diagrammed in Figure 7A. The current is sampled near the end of each pulse at a time τ, just before the potential is dropped back to the base potential. The current vs. time profile is diagrammed in Figure 7B.

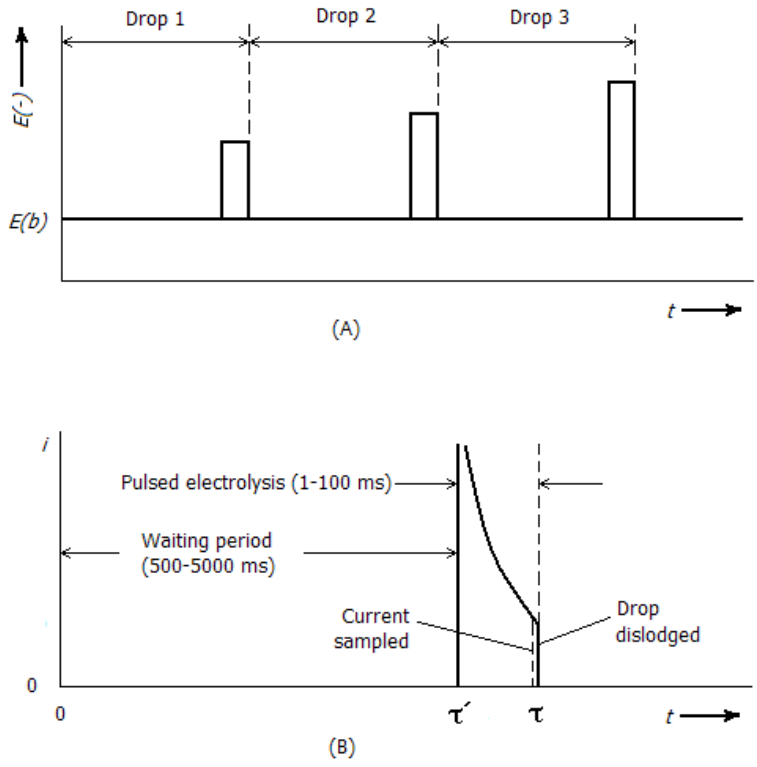


**Figure 7. Normal Pulse Voltammetry sampling scheme. (A) Potential profile. (B) Current vs. time.**

The current produced during each step is proportional to the concentration of the analyte, which is the same for chronoamperometry. Normal pulse voltammetry, however, has an additional benefit of limiting electrode fouling or filming and preventing compilation of the remaining oxidized analyte, which can be neurotoxic. This benefit comes from the stepping down back to the base potential at each pulse, which decreases the newly produced oxidized analyte species. NPV can also be used to help identify the electrolyzed species through the observation of the ratio of oxidation to reduction currents.

Yet another potential pulse electrochemical technique is the differential pulse voltammetry (DPV) method. DPV can provide greater sensitivity and more efficient resolution and differentiation of various species. This technique is comparable to normal pulse voltammetry, but with some major differences. The potential profile for DPV is diagrammed in Figure 8A. The major differences are: (1) The base potential is not the same potential throughout the experiment, but changes steadily with each step in small increments. (2) The height of each step is maintained at a constant height with respect to the base potential and ranges from only 10-100 mV. (3) The current is sampled twice during each cycle, once immediately before the potential is stepped up ($i_a$) and again immediately before the potential drops down ($i_b$). (4) The experiment is recorded in a plot of the difference between current responses ($i_b - i_a$) versus the base potential to produce a voltammogram. This voltammogram, diagrammed in Figure 8B, provides a better qualitative characterization of the electrolyzed species and gives the greater selectivity for easier identification of the species of interest.

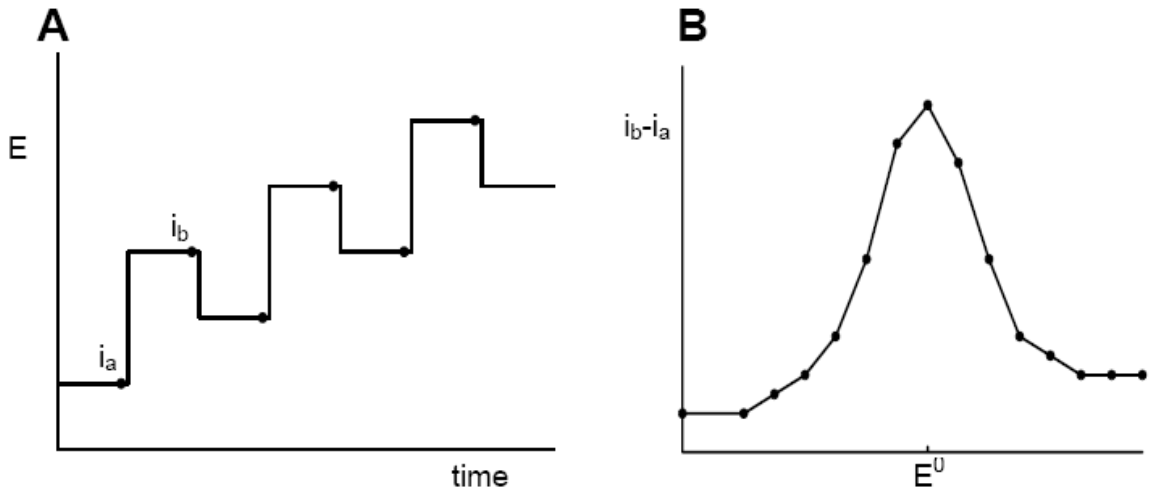


**Figure 8 (Bard & Faulkner, 2000). (A) Differential pulse voltammetry potential profile. (B) Differential pulse voltammogram.**

## 4.4 CYCLIC VOLTAMMETRY

Chronoamperometry, NPV, and DPV are all potential pulse techniques that measure current with respect to time. There are other kinds of electrochemical methods, however, that can improve sensitivity and selectivity- potential sweep methods. Cyclic voltammetry (CV) is a widely used potential sweep method for neuroscience applications. This method is different than those previously mentioned in that the applied potential is progressively increased from the base potential over a specified time interval, not stepped up, and then progressively decreased back to the base potential, typically using a constant sweep rate throughout. The increased sweeping oxidizes the analyte while the decreased sweeping reduces the newly produced oxidant. A waveform diagram is shown in Figure 9A. The current is measured continuously during the

cyclic sweeping in a CV experiment and is plotted versus the applied potential in a voltammogram as shown in Figure 9B.

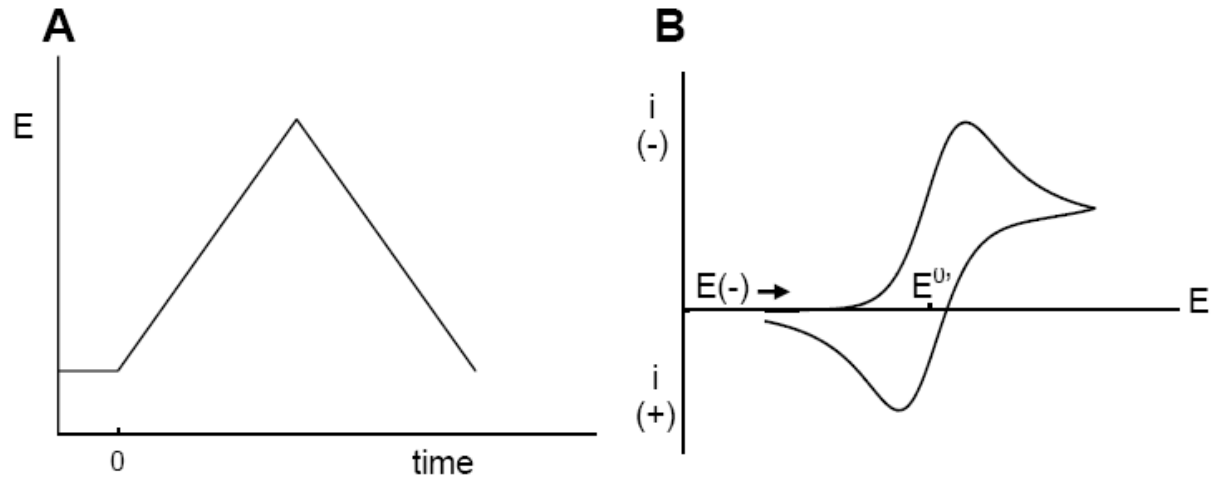


**Figure 9 (Bard & Faulkner, 2000). (A) Potential waveform diagram for a cyclic voltammetry experiment. (B) A voltammogram of a CV experiment.**

This method can provide useful voltammetric information of *in vivo* studies for the purposes of qualitative identification of a single species among the plethora of other substances without the need for further separation. The voltammogram of a species generally has a unique position along the potential axis and a unique shape. For example, a voltammogram of dopamine can easily be distinguished from a voltammogram of serotonin. In addition, by increasing the potential scan rate, the current response of ascorbic acid for instance, which normally overlaps with that of the catecholamines (e.g. dopamine), is eliminated.

A slight variation on cyclic voltammetry, called fast scan cyclic voltammetry (FSCV), utilizes the same basics as classic cyclic voltammetry only at a much faster scan rate. These sweep rates are typically accelerated by many orders of magnitude to several hundred volts per second (e. g. 300 V/s) and are very feasible experiments using small carbon fiber electrodes

which can charge to the new applied potential very rapidly. This means that an entire cyclic voltammetry experiment can be recorded within milliseconds. This is approximately the same amount of time that it takes to record a single chronoamperometric pulse. People such as Julian Millar and R. Mark Wightman have been the fore-runners in this field of FSCV, using it for their experiments with brain slices as well as anesthetized and freely-moving animals (Millar et al., 1985; Wightman et al., 1988; Bull et al., 1990). FSCV is a very desirable method because it is the fastest voltammetric recording technique available to date. Since this temporal resolution is one of the great traits of electrochemistry techniques compared to microdialysis, it is being used more and more in the neurosciences. Cyclic voltammetry in general is also appealing because of the other useful information it can obtain, such as electrode properties, reaction kinetics and quantitative measurement of a number of substances.

It may be of interest to also consider how these methods might apply to carbon nanotubes since they may play a major role in the future of microelectrodes. Because carbon nanotubes have such a large surface area, their voltammetric characteristics are slightly different. Chen and colleagues have seen that measured background currents of an electrode modified with carbon nanotubes are much larger compared to bare carbon fiber electrodes (Chen et al., 2003). As another example, at scan rates between 0.01-0.1 V/s, the cyclic voltammograms looked slightly different for the electrodes modified with carbon nanotubes whereas there were no big differences in the CV's for the unmodified electrodes. They do, however, take on the sigmoidal shape, characteristic of steady-state radial diffusion ( Campbell et al., 1999; Chen et al., 2003).

# 5.0 CHARGING CURRENTS

It should be noted that the other type of current produced at the electrode surface, other than faradaic current, is known as the charging current which comes from the solution itself. The charging current comes from all the other electrochemical reactions occurring at the electrode surface, in particular the build up of differences in potential between the electrode and the solution. The major consequence of charging currents is that all electrochemical measurements have a background signal that is nonzero. This makes measuring static concentrations of the analyte of interest virtually unobtainable by way of electrochemical methods because knowing what portion of the signal is due to the charging current and what portion of the signal is due to faradaic current is usually unattainable. For this reason, electrochemical methods, like those mentioned previously, are best fitted for the monitoring of dynamic occurrences such as the change in extracellular serotonin concentration during electrical stimulation of a serotonin axon. Electrochemical methods used in neuroscience are not well fitted for an analyte's resting or basal concentration measurements in any fashion.

# 6.0 CONCLUSION

From the birth of *in vivo* neurochemical monitoring to the present day, carbon electrodes have been extremely useful analytical tools. Even though the complexity of the brain is somewhat intimidating, while at the same time very fascinating, the carbon fiber electrode has the ability to overcome many obstacles. A carbon fiber electrode utilizes the electrochemical principles to detect species that can become oxidized or reduced on its surface, which expels or takes up electrons. This exchange of electrons creates a change in current, something measurable. This measured signal is used to calculate the concentration of the analyte of interest, as well as determine the species that caused the signal by means of electrochemical methods. In the neurosciences, the most widely used methods are chronoamperometry, normal pulse voltammetry, differential pulse voltammetry, and cyclic voltammetry.

The many benefits that carbon fiber electrodes offer surpass other leading investigative tools in several aspects. Superior spatial and temporal resolutions make the use of CFE's in conjunction with electrochemical methods an ideal way to study *in vivo* chemical events. The subsecond recordings can give insight into what is going on during these events in real-time. Due to its small size, a carbon fiber electrode is also an ideal tool to explore environments previously too small or too delicate to be explored in other ways. Upon implantation, the tissue surrounding the CFE appears to sustain minimal damage, another benefit for examination of neurochemical events. Also, the CFE has the ability not only to detect electroactive species such as dopamine,

but to detect non-electroactive species such as glucose by altering the surface of the electrode, for example via thin film coating of a polymer or well-chosen enzyme (glucose oxidase).

The carbon fiber electrode, when used with electrochemical methods, thus provides a relatively easy and adaptable way to study physiological environments in the brain. It has proven to be a successful and reliable tool in the field of neuroscience. Hence, carbon fiber electrodes will continue to provide insights into the intricacies of the inner workings of the brain and take part in unraveling some of the mysteries of neurochemical activities.

## BIBLIOGRAPHY


Adams, R. N. (1976). "Probing brain chemistry with electroanalytical techniques." Anal. Chem. **48**: 1126A-1138A.

Avshalumov, M. V., B. T. Chen, S. P. Marshall, D. M. Pena, M. E. Rice (2003). "Glutamate-Dependent Inhibition of Dopamine Release in Striatum Is Mediated by a New Diffusible Messenger, H2O2." J. Neurosci. **23**(7): 2744-2750.

Bard, A. J. and L. R. Faulkner (2000). Electrochemical Methods: Fundamentals and Applications, 2nd Edition. Hoboken, NJ, John Wiley & Sons, Inc.

Bartlett, P. N. and J. M. Cooper (1993). "A review of the immobilization of enzymes in electropolymerized films." Journal of Electroanalytical Chemistry **362**(1-2): 1-12.

Borland, L. M., G. Shi, H. Yang, A. C. Michael (2005). "Voltammetric study of extracellular dopamine near microdialysis probes acutely implanted in the striatum of the anesthetized rat." Journal of Neuroscience Methods **146**(2): 149-158.

Boudko, D. Y., L. L. Moroz, P. J. Linser, J. R. Trimarchi, P. J. S. Smith, W. R. Harvey (2001). "In situ analysis of pH gradients in mosquito larvae using non-invasive, self-referencing, pH-sensitive microelectrodes." J Exp Biol **204**(4): 691-699.

Bull, D. R., P. Palij, M. J. Sheehan, J. Millar, J. A. Stamford, Z. L. Kruk, P. P. A. Humphrey (1990). "Application of fast cyclic voltammetry to measurement of electrically evoked dopamine overflow from brain slices in vitro." Journal of Neuroscience Methods **32**(1): 37-44.

Campbell, J. K., L. Sun, R. M. Crooks (1999). "Electrochemistry using single carbon nanotubes." J. Am. Chem. Soc. **121**(15): 3779-3780.

Cheer, J. F., K. M. Wassum, M. L.A.V. Heien, P. E. M. Phillips, R. M. Wightman (2004). "Cannabinoids Enhance Subsecond Dopamine Release in the Nucleus Accumbens of Awake Rats." J. Neurosci. **24**(18): 4393-4400.

Chen, R. S., W. H. Huang, H. Tong, Z. L. Wang, J. K. Cheng (2003). "Carbon fiber nanoelectrodes modified by single-walled carbon nanotubes." Anal. Chem. **75**(22): 6341-6345.

Clapp-Lilly, K. L., R. C. Roberts, L. K. Duffy, K. P. Irons, Y. Hu, K. L. Drew (1999). "An ultrastructural analysis of tissue surrounding a microdialysis probe." Journal of Neuroscience Methods **90**(2): 129-142.

Clark, L. C., C. Lyons (1962). Jrn. Ann. NY Acad. Sci. **102**: 29-45.

Colliver, T. L., S. J. Pyott, M Achalabun, A. G. Ewing (2000). "VMAT-Mediated Changes in Quantal Size and Vesicular Volume." J. Neurosci. **20**(14): 5276-5282.

Daws, L. C., G. M. Toney, G. A. Gerhardt, A. Frazer (1998). "In Vivo Chronoamperometric Measures of Extracellular Serotonin Clearance in Rat Dorsal Hippocampus: Contribution of Serotonin and Norepinephrine Transporters." J Pharmacol Exp Ther **286**(2): 967-976.

Gerhardt, G. A., A. F. Oke, G. Nagy, B. Moghaddam, R. N. Adams (1984). "Nafion-coated electrodes with high selectivity for CNS electrochemistry." Brain Research **290**(2): 390-395.

Gonon, F., M. Buda, R. Cespuglio, M. Jouvet, J. F. Pujol (1980). "In vivo electrochemical detection of catechols in the neostriatum of anaesthetized rats: dopamine or DOPAC?" Nature **286**(5776): 902-904.

Gonon, F. G., C. M. Fombarlet, M. Buda, J. F. Pujol (1981). "Electrochemical treatment of pyrolytic carbon fiber electrodes." Anal. Chem. **53**(9): 1386-1389.

Greco, P. G. and P. A. Garris (2003). "In vivo interaction of cocaine with the dopamine transporter as measured by voltammetry." European Journal of Pharmacology **479**(1-3): 117-125.

Gregg, B. A. and A. Heller (1990). "Cross-linked redox gels containing glucose oxidase for amperometric biosensor applications." Anal. Chem. **62**(3): 258-263.

Heller, A. (1992). "Electrical connection of enzyme redox centers to electrodes." J. Phys. Chem. **96**(9): 3579-3587.

Heller, A., Q. Chen, G. Kenausis (1997). "Electrochemical Glucose and Lactate Sensors Based on "Wired" Thermostable Soybean Peroxidase Operating Continuously and Stably at 37$^{o}$C." Anal. Chem. **69**(6): 1054-1060.

Jones, S. R., P. A. Garris, R. M. Wightman (1995). "Different effects of cocaine and nomifensine on dopamine uptake in the caudate-putamen and nucleus accumbens." J Pharmacol Exp Ther **274**(1): 396-403.

Justice, J. B. J. (1987). Voltammetry in the Neurosciences: Principles, Methods, and Applications. Clifton, NJ, USA, Humana Press.

Kennedy, R. T., S. R. Jones, R. M. Wightman (1992). "Simultaneous measurement of oxygen and dopamine: Coupling of oxygen consumption and neurotransmission." Neuroscience **47**(3): 603-612.

Kozminski, K. D., D. A. Gutman, V. Davila, D. Sulzer, A. G. Ewing (1998). "Voltammetric and Pharmacological Characterization of Dopamine Release from Single Exocytotic Events at Rat Pheochromocytoma (PC12) Cells." Anal. Chem. **70**(15): 3123-3130.

Kulagina, N. V. and A. C. Michael (2003). "Monitoring Hydrogen Peroxide in the Extracellular Space of the Brain with Amperometric Microsensors." Anal. Chem. **75**(18): 4875-4881.

Kulagina, N. V. Shankar, L. and Michael, A. C. (1999). "Monitoring Glutamate and Ascorbate in the Extracellular Space of Brain Tissue with Electrochemical Microsensors." Anal. Chem. **71**(22): 5093-5100.

Kumar, S. M., D. M. Porterfield, K. J. Muller, P. J. S. Smith, C. L. Sahley (2001). "Nerve Injury Induces a Rapid Efflux of Nitric Oxide (NO) Detected with a Novel NO Microsensor." J. Neurosci. **21**(1): 215-220.

Leszczyszyn, D. J., J. A. Jankowski, O. H. Viveros, E. J. Diliberto Jr., J. A. Near, R. M. Wightman (1991). "Secretion of catecholamines from individual adrenal medullary chromaffin cells." Neurochem. **56**(6): 1855-1863.

Millar, J., J. A. Stamford, Z. L. Kruk, R. M. Wightman (1985). "Electrochemical, pharmacological and electrophysiological evidence of rapid dopamine release and removal in the rat caudate nucleus following electrical stimulation of the median forebrain bundle." European Journal of Pharmacology **109**(3): 341-348.

Peters, J. L., L. H. Miner, A. C. Michael, S. R. Sesack (2004). "Ultrastructure at carbon fiber microelectrode implantation sites after acute voltammetric measurements in the striatum of anesthetized rats." Journal of Neuroscience Methods **137**(1): 9-23.

Phillips, P. E. M. and R. M. Wightman (2003). "Critical guidelines for validation of the selectivity of in-vivo chemical microsensors." TrAC Trends in Analytical Chemistry **22**(8): 509-514.

Ponchon, J. L., R. Cespuglio, F. Gonon, M. Jouvet, J. F Pujol (1979). "Normal pulse polarography with carbon fiber electrodes for in vitro and in vivo determination of catecholamines." Anal. Chem. **51**(9): 1483-1486.

Pothos, E. N., K. E. Larsen, D. E Krantz, Y. Lui, J. W. Haycock, W. Setlik, M. D. Gershon, R. H. Edwards, D. Sulzer (2000). "Synaptic Vesicle Transporter Expression Regulates Vesicle Phenotype and Quantal Size." J. Neurosci. **20**(19): 7297-7306.

Rebec, G. V., R. C. Pierce (1994). "A vitamin as neuromodulator: ascorbate release into the extracellular fluid of the brain regulates dopaminergic and glutamatergic transmission." Progress in Neurobiology. **43**(6): 537-565.

Rebec, G. V., Z. Wang (2001). "Behavioral activation in rats requires endogenous ascorbate release in striatum." Journal of Neuroscience **21**(2): 668-675.

Robinson, D. L., B. J. Venton, M. L.A.V. Heien, R. M. Wightman (2003). "Detecting Subsecond Dopamine Release with Fast-Scan Cyclic Voltammetry in Vivo." Clin Chem **49**(10): 1763-1773.

Runnels, P. L., J. D. Joseph, M. J. Logman, R. M. Wightman (1999). "Effect of pH and surface functionalities on the cyclic voltammetric response of carbon-fiber microelectrodes." Anal. Chem. **71**(14): 2782-2789.

Turner, A. P. F. (1993). Advances in Biosensors. Supplement 1: Chemical Sensors for *In Vivo* Monitoring, Greenwich, CT, JAI Press Inc.

Valcarcel, M., S. Cardenas, B. M. Simonet (2007). "Role of carbon nanotubes in analytical science." Anal. Chem. **79**(13): 4788-4797.

Wightman, R. M., L. J. May, A. C. Michael (1988). "Detection of Dopamine Dynamics in the Brain." Anal. Chem. **60**: 769A-779A.

Wightman, R. M., J. A. Jankowski, R. T. Kennedy, K. T. Kawagoe, T. J. Schroeder, D. J. Leszczyszyn, J. A. Near, E. J. Diliberto Jr., O. H. Viveros (1991). "Temporally resolved catecholamine spikes correspond to single vesicle release from individual chromaffin cells." Proc. Natl. Acad. Sci. **88**(23): 10754-10758.

Zhang, X., L. Cardosa, M. Broderick, H. Fein, J. Lin (2000). "An Integrated Nitric Oxide Sensor Based on Carbon Fiber Coated with Selective Membranes." Electroanalysis **12**(14): 1113-1117.

Zhou, F., X. Zhu, R. J. Castellani, R. Stimmelmayr, G. Perry, M. A. Smith, K. L. Drew (2001). "Hibernation, a Model of Neuroprotection." Am J Pathol **158**(6): 2145-2151.