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***Chapter 12***

# ELECTROCHEMICAL DETECTION OF NEUROTRANSMITTERS A STRUCTURALLY SMALL ELECTRODES

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## ABSTRACT

Electroanalytical chemistry has been widely developed and applied to the study of neurochemical systems. This then leads to a better understanding of many aspects of neurotransmission, for example, neural circuitry and neural substrates of compulsive drug use. This feasibility partly stems from the ease of oxidative detection of many neurotransmitters including dopamine, acetylcholine, norepinephrine, serotonin, glutamic acid and γ–aminobutyric acid. At the same time, this has also stimulated the development of structurally small electrodes for applications to the detection of neurotransmitters in biological microenvironments. In this respect, the small dimension of such electrodes permits minimal tissue damage upon implantation and, of equal importance, permits very careful selection of the region of tissue where measurements can be performed. In addition, the inherent fast response time of structurally small electrodes makes it feasible to follow biochemical events frequently taking place on a millisecond time scale (e.g. neuronal firing).

Various electrode materials used to construct structurally small electrodes of different geometries and sizes have hitherto been reported. Common electrode materials both modified and otherwise, include metals such as tungsten and aluminium, gold nanoparticledeposited aluminium, various forms of carbon e.g. doped diamond, nanocrystalline diamond, pyrolysed carbon, carbon fibres, and gold nanoparticles deposited onto glassy carbon.

A common problem encountered while performing in vivo electrochemical analyses of neurotransmitters is the adsorption of lipids, peptides and high molecular weight proteins present in biological matrices on the electrode surface. Formation of these layers leads to electrode fouling which distorts the voltammetric signal and suppresses the

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sensitivity of the electrode. Considerable research effort has been devoted to addressing electrode fouling problems. Approaches ranging from fast scan voltammetry, immobilising a protective organic film on the electrode surface, completely altering the surface termination, fabrication of nanocrystalline diamond coated electrodes, or of doped diamond electrodes, to gold electrodes modified with gold nanorod and gold nanoparticles have been developed. Apart from overcoming fouling, the latter methods have also demonstrated other advantages such as wider potential windows, greater durability, increased robustness and enhanced sensitivity.

In this paper, we aim to thoroughly review the techniques used in developing structurally small electrodes of different geometries, which were then applied to the detection of neurotransmitters. We will also pay special emphasis on the strategies used to minimize electrode fouling during electrochemical detection of neurotransmitters at these electrodes. A comparison of these methods and possible future directions in the development of structurally small electrodes for detection of neurotransmitters will conclude the review.

## 1. INTRODUCTION

Electroanalytical chemistry has been widely applied to the study of neurochemical systems. The outcomes of such a study are expected to contribute to a better understanding of many aspects of neurotransmission, for example, neural circuitry and neural substrates of compulsive drug drug use[1-3]. This feasibility partly stems from the ease of oxidative detection of many neurotransmitters including dopamine, acetylcholine, norepinephrine, serotonin, glutamic acid and γ–aminobutyric acid. The structures of these molecules are shown in Figure 1. In addition, the development of structurally small electrodes has made in vivo detection neurotransmitters possible in biological biological microenvironments[1-3]. In this respect, the small dimension of such electrodes permits minimal tissue damage upon implantation and, of equal importance, permits very careful selection of the region of tissue where measurements can be performed. Moreover, the inherent fast response time of structurally small electrodes makes it feasible to follow biochemical events frequently taking place on a ms time scale (e.g. neuronal firing).

Various electrode materials have been reported for use in constructing structurally small electrodes of different geometries and sizes have hitherto been reported[4-7]. Common electrode materials, both modified and otherwise, include metals such as tungsten and aluminium, gold nanoparticle-deposited aluminium, various forms of carbon e.g. doped diamond, nanocrystalline diamond, pyrolysed carbon, carbon fibres, and gold nanoparticles deposited on glassy carbon[1-5].

A common problem encountered during in vivo detection of neurotransmitters is the adsorption of lipids, peptides and high molecular weight proteins present in biological matrices on the electrode surface. Formation of these layers leads to electrode fouling which distorts the voltammetric signal and suppresses the sensitivity of the electrode. Considerable research effort has been devoted to addressing electrode fouling problems. Approaches ranging from fast scan voltammetry, immobilising a protective organic film on the electrode surface, completely altering the surface termination, fabricating nanocrystalline diamond coated electrodes or doped diamond electrodes, to gold electrodes modified with gold nanorod and gold nanoparticles have been developed[1]. Apart from overcoming fouling, these

methods have also demonstrated other advantages such as wider potential windows, greater durability, increased robustness and enhanced sensitivity.

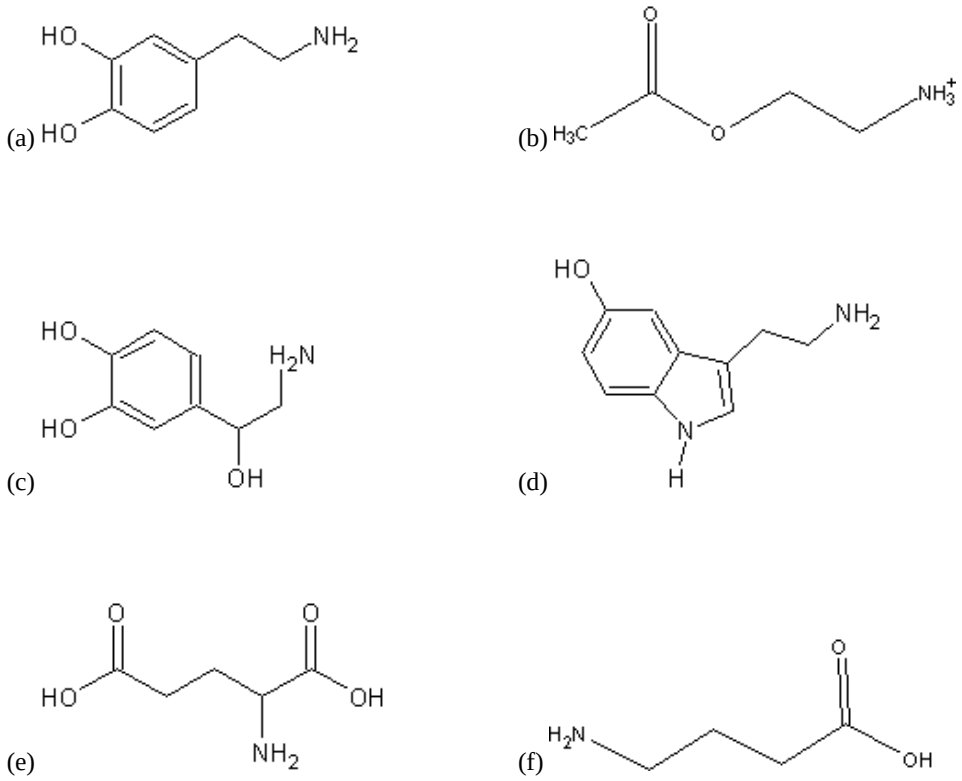


Figure 1. Common neurotransmitters: (a) dopamine; (b) acetylcholine; (c) norepinephrine; (d) serotonin; (e) glutamic acid; (f) γ–aminobutyric acid.

In this chapter, we aim to review the techniques used in developing structurally small electrodes of different geometries, which were then applied to the detection of neurotransmitters. We will also pay special emphasis on the strategies used to minimise electrode fouling during electrochemical detection of neurotransmitters at these electrodes. A comparison of these methods and possible future directions in the development of structurally small electrodes for detection of neurotransmitters will also be presented.

## 2. NEUROTRANSMITTERS AND THEIR DYNAMICS

In the mammalian brain, neuronal networks process vast amounts of information received from a subject's environment through the senses. Much of the signalling within the brain uses small molecules called neurotransmitters as messengers between neurons. During neuronal communication, neurotransmitters are released from the axon end of a neuron, usually followed by uptake of the released neurotransmitter by receptors on an adjacent neuron (i.e. the dendrites). The process of uptake involves interaction between the released neurotransmitters with membrane-bound proteins called transporters, which transport the

extracellular neurotransmitter back into the cell. The remaining neurotransmitters can diffuse out of the neuronal region and be subsequently metabolized[2]. The processing in the brain networks eventually manifests as animal behaviour. The brain is a challenging environment for chemical sensing because low concentration of analytes must be detected in the presence of interferences with yet minimal tissue damage. To conduct meaningful measurements, the properties of the analytical sensor and the general characteristics of the biological system must be understood.

Catecholamines is a group of biogenic monoamine neurotransmitters containing a nucleus of catechol, which is the aromatic portion comprising of a benzene ring with two adjacent hydroxyl groups and an aliphatic side chain of ethylamine or one of its derivatives. The immunomodulatory functions of catecholamines acting as chemical messengers transporting information between cells have been documented as early as the beginning of the last century[3]. Between cells, catecholamines act as chemical messengers that transport information[4]. This has been an area of interest to researchers as is evidenced by numerous publications in literature aimed at understanding catecholamine and quinone electrochemistry[4-8].

Among the catecholamines, dopamine has long been of interest to both chemists and neuroscientists. It is one of the most important neurotransmitters and is ubiquitous in the mammalian central nervous system[5]. It modulates many aspects of brain circuitry in a major system of the brain including the extra pyramidal and mesolimbic system, as well as the hypothalamic pituitary axis[6]. It also plays a crucial role in the functioning of the central nervous, cardiovascular, renal and hormonal systems[7]. A loss of dopamine containing neurons or its transmission is also related to a number of illnesses and conditions including Parkinson's disease, schizophrenia, motivational habit, reward mechanisms and the regulation of motor functions and in the function of the central nervous, hormonal and cardiovascular system[5, 8, 9]. It is therefore of interest to measure dopamine in the extracellular fluid in animals to order to monitor neurotransmission processes and correlate neurochemistry with behaviour[8].

## 3. MEASUREMENT OF DOPAMINE CONCENTRATIONS

The dynamics of the release and uptake of dopamine into brain extracellular space are currently under intense investigation[25-27]. Dopamine is a well-known extrasynaptic messenger that functions *via* volume transmission, escaping the synaptic cleft to bind to extrasynaptic receptors and transporters. High sensitivity, chemical selectivity, and fast temporal resolution are all desirable characteristics in detecting neurotransmitters *in vivo*. In practice, it is difficult to achieve all of these with one method.

Two techniques that have evolved to accomplish this are microdialysis and electrochemistry[5]. For measurements of basal concentration, microdialysis techniques with superb chemical specificity and sensitivity are often employed. However, the main limitations to microdialysis are spatial resolution due to the large probe size ($\geq$200 μm), resulting in significant damage to the region of the probe insertion and poor temporal resolution of 5–20 min per sample[10, 11]. On the other hand, electrochemical techniques are well suited for the measurement of transient changes in concentration. Such techniques are concerned with the

interplay between electricity and chemistry, namely the measurement of electrical quantities such as current, potential or charge, and their relationship to chemical parameters[10,12]. Electroanalytical techniques have been widely developed and, more recently, applied to the investigation of neurochemical systems, leading to a better understanding of neurotransmission through the detection of several compounds including acetylcholine, dopamine, norepinephrine, serotonin, γ–aminobutyric acid, and glutamic acid[13]. They provide a platform for the construction of sensors of the concentration fluctuations of easily oxidised neurotransmitters in the extracellular fluid of the brain[12]. An overview of the development of analytical chemistry demonstrates that electrochemical sensors represent the most rapidly growing class of chemical sensors[14, 15].

## 4. APPLICATIONS OF ELECTROANALYTICAL CHEMISTRY TO STUDY NEUROCHEMICAL SYSTEMS

For the detection of dopamine, controlled-potential (potentiostatic) techniques, which are concerned with the study of charge transfer processes at the electrode-solution interface, are favoured due to a number of advantages. These include high sensitivity, selectivity towards electroactive species, wide linear range, portability and low cost of instrumentation, speciation capability and a wide range of electrodes which allow assays of unusual environments[16].

Although multiple electrochemical techniques exist, those used in freely moving animals are chronoamperometry, differential normal-pulse voltammetry, and fast-scan cyclic voltammetry. Excellent comparisons between these can be found in literature, particularly Troyer *et al*.[17, 18, 27] and Robinson *et al*.[17] and therefore will not be discussed here.

Fast-scan cyclic voltammetry has been used extensively to investigate the rapid events associated with neurotransmission *in vivo* and *in vitro*. It is a valuable preclinical tool to evaluate both drug mechanisms and animal models of disease associated with dopaminergic transmission. Relative to other available techniques, fast-scan cyclic voltammetry offers several advantages including real time measurements of dopamine concentration on a subsecond timescale, quantification of the increases and decreases in dopamine concentrations in the nM to μM range, and positive identification of dopamine *via* the cyclic voltammograms. Detection of dopamine is further enhanced when fast-scan cyclic voltammetry is conducted at probes with a micrometer-dimension that give fine spatial resolution with minimal tissue damage[18].

Additionally, electroanalytical techniques coupled with microelectrodes offer further advantages such as enhanced current densities due to the hemispheric diffusion field around the electrodes, a lack of sensitivity to solution flow, reduced double-layer charging effects and the ability to be used in highly resistive media as the ohmic drop is small[19]. Further, the small size of microelectrodes *in vivo* imparts only minimal physical damage in living tissues while implanting into the specimen, as well as permitting a careful selection of the neural region to be investigated[20].

## 5. ULTRAMICROELECTRODE GEOMETRIES

As low concentrations of dopamine are released and rapidly cleared from the extracellular space, the sensing electrodes must be sensitive, and selective and respond quickly[6]. For *in vivo* detection of neurotransmitters such as dopamine, physically small electrodes are advantageous due to their small size and high sensitivity to catecholamines[21]. There are currently no electrodes small enough to measure dopamine concentrations within the approximately 100-nm synapse, but considerable developments are being made in minimising electrode size to approach synapses as closely as possible and also to minimise tissue damage[22]. In addition, as there are other electroactive species present at a much higher concentration than dopmaine in the extracellular medium, chemical selectivity on the electrode is absolutely essential. Furthermore, because dopamine conveys information on a subsecond time scale, fast temporal response is needed to follow these changes[21]. Electrochemical methods using ultramicroelectrodes have been proven to be rapid, simple and sensitive in the determination of dopamine[22]. In general, ultramicroelectrodes are defined as electrodes with a characteristic length that is less than 20 μm. For example, this can be an electrode with μm length in one direction and with mm length in another direction.

Electrodes of different materials have been miniaturised in many geometric shapes with the common characteristic that the electrode is significantly smaller than the diffusion layer at the electrode surface for ordinary voltammetric time scales (e.g. 1-10 s)[23]. According to Koichi[24], if the characteristic length of a small electrode, such as an ultramicroelectrode, is made infinitesimally small, it tends to adopt the geometry of either a point, a line, or a plane. On this basis, ultramicroelectrodes can generally be classified into a point electrode, a line electrode, and a plane electrode.

A point electrode resembles a spot. It adopts a spherical-shaped concentration profile and potential distribution in the solution. As a result, such electrodes easily achieve a steady state and yield a steady-state current. This current is expected to be proportional to the characteristic length (radius) of the electrode. A typical point electrode is a disc electrode inlaid on an insulating plane. On the other hand, an ultrathin ring electrode shares characteristics of the point electrode and the line electrode. It appears as a point from a position distal from the electrode, but it resembles a curved line upon closer inspection. It exhibits a steady-state current because of the feature of the point electrode. Next, a plane electrode of interest is a microarray electrode, which is composed of point electrodes and line electrodes on a planar insulator. It is versatile in functionality by designing the geometrical arrangement. A mode of mass transport depends on whether elementary electrodes are a point or a line electrode.

In the following sections, different geometries of electrodes will be discussed, and categorised as point or line electrodes. Most of these are based on carbon. This is often due to its broad potential window, low background current, rich surface chemistry, low cost, chemical inertness and suitability for various sensing and detection applications. While all common carbon electrodes share the basic structure of a six-member aromatic ring and $sp^2$ bonding, they differ in the relative density of the edge and basal planes at their surfaces. The edge orientation tends to be more reactive than the basal plane towards electron transfer and adsorption. As a result, materials with different edge to basal plane ratios display different electron transfer kinetics for a given redox analyte[25].

## 5.1. Disc-Shaped Point Electrodes

In general, a disc electrode consists of a short cylindrical rod of the electrode material embedded in a tightly fitting tube of an insulating material (e.g. Teflon). Electrical contact is made at the rear end. Disc-shaped nanometer-sized electrodes are often used because they are relatively simple and can attain true steady-state current[11]. Another approach to fabricate nanometer sized disc electrodes is the glass-sealed approach[26], in which a metal wire is sealed into a glass pipette before it is pulled into a nanometer-sized tip with the help of a laser pipette puller. Finally, the tip covered with glass is exposed either by etching away or by micropolishing a small portion of glass insulator. Similarly, Wong and Xu[27] fabricated ultrasmall carbon disc electrodes constructed by pyrolysing methane gas at a pressure of approximately 900 kPa in pulled quartz capillaries. This was found to be sufficient to form a carbon deposit at the tip of the capillary. Electrical contact to the carbon deposit was accomplished with mercury and a nichrome wire. The electrodes were estimated to exhibit structural diameters of 500–1000 nm with a fabrication success rate of 85%. Favourable stability was also observed by having current deterioration of 10% over a period of 5 days. More recently, disc microelectrode fabrication has been extended to dual-disc electrodes. This is because two micrometer-sized electrodes are very convenient for detection of two electroactive species and for acquirement of dual information in single cells[9].

## 5.3. Carbon Ultra Thin Ring

Investigations for nonplanar electrodes are important because it is easier to construct spherical or conical-shaped microelectrodes than disc-shaped microelectrodes, especially those with a very small tip[28].

Most often, ring electrodes are fabricated by applying a conductor to the walls of an insulating cylindrical support. This is often a glass rod, or for smaller diameter rings, a flame/laser heat drawn glass rod. To fabricate a metal ring, the support can be either painted with organometallic compounds or coated by vapour deposition, or sputtering of metal onto a rotating glass rod. However, the vapour deposition method ensures a uniform metal coating and permits rings of thickness ranging from 10 nm to 5 μm. The coated support is then insulated from solution by sealing into a larger glass tube with resin or collapsing the glass around the rod. The structure is then sectioned and polished to expose the inlaid ring[29].

## 5.4. Carbon Fibre Line Electrodes

The first carbon fibre microelectrode reported in literature was that fabricated by Ponchon and co-workers in 1979[30]. This procedure involved pulling a glass tube to obtain a diameter of few micrometers. Then the carbon fibre (outside diameter 8 μm, length 20 to 40 mm) was threaded into the capillary, thus enabling the fibre to be pushed a few mm through the capillary. The authors reported that this method minimised the interstitial space between the capillary and the carbon fibre. Then, the capillary was inverted into a mixture of graphite powder and polyester resin to fill 4-5 mm of the body with the paste. A contact wire was then

pushed as far as possible into the barrel filled with the paste. Immediately before use, the electrodes were cut to a length of 0.5 mm[31].

The present conventional method for fabricating carbon fibre microelectrodes is as follows. The carbon fibre is aspirated into the glass capillary that is then pulled to the dimensions of the fibre using a vertical puller. The fibre is then sealed in the glass capillary with epoxy and the electrical junction made by back filling the capillary with graphite and inserting a chrome wire for contact. In this method, poor sealing between the fibre/glass interface can often arise from unavoidable bad sealing and leakage of the epoxy. This results in high noise, low sensitivity, short electrode life and sometimes pollution of the solution in which the electrode is immersed. In addition, owing to difficulty in ensuring a successful back filling procedure with graphite, the fabrication efficiency of the method is low. Finally, with most epoxies being organic based, electrode modification or even application in organic solvents can be a challenge[32].

As the diameter of the carbon fibre determines the electrode area, electrodes of such geometry have response times greatly different from conventional electrodes. In addition, they have been found to conform to the Cottrell Equation, are linear in response to concentration, they provide a method for immediate residual current correction and they avoid the complication of competing chemical and electrochemical reactions. Furthermore, they are virtually indestructive[33].

Carbon fibre electrodes tend to have a relatively larger cylindrical surface area, compared to, for example, that of ultrasmall carbon ring electrodes. They are readily accessible to the diffusing species, giving rise to a larger detection current at carbon fibre electrodes. However, owing to the soft mechanical strength of carbon fibres, penetration into soft tissue or frequent vibrations under a microscope often make it a demanding task to manipulate the electrode into the in vivo microenvironment.

## 5.5. Microelectrode Arrays

As the electrode size decreases, especially for point electrodes, Faradaic current generated decreases proportionally to the disc radius, leading to a diminishing ohmic potential drop. In fast experiments, radial diffusion contributes little to the flux of reactant at the electrode. Thus the cell current, which is proportional to the disc area, plummets rapidly as smaller and smaller discs are used. Consequently, it is necessary to use a high-gain current-to-voltage converter, often with two or more stages of amplification, and careful attention must be paid to noise and bandwidth considerations. A direct way of increasing the current to be measured is to use more than one microelectrode, i.e., arrays of $N$ widely separated and non-interacting discs that will provide $N$ times the current from a single disc[25]. This enables exploitation of the advantages of microelectrodes whilst ensuring large total currents by using microelectrode arrays, where each microelectrode has the same function. If these microelectrodes are sufficiently spaced apart, then the array can act as the sum of the individual responses. On the other hand, if they are very close, then the array behaves as a macroelectrode with dimensions equal to that of the assembly. Signal-to-noise ratios can be improved by using such arrays, since the noise levels depend on the active area of the electrodes whereas the signal depends on the total area of the diffusion field[11].

Xiao et al[26] have reported the construction of a random array of boron doped diamond nano-disc electrodes, formed by a simple three-step method. Initially, molybdenum(IV) dioxide nanoparticles were electrodeposited on a boron doped diamond substrate. This was then covered in an insulating polymer film by electropolymerising a 4-nitrophenyldiazonium salt. Next, molybdenum dioxide nanoparticles were dissolved from the boron doped diamond surface (removing the polymer layer directly above them only) using dilute hydrochloric acid etching. This resulted in the exposure of nano-discs of boron doped diamond of approximately $20 \pm 10$ nm in diameter surrounded by a polymer insulating the remainder of the boron doped diamond. This method produced up to $650 \pm 25$ million boron doped diamond nano-disc electrodes per $cm^2$. Various random arrays of boron doped diamond nanodisc electrodes were produced using this method with a similar distribution of nano-disc size and number density, confirming that this was a reliable and reproducible method of manufacturing such nanoelectrode arrays. At modest scan rates ($10 – 1000$ mV $s^{-1}$) the array was found to produce peak currents approaching that of the Randles-Ševčík limit for the equivalent geometric electrode area despite the fact that most of the surface was insulated by the polymer as shown by voltammetry and atomic force microscopy. The experimental results were compared with simulations of both ordered and random arrays of nano-disc electrodes, the results of which demonstrated that the maximum current obtainable at such arrays was that predicted by the Randles-Ševčík equation. The array of boron doped diamond nano-discs also showed a significantly reduced capacitive background current compared to the bare boron doped diamond electrode, suggesting that such devices may offer improved signal resolution in electroanalytical measurements.

## 6. CHALLENGES IN DOPAMINE DETECTION

Detection of dopamine in a physiological environment with selectivity and sensitivity has been an important topic of electroanalytical research but one that has also experienced great challenges. Direct voltammetric detection of dopamine at naked electrodes such as carbon and metallic electrodes (such as Au, Pt) is ineffective partly because of overlapping signals from interferents in a biological environment such as the brain. These interferents include serotonin, 3,4-dihydroxyphenylacetic acid (DOPAC), uric acid and ascorbic acid. Ascorbic acid is the most commonly encountered interferent and an electroactive species that coexists with dopamine in the central nervous system. In general, dopamine is oxidised at 400 mV versus saturated calomel electrode[34,35], whereas ascorbic acid is at 500 mV[34,3, 36] and both species have comparable sensitivities on known bare electrodes[37]. In the extracellular fluid of the central nervous system, dopamine is present in the concentration range of $0.2 – 2.0$ μM[24,38], whereas ascorbic acid level is very much higher at $125 – 420$ μM[6,39]. All these make it very difficult to selectively detect dopamine in the presence of ascorbic acid by electrochemical methods.

Another common challenge in electrochemical analysis of dopamine is the phenomenon of fouling. Electrode fouling is the passivation of the electrode surface by the adsorption of non-electroactive species, particularly in the analysis of biological samples. Species such as lipids, peptides and high molecular weight proteins present in biological matrices are major

sources of fouling, which results in a decreasing electrode response over time, distorts the voltammetric signal and suppresses the sensitivity of the electrode[40,41].

## 6.1. Fast Scan Cyclic Voltammetry

In order to minimise fouling, it is essential that the electrochemical technique be fast enough to detect the analyte and quantify it before severe fouling can take place. One such technique is fast-scan cyclic voltammetry[42]. Voltammetric measurements allow the rapid concentration dynamics of redox-active species to be followed *in situ*. No other method offers this quantitative and qualitative information concerning endogenous substances on a ms time scale. Other electrochemical methods have either less chemical resolution or low time resolution[43]. In particular, dopamine released by short stimulations (<1 s) can be monitored and fast-scan cyclic voltammetry provides a good method for the evaluation of drug actions on dopamine neurons. This, with the added high time resolution of the technique, also allows the kinetics of dopamine release to be followed in greater detail[44]. Fast-scan cyclic voltammetry has been particularly useful for monitoring fluctuations of neurotransmitter concentrations both *in vivo* and *in vitro*[45]. However, fast-scan cyclic voltammetry also suffers from the drawback that the very high potential scan rates reduce the sensitivity of the method compared to that of other techniques. This is primarily due to the high background current that exceeds the Faradaic current from redox reactions of dopmaine. The background is composed of current required to charge the double layer and current arising from redox reactions of surface-attached functional groups. The magnitude of both of these is directly proportional to the potential scan rate, whereas the current arising from a diffusion-controlled electrochemical reaction is proportional to the square root of the potential scan rate. Thus optimum ratios of the Faradaic to background current are not achieved with fast-scan cyclic voltammetry[45]. As an example, fast-scan cyclic voltammetry is unable to detect concentrations much below 200 nM[46].

Fast scan cyclic voltammetry also provides only limited chemical resolution. A substance's redox potential ($E^0$) is insufficiently unique for molecular identification. In addition, to distinguish between chemical species that are involved in diffusion-controlled one-electron electrolysis processes, their $E^0$'s need to differ by at least 0.118 V. in aqueous solution, the potential limits are less than 2.0 V and so, even under optimum conditions, less than 15 compounds could be resolved[43]

To overcome these issues, as well as to detect dopamine in the presence of interferents such as ascorbic acid and uric acid, several means to improve the sensitivity and selectivity of fast-scan cyclic voltammetry have been adopted. An extension of the anodic scan limit to 1400 mV has been reported to result in a dramatic increase in the sensitivity of the electrodes to dopamine[46]. The electrodes were found to retain their sensitivity in brain tissue and were capable of measuring dopamine concentrations of 50 nM in the presence of DOPAC or ascorbic acid. Recently, an analogue method to subtract the background currents that occur during cyclic voltammetry at high scan rates has been reported[45]. This subtraction enables the use of higher gains before the analogue-to-digital conversion. Furthermore, using principal component regression to account for background changes permitted fast-scan cyclic voltammetric measurements to be made for longer times. This has enabled the monitoring of dopamine over time windows that previously were accessible only to microdialysis

experiments but with a 600 times greater time resolution. With such high time resolution, short-term dopamine fluctuations in dopamine concentrations can also be measured.

The most common approach to selective determination of dopamine in the presence of ascorbic acid and other interferents using fast-scan cyclic voltammetry is to prevent the interfering species from accessing the electrode surface. This has been achieved by many studies and approaches ranging from application of selective layers of organic films that repel the interferents, to methods of enhancing the dopamine signal while suppressing that of others.

## 6.2. Film Coated Electrodes

In 1984, the use of Nafion was as a permselective film coating on small graphite electrodes was reported by Gerhardt and co-workers[47]. This polymer is an ion-exchange perfluoronated derivative film of Teflon, which are highly permeable to cations but almost impermeable to anions. A Nafion-coated electrode will respond minimally to ascorbic acid in extracellular fluid. The membrane strongly rejects passage of anionic metabolites such as DOPAC and 5-hydroxyindoleacetic acid (5-HIAA). It is also insensitive to natural metabolites such as 3,4-dihydroxyphenylethyleneglycol. Thus, it is highly selective for only cationic species such as the primary neurotransmitters dopamine, norepinephrine, and 5-hydrocytryptamine, which are all cationic at physiological pH of ~7.4[47]. Since then, a number of studies have emerged on Nafion-modified electrodes[48,49,50,4,51,52].

However, Nafion-modified electrodes also exhibited several disadvantages. For example, the response time of the Nafion-coated sensors increases due to a reduced diffusion coefficient value in the film[4]. This can pose a serious disadvantage for *in vivo* work where dopamine and other neurotransmitter releases often occur on a sub-second time scale. In addition, Nafion coatings perform well for applications such as stripping analysis, but their use for direct voltammetric analysis is complicated by slow equilibration of the film with solution species[53]. Therefore, there is a need for a modification system that can allow for rapid and selective permeation of the ions of interest.

## 6.3. Electrochemically Grafted Aryl Films

In recent years, many carbon electrodes were modified by an oxidative procedure that generated oxygen functionalities that are useful for further chemistries[54]. In 1990, Barbier *et al*.[55] argued that electrochemical or chemical oxidation can often damage the carbon surface and oxidation tends to lead to the generation of superficial carboxylic, quinonic, ketonic or hydroxylic groups that then further react with the substance to be attached. The exact nature and number of oxygenated functional groups were thus difficult to ascertain and control, and corrosion of the carbon surface was observed, leading to large background currents. Their study provided an alternative method that was based on the electrochemical reduction of a phenyldiazonium derivative. This carbon surface modification procedure involved the formation of a diazonium radical that forms a covalent bond to the glassy carbon electrode surface. The technique was based on the electrochemical reduction of diazonium

salts, which leads to very solid and non-corrosive covalent attachment of aryl groups onto carbon surfaces.

The versatility of the method is founded on the possibility of grafting a variety of functionalised aryl groups. This allows the attachment of a wide spectrum of substances[61, 62]. In 1992, a study by Delamar and co-workers[56] demonstrated that reduction of diazonium salts at carbon surfaces resulted in a strongly attached surface layer. They attributed this to covalent bond formation between the aryl radical and the carbon surface[56, 57]. One electron reduction of aryl diazonium salts at carbon electrodes leads to grafting of aryl groups to the surface. Acetonitrile is often used as the modification medium. Reduction of the diazonium salt can be achieved by cyclic voltammetry or controlled potential electrolysis. The coupling reaction is favoured both by the adsorption of the diazonium prior to its reduction and by the relatively positive potential of the diazonium prior to its reduction[57].

Numerous studies have now focussed on this technique of using diazonium salts for modifying electrode surfaces for a whole host of applications[10,59,60,64-68]. For example, Hong and Porter[58] have reported the electrochemical reduction of benzenediazonium tetrafluoroborate in acetonitrile containing tetrabutylammonium tetrafluoroborate to incorporate a phenyl layer on glassy carbon electrodes. The phenyl modifier is reported to show strong hydrophobicity and produce the thinnest film, hence the choice of phenyl layer as a film. More recently, Pellissier *et al.*[59] reported the modification of glassy carbon electrodes with phenyl-$C_n$-$H_{2n}$-COOH moieties by electrochemical reduction of *in situ* generated diazonium salts bearing carboxylic acid groups. These groups then served as a precursor to grafting of an enzyme layer.

Downard et al[60] have reported the application of a phenylacetate layer to glassy carbon macroelectrodes. Their study determined dopamine levels in the presence of ascorbic acid. Differential pulse voltammetry of dopamine and ascorbic acid at both modified and unmodified electrodes showed almost a six-fold enhancement of dopamine anodic peaks at the modified electrodes. For ascorbic acid, while the magnitude of its anodic current remained similar at modified electrodes, the peaks were no longer as well-resolved as for unmodified electrodes.

## 6.4. Other Modifying Coatings

In addition to films such as Nafion and phenylacetate, conducting polymers[6,61] including polypyrrole, polythiophene, polyaniline, polyacetylene, and polyindole have attracted considerable attention. Among these, polypyrrole and its derivatives play the leading role because of their versatile applicability and the wide variety of molecular species covalently linked to a pyrrole group[61]. Other polymeric molecules applied to microelectrodes include polycarbazole and poly(carbazole-co-*p*-tolylsulfonyl pyrrole)[62]. Unfortunately, while improving sensor selectivity, the incorporation of conducting polymers render the electrode surface hydrophobic. With high molecular proteins being hydrophobic as well, there is subsequent adsorption of these proteins onto the electrode surface[4]. In addition, considering that covering the electrode with such protective layers is neither reproducible nor effective[63], alternative surface modifications are clearly required.

## 6.5. Nanoparticle-Modified Electrodes

Jia *et al.*[1] fabricated glassy carbon electrodes (GC) modified with gold nanorods (GN) and gold nanoparticles (GNP) *via* template technique and then dispersed the electrodes in a saturated sodium citrate solution by ultrasonication to form a gold nanorod and gold nanoparticle suspension, respectively. The electrodes were labelled as GNR/GC and GNP/GC, respectively. For comparison, glassy carbon electrodes were subjected to the same procedure but in a sodium citrate solution without any gold particles. These were labelled as activated electrodes. Dopamine was detected at all the four types of electrodes (GNR/GC, GNP/GC, activated GC and bare GC electrodes) and the resulting anodic peak currents were compared. At the GNR/GC electrode, the dopamine anodic peak current was 5 times larger than that at the GNP/GC electrodes, and 26 times larger than that at the bare GC electrodes. Peak currents similar to the bare GC electrodes were obtained at the activated GC electrodes, indicating that any increase in the peak current was due to the gold nanorods and not activation alone. The study also found that the increase in electrode surface area resulting from the gold nanorod modification was linearly related to the increase in currents. The detection of dopamine in the presence of 1000 fold ascorbic acid was found to be unhindered by the ascorbic acid at GNR/GC electrodes. This selectivity for dopamine over ascorbic acid by GNR/GC electrodes was attributed to the positively charged amine group of dopamine ($pK_a$ = 8.9), whereas the hydroxyl next to the carbonyl group of ascorbic acid ($pK_a$ = 4.10) is negatively charged at pH 7.4, which is similar to the pH of extracellular fluid. As the dispersed gold nanorods are stabilised by citrate ions and thus hold the negative charges, the gold nanorod-modified glassy carbon electrode was electrostatically repelling ascorbic acid and attracting dopamine. Therefore, the oxidation of ascorbic acid is inhibited and the oxidation of dopamine is promoted at the gold nanorod-modified glassy carbon electrode, which improves the selectivity of detection.

## 6.6. Hydrogenated Electrodes

A strategy to promote the formation of a hydrophobic surface is to directly introduce a hydrogen-terminated layer on carbon electrodes. Moreover, compared to polymeric membranes, hydrogenation reaction is more likely to yield a low-capacitance film with much less severe coverage problems. Recently, Alwarappan *et al.*[42] introduced a hydrogenated film on physically small carbon cylinder electrodes by remote plasma hydrogenation. The modified electrode clearly indicated a minimal fouling effect of 5% at hydrogenated carbon cylinder electrodes. This anti-fouling property is attributed to the hydrophobic hydrogenated layer that is free from oxygen bearing functionalities and other essential sites that facilitate the adsorption of high-molecular weight proteins, peptides and lipids.

## 6.7. Diamond Electrodes

Diamond is a material exhibiting unique properties such as extraordinary high atomic density, hardness, insulating ability, thermal conductivity, and chemical inertness. Diamond

became an object of electrochemical investigation only two decades ago because of serious handicaps including its non-conductivity and accessibility[64]. Since the first article on diamond as an electrochemical material published in 1983 by Iwaki and co-workers[65], and subsequent extensive work by Pleskov and co-workers in 1987[37], research into diamond has attracted a lot of attention. This is because the progress in the technology, deposition of diamond films from gas phase at a sub-atmospheric pressure became possible[64]. Furthermore, the performance of doped diamond electrodes can be vastly superior to that of alternative material such as glassy carbon[30]. Notable advantages include an excellent stability and reproducibility as a result of the chemical inertness, a wide potential working window in aqueous solution due to high overpotential for hydrogen and oxygen evolution and fast reaction kinetics for simple electron transfer processes[66]. Another strong factor fuelling the turn towards diamond electrodes was their ultimately strong resistance to surfactant fouling effects reflecting the surface properties of diamond electrodes, particularly the minimal number of oxygen functional groups and other surface sites that are commonly responsible for the adsorption of surface-active agents[63\. Most characterisation studies on films have used either a combination of Raman spectroscopy[10, 36], scanning electron microscopy[10], X-ray diffraction[67], atomic force microscopy[68], or X-ray photoelectric spectroscopy[4], in addition to chemical characterisation for surface studies.

There are divergent views among researchers on the role of surface termination on diamond electrode sensitivity and/or background current levels. For example, Park *et al*.[1] have reported that diamond microelectrodes do not possess surface oxides when they are hydrogen-terminated. Therefore, there are no redox waves present and the background is relatively insensitive to changes in solution pH at constant ionic strength. On the other hand, according to Suzuki *et al*.[69] oxygen-terminated boron-doped diamond is more stable than hydrogen-terminated boron-doped diamond.

Although often containing low levels of nitrogen (yellow colouration), boron (blue colouration), and other elements as dopants, natural diamond is essentially electrically insulating. Synthetic high purity diamond is one of the best insulator materials known with a break down voltage of up to $10^9$ V $m^{-1}$[74]. However, enhancing its conductivity by doping with a conducting species allows diamond to be turned into a good electrical conductor.

Boron-doped diamond electrodes have attracted much attention in the past due to their superior properties including low background currents, a wide working potential window, favourable electron transfer kinetics and surface inertness which results in high resistance to deactivation[29]. Boron-doped diamond is a near-ideal electrode material for analytical chemistry because it interferes very little with the electrochemistry of the species being measured. The typically used boron-doped diamond films prepared *via* chemical vapour deposition or hot filament deposition are hydrogen terminated. This surface provides relatively high electron transfer rates to many redox couples which involve a single electron transfer[70]. Highly boron-doped diamond couples metallic conductivity with desirable intrinsic material properties of diamond; it is robust, hard, and inert. Boron-doped diamond exhibits an impressive resistance to fouling and electrode deactivation in comparison to other electrode materials[29-71,30].

As recently as 2007, few reports on boron-doped diamond microelectrodes in biological tissue had been published. This mainly arises from the difficulties in making boron-doped diamond microelectrodes with very small tips that are less invasive of tissue. The diameter of

reported boron-doped diamond microelectrodes (10-30 μm) is still too big for applying them to *in vivo* detection, when a diameter of ~10 μm with a length of 25-500 μm is generally required for minimal tissue damage[72].

A boron-doped diamond microelectrode fabricated for application in *in vivo* detection has been reported in literature[33]. The boron-doped diamond was deposited on tungsten wires through the following procedure. Boron-doped diamond was initially deposited on tungsten wires that were electrochemically etched in 2 M NaOH at 3.0 V (vs Ag|AgCl) for 20 s to produce conical tips with very small tip diameters (~5 μm). Then seeding in an ultrasonic bath containing 2-propanol suspension of diamond particles was conducted for 1 hour. Deposition of diamond was achieved using a microwave plasma assisted chemical vapour deposition system. The carbon source was a mixture of acetone and ethanol (ratio 9:1), while $B_2O_3$ was the boron source. The diamond grain size was ~ 2 μm, while the average tip length was ~ 250 μm. In a hydrogen plasma chemical vapour deposition chamber, electrodes tend to be initially H-terminated, but this procedure went a step further involving an anodic oxidation of the boron-doped diamond electrodes, which resulted in C–O surface bonds, to facilitate peak separation between ascorbic acid and dopamine. The electrostatic repulsion between the negatively charged ascorbic acid and the negative potential of the anodic-oxidised electrode surface was reported to shift the potential to more positive values (>1.4 V versus Ag|AgCl). For *in vivo* analysis, the boron-doped diamond electrode exhibited low noise and low standard deviaiton between analyses[33].

Alternative methods for introducing electrical conductivity into diamond have been developed, which include dopants such as nitrogen[36, 37, 80], metal and metal cluster inclusions, $sp^2$ carbon inclusions in grain boundaries and subsurface hydrogen[29]. Other forms of conductive diamond, such as surface conductive or ultracrystalline diamond have also been reported in literature[29], suggesting that several types of chemically vapour-deposited diamond may find electrochemical applications[29, 73].

Nitrogenated nanocrystalline diamond with grain sizes ~10 nm is also in use as electrode material in neurotransmitter detection. The nanocrystalline films can be grown in methane (up to 30% volume with nitrogen) and argon microwave plasma. This material is an intermediate between microcrystalline diamond and amorphous diamond-like carbon. The intergrain zones consist of disordered carbon with high mixture of nitrogen. It is these zones (disordered carbon of intergrain boundaries) that impart conductivity to the material[64].

Gruen and co-workers[74] and Fausett *et al*.[29] also discussed about manufacturing smoother diamond films by modifying the growth conditions, but these films usually contain nondiamond intergranular phases. They found that nanocrystalline diamond films produced from $C_{60}$|Ar gas mixtures demonstrated basic electrochemical properties that were similar to boron-doped microcrystalline diamond films. These include a wide working potential window (~ 3 V), a low voltammetric background current (~1 order of magnitude lower than freshly polished glassy carbon), and a high degree of electrochemical activity for several inorganic redox systems without any conventional pretreatment. Nanocrystalline diamond films produced from such gas mixtures were found to be undoped, yet they possessed semi-metallic electronic properties over a potential range of at least 1.0 to -1.5 V (versus saturated calomel electrode). The conductivity of the film was attributed to charge carriers introduced by grain boundary carbon. Any resistance found in the films was recommended as being possibly

reduced through doping. As a consequence of the non-diamond intergranular phases, such films are not as hard, as chemically resistant or as thermally stable as pure diamond.

Gaudin *et al*.[75] have suggested that conductivity in polycrystalline diamond films is due to migration of adsorbates such as water, hydrocarbons, $NO_2$ and $NH_3$ through the film grain boundaries. These are believed to be capable of influencing the properties of the near surface of the film.

As Hian *et al*.[30] demonstrated, sub-micron grain sized nanocrystalline diamond films, which display conductivity as a result of graphitic inclusions within the grain boundaries, may be preferred over boron-doped diamond films as a choice in electrochemical applications. Compared to boron-doped diamond, nanocrystalline diamond films demonstrate superior advantages including wider working potential window, more robust nature of electrode, good and reproducible activity, greater activity towards aqueous systems[37].

Diamond-coated metallic microprobes of cylindrical geometry, fabricated by chemical vapour diamond deposition on tungsten wires, using selective growth techniques have also been reported[76]. The tungsten wires (130 μm diameter, 5.5 cm long) were electrochemically sharpened to a tip diameter of approximately 0.5 μm. The chemical vapour diamond deposition was performed on these sharpened wires. Scanning electron microscopy showed the surface to have a texture with nanoscale features, which increased the surface area. The actual surface exposed per length of microprobe was much greater than as smooth surface, which was attributed as one of the reasons for higher sensitivity of the microprobe in the analyte. The nano-diamond microprobe also demonstrated a large potential window of 3 V suggesting it could be a replacement for metal electrodes for electrochemical analysis and neuron imaging in brains. The sharper the tip the more sensitive the response is, with less background current.

Park *et al*.[77] have shown that a microelectrode for electroanalytical measurements can be formed by depositing boron-doped diamond thin film on a sharpened Pt wire. Response stability, fouling resistance, and low and pH independent background current were highlighted as characteristic features of this new microelectrode. These attractive properties were attributed to the absence of carbon–oxygen functional groups on the hydrogen-terminated diamond surface. Electrochemical and video imaging techniques were used to simultaneously monitor norepinephrine released from sympathetic nerves supplying rat mesenteric artery and vasoconstriction.

A comparative study between a boron-doped diamond coated Pt wire and carbon fibre electrodes to study serotonin and melatonin has recently been reported[78]. In this study, the boron-doped diamond thin film was deposited on a Pt wire by microwave-assisted chemical vapour deposition. The tapered end of this diamond-coated Pt wire was carefully heated inside a polypropylene micropipette tip, which softened the polypropylene and caused it to conformally coat the rough, polycrystalline diamond surface. This procedure resulted in a microelectrode that was cylindrical with a diameter of ~40 μm. The length of the exposed electrode was 100–200 μm. According to the study, this method of fabrication lacks precise control of the exposed electrode length. However, when applied to *in vitro* work in the mucosa in rabbit iliem, the electrodes provided extremely stable responses, with excellent sensitivity and a low limit of detection. There was no significant electrode fouling observed during the experiments, which allowed for long-term repeated measurements compared to carbon fibre electrodes from the same study.

In another study by Bhavik Patel[79], comparisons were again made in the *in vitro* electrochemical behaviour of 5-hydroxytryptamine and serotonin (neurotransmitter regulating feeding patterns) at boron-doped diamond coated Pt electrodes and carbon fibre electrodes[78], The diamond microelectrode was found to be attractive for the measurement of these neurotransmitters, and clearly outperformed a bare carbon fibre because of its resistance to fouling by the 5-hyroxytryptamine oxidation reaction products at low analyte concentrations. This is in contrast to the strong adsorption that occurs on the oxygen terminated, $sp^2$ bonded (i.e., extended *p* electron system) carbon fibre surface. This was attributed to the absence of strong molecular adsorption on the H-terminated, $sp^3$-bonded diamond surface

Halpern *et al*.[80] have reported their studies of neurons from the marine mollusc, *Aplysia californica*. In this study, the electrode of choice was a 30-µm diameter diamond microdisc electrode to study feeding patterns in the animal model based on extracellular measurements of 5-hydroxytryptamine. Apart from stable oxidation currents for the electrically-evoked release of serotonin from metacerebral cells, the key finding from this work was that the diamond electrode could be employed in both stimulation and recording of neurotransmitter release.

In another recent study, Suzuki *et al*.[33] reported the application of boron-doped diamond films on tungsten wire. The wire was electrochemically etched while simultaneously being lifted up from the etching solution. Finally, the tip of the wire was conically shaped to leave a tip with a diameter of 3 µm. The sharpened wire was then subjected to a seeding process in an ultrasonic bath containing a 2-propanol suspension of diamond nanoparticles before a boron-doped diamond film was deposited using a microwave. This electrode was used in the *in vivo* detection of dopamine in a rat. It demonstrated a larger electroactive area with lower background current, higher sensitivity, and selectivity for dopamine oxidation was demonstrated. Moreover, the different behaviour of the potential dependence between dopamine and ascorbic acid measured by different pulse voltammetry methods suggests a new method for selective detection of dopamine in the presence of ascorbic acid. As an example of an application, *in vivo* detection of dopamine in a mouse brain was also performed. High sensitivity and stability of the peak currents were found following medial forebrain stimulation. Selective *in vivo* detection of dopamine was confirmed by the inhibition of the dopamine uptake process by nomefensine.

## 7. CONCLUDING REMARKS

In a complex environment such as the brain, analysis of selected neurotransmitters present at a lower concentration than most other species has its challenges. While electrochemistry is not without limitations, it does present significant advantages over other techniques. It provides a platform for neurotransmitter sensing and monitoring *via* a wide range of techniques depending on the information needed. However, its greatest contribution is the continuously and rapidly improving advancements in sensor fabrications and design, facilitating *in vivo* chemistry in continually smaller environments.

The various designs and sizes of microelectrodes in existence today and the improvements being made to them are the foundation supporting these advancements in *in*

*vivo* and *in vitro* analyses. Each design has its strengths and weaknesses, and research is continually exploring ways to make smaller, more sensitive and selective sensors that can deliver information at a similar rate to the release and flux of the neurotransmitters in the brain. Fast scan cyclic voltammetry is often used to accomplish these rapid analyses. However, the high potential scan rates themselves lead to reduced sensitivities, high background current and limited chemical resolution. Then, the interference at a bare carbon surface from other neurotransmitters masks the signal from the neurotransmitter of choice. To overcome these difficulties, the electrode surface can be coated with protective coatings such as that of permselctive polymers and conducting polymers.

However, while electrodes modified with various films, such as Nafion, clay, conducting polymers, and others, at a physiological pH of 7.4 could absorb and even preconcentrate the cationic dopamine while effectively rejecting the negatively charged ascorbic acid and other anionic interfering agents, some disadvantages exist in the previously reported modified electrodes. For example, the response time of the Nafion-coated sensors increases due to a smaller diffusion coefficient value in the film, whereas conducting polymer-modified sensors have hydrophobic surfaces that would adsorb proteins easily. The adsorption of protein on the electrode surface is undesirable because the sensors would need renewal frequently due to the fouling effect. In most cases, a simple polymer or polymer-complex layer have been coated on the electrode surface, which plays the role of separating the voltammetric peaks other than showing electrocatalytic activity to these interested species.

Alternatively, hydrogenated electrodes have been successfully applied to electrode surfaces to suppress interferents of some unwanted neurotransmitter signals (such as that of ascorbic acid when analysing for DA). More recently, attention has turned to doped diamond as an electrode material with dopants ranging from boron, nitrogen, to intergranular $sp^2$ carbon in the film. Apart from retaining its uniquely advantageous properties such as high atomic density, hardness and chemical inertness, doped-diamond is an excellent electrical conductor and has a H-terminated $sp^3$-bonded surface that leads to absence of strong molecular adsorption of fouling species on its surface. With anodic oxidation of the H-terminated surface, the introduction of C—O surface bonds has been employed to facilitate separation of ascorbic acid and dopamine peaks. Perhaps, the next course of research should focus on preparing a surface that can repel both ascorbic acid (in the case of dopamine) and fouling species at a diamond electrode. With its versatility, doped diamond can be applied to a range of substrates such as carbon, metals, and in cases of bigger electrodes, even silicon wafers. This new electrode material has significant promise for even greater capabilities such as complete resistance to interferents. Future research is likely to engage in this pursuit for some time to come yet.

## REFERENCES

[1] Jia, Z.; Liu, J.; Shen, Y. *Electrochemistry Communications* 2007, *9*, 2739-2743.

[2] Michael, D. J.; Wightman, R. M. *Journal of Pharmaceutical and Biomedical Analysis* 1999, *19*, 33-46.

[3] Venton, B. J.; Troyer, K. P.; Wightman, R. M. *Anal. Chem.* 2002, *74*, 539-546.

[4] Xiao, Y.; Guo, C.; Li, C. M.; Li, Y.; Zhang, J.; Xue, R.; Zhang, S. *Analytical Biochemistry* 2007, *371*, 229-237.
[5] Troyer, K. P.; Heien, M. L. A. V.; Venton, B. J.; Wightman, R. M. *Current Opinion in Chemical Biology* 2002, *6*, 696-703.
[6] Wang, J. *Analytical Electrochemistry*, Third Edition ed.; John Wiley & Sons: Hoboken, 2006.
[7] Arya, S. K., Singh, S. P. and Malhotra, B. D., Ed. *Electrochemical techniques in biosensors.*; John Wiley & Sons, Ltd.: Chichester, 2007.
[8] Hermans, A.; Seipel, A. T.; Miller, C. E.; Wightman, R. M. *Langmuir* 2006, *22*, 1964-1969.
[9] Ponchon, J.; Cespuglio, R.; Gonon, F.; Jouvet, M.; Pujol, J. *Anal. Chem.* 1979, *51*, 1483-1486.
[10] McNally, M.; Wong, D. K. Y. *Anal. Chem.* 2001, *73*, 4793-4800.
[11] Fungaro, D., A.; Brett, C. M. A. *Analytica Chimica Acta* 1999, *385*, 257-264.
[12] Xu, G.-R.; Xu, M.-L.; Zhang, J.-M.; Kim, S.; Bae, Z.-U. *Bioelectrochemistry* 2008, *72*, 87-93.
[13] Sun, W.; Yang, M.; Jiao, K. *Anal. Bioanal. Chem.* 2007, *389*, 1283-1291.
[14] Laschi, S.; Mascini, M. *Medical Engineering & Physics* 2006, *28*, 934-943.
[15] Wang, J. *Electroanalysis* 1991, *3*, 255-259.
[16] Ambrosi, A.; Morrin, A.; Smyth, M. R.; Killard, A. J. *Analytica Chimica Acta* 2008, *609*, 37-43.
[17] Lane, R. F.; Blaha, C. D.; Hari, S. P. *Brain Research Bulletin* 1987, *19*, 19-27.
[18] Schenk, J. O.; Miller, E.; Rice, M. E.; Adams, R. N. *Brain Research* 1983, *277*, 1-8.
[19] Brazell, M. P.; Marsden, C. A. *Brain Research* 1982, *249*, 167-172.
[20] Miele, M.; Fillenz, M. *Journal of Neuroscience Methods* 1996, *70*, 15-19.
[21] Gao, N.; Lin, X.; Jia, W.; Zhang, X.; Jin, W. *Talanta* 2007, *73*, 589-593.
[22] Wong, D. K. Y.; Xu, L. Y. F. *Anal. Chem.* 1995, *67*, 4086-4090.
[23] Lee, Y.; Amemiya, S.; Bard, A. J. *Anal. Chem.* 2001, *73*, 2261-2267.
[24] Zoski, C. G. *Electroanalysis* 2002, *14*, 1041-1051.
[25] Strohben, W. E.; Smith, D. E.; Evans, D. H. *Anal. Chem.* 1990, *62*, 1709-1712.
[26] Xiao, L.; Streeter, I.; Wildgoose, G. G.; Compton, R. G. *Sensors and Actuators B: Chemical* 2008, *133*, 118-127.
[27] Wightman, R. M. *Anal. Chem.* 1981, *53*, 1125-1134.
[28] Chen, L.-C.; Chang, C.-C.; Chang, H.-C. *Electrochimica Acta, In Press, Accepted Manuscript*.
[29] Fausett, B.; Granger, M. C.; Hupert, M. L.; Wang, J.; Swain, G. M.; Gruen, D. M. *Electroanalysis* 2000, *12*, 7-15.
[30] Hian, L. C.; Grehan, K. J.; Compton, R. G.; Foord, J. S.; Marken, F. *Diamond and Related Materials* 2003, *12*, 590-595.
[31] Holt, K. B.; Hu, J.; Foord, J. S. *Anal. Chem.* 2007, *79*, 2556-2561.
[32] Spataru, T.; Spataru, N.; Fujishima, A. *Talanta* 2007, *73*, 404-406.
[33] Suzuki, A.; Ivandini, T. A.; Yoshimi, K.; Fujishima, A.; Oyama, G.; Nakazato, T.; Hattori, N.; Kitazawa, S.; Einaga, Y. *Anal. Chem.* 2007, *79*, 8608-8615.
[34] Venton, B. J.; Wightman, R. M. *Anal. Chem.* 2003, *75*, 414A-421A.
[35] Stefan, R., van Staden, J. F. and Abdul-Enein, H. Y. *Electrochemical Sensors in Bioanalysis*; Marcel Dekker Inc.: New York, 2001.

[36] Heien, M. L. A. V.; Phillips, P. E. M.; Stuber, G. D.; Seipel, A. T.; Wightman, M. M. *Analyst* 2003, *128*, 1413-1419.
[37] Chiku, M.; Ivandini, T. A.; Kamiya, A.; Fujishima, A.; Einaga, Y. *Journal of Electroanalytical Chemistry, In Press, Corrected Proof*.
[38] Liu, A.; Honma, I.; Zhou, H. *Biosensors and Bioelectronics* 2007, *23*, 74-80.
[39] Koichi, A. *Electroanalysis* 1993, *5*, 627-639.
[40] Geise, R. J.; Adams, J. M.; Barone, N. J.; Yacynych, A. M. *Biosensors and Bioelectronics* 1991, *6*, 151-160.
[41] Dale, N.; Hatz, S.; Tian, F.; Llaudet, E. *Trends in Biotechnology* 2005, *23*, 420-428.
[42] Alwarappan, S.; Butcher, K. S. A.; Wong, D. K. Y. *Sensors and Actuators B: Chemical* 2007, *128*, 299-305.
[43] Heien, M. L. A. V.; Johnson, M. A.; Wightman, R. M. *Anal. Chem.* 2004, *76*, 5697-5704.
[44] Stamford, J. A.; Kruk, Z. L.; Millar, J. *Brain Research* 1986, *381*, 351-355.
[45] Hermans, A.; Keithley, R. B.; Kita, J. M.; Sombers, L. A.; Wightman, R. M. *Anal. Chem.* 2008, *80*, 4040-4048.
[46] Hafizi, S.; Kruk, Z. L.; Stamford, J. A. *Journal of Neuroscience Methods* 1990, *33*, 41-49.
[47] Gerhardt, G. A.; Oke, A. F.; Nagy, G.; Moghaddam, B.; Adams, R. N. *Brain Research* 1984, *290*, 390-395.
[48] Santos, R. M.; Lourenço, C. F.; Piedade, A. P.; Andrews, R.; Pomerleau, F.; Huettl, P.; Gerhardt, G. A.; Laranjinha, J.; Barbosa, R. M. *Biosensors and Bioelectronics* 2008, *24*, 704-709.
[49] Xiang-Qin, L.; Guang-Feng, K.; Ying, C. *Chinese Journal of Analytical Chemistry* 2008, *36*, 157-161.
[50] Wiedemann, D. J.; Kawagoe, K. T.; Kennedy, R. T.; Ciolkowski, E. L.; Wightman, R. M. *Anal. Chem.* 1991, *63*, 2965-2970.
[51] Selvaraju, T.; Ramaraj, R. *Journal of Electroanalytical Chemistry* 2005, *585*, 290-300.
[52] Wiedemann, D. J.; Basse-Tomusk, A.; Wilson, R. L.; Rebec, G. V.; Wightman, R. M. *Journal of Neuroscience Methods* 1990, *35*, 9-18.
[53] Alison J. Downard, A. D. R. *Electroanalysis* 1995, *7*, 376-378.
[54] Saby, C.; Ortiz, B.; Champagne, G. Y.; Belanger, D. *Langmuir* 1997, *13*, 6805-6813.
[55] Barbier, B.; Pinson, J.; Desarmot, G.; Sanchez, M. *Journal of The Electrochemical Society* 1990, *137*, 1757-1764.
[56] Delamar, M.; Hitmi, R.; Pinson, J.; Saveant, J. M. *J. Am. Chem. Soc.* 1992, *114*, 5883-5884.
[57] Alison, J. D. *Electroanalysis* 2000, *12*, 1085-1096.
[58] Hong, H.-G.; Porter, M. D. *Journal of Electroanalytical Chemistry* 2005, *578*, 113-119.
[59] Pellissier, M.; Barrière, F.; Downard, A. J.; Leech, D. *Electrochemistry Communications* 2008, *10*, 835-838.
[60] Downard, A. J.; Roddick, A. D.; Bond, A. M. *Analytica Chimica Acta* 1995, *317*, 303-310.
[61] Cosnier, S. In *Handbook of Biosensors and Biochips*; Marks, R. S., Cullen, D.C., Karube, I. Lowe, C.R. and Weetall, H.H., Ed.; John Wiley & Sons Ltd.: Chichester, 2007; Vol. 1, pp 237-249.

[62] Ates, M.; Castillo, J.; Sezai Sarac, A.; Schuhmann, W. *Microchimica Acta* 2008, *160*, 247-251.
[63] Shin, D.; Tryk, D. A.; Fujishima, A.; Merkoçi, A.; Wang, J. *Electroanalysis* 2005, *17*, 305-311.
[64] Pleskov, Y. V. In *Electroanalytical Chemistry Research Developments*; Jiang, P. N., Ed.; Nova Science Publishers Inc: Hauppauge, 2007, pp 183-227.
[65] Iwaki, M.; Sato, S.; Takahashi, K.; Sakairi, H. *Nuclear Instruments and Methods in Physics Research, 209-210*, 1129-1133.
[66] Hiramatsu, M.; Lau, C. H.; Bennett, A.; Foord, J. S. *Thin Solid Films* 2002, *407*, 18-25.
[67] Jiang, L.; Jones, T. G. J.; Hall, C. E.; (Schlumberger Holdings Limited, Virgin I. (Brit.)). Application: GB, 2005, pp 40 pp.
[68] Wen, X.-L.; Jia, Y.-H.; Liu, Z.-L. *Talanta* 1999, *50*, 1027-1033.
[69] Yan, W.; Feng, X.; Chen, X.; Li, X.; Zhu, J.-J. *Bioelectrochemistry* 2008, *72*, 21-27.
[70] Tryk, D. A.; Tachibana, H.; Inoue, H.; Fujishima, A. *Diamond and Related Materials* 2007, *16*, 881-887.
[71] Park, J.; Show, Y.; Quaiserova, V.; Galligan, J. J.; Fink, G. D.; Swain, G. M. *Journal of Electroanalytical Chemistry* 2005, *583*, 56-68.
[72] Ivandini, T. A.; Sarada, B. V.; Terashima, C.; Rao, T. N.; Tryk, D. A.; Ishiguro, H.; Kubota, Y.; Fujishima, A. *Journal of Electroanalytical Chemistry* 2002, *521*, 117-126.
[73] Rezek, B.; Nebel, C. E.; Stutzmann, M. *Diamond and Related Materials* 2004, *13*, 740-745.
[74] Gruen, D. M.; Shengzhong, L. *Applied Physics Letters* 1994, *64*, 1502.
[75] Gaudin, O.; Whitfield, M. D.; Foord, J. S.; Jackman, R. B. *Diamond and Related Materials* 2001, *10*, 610-614.
[76] Soh, K. L.; Kang, W. P.; Davidson, J. L.; Basu, S.; Wong, Y. M.; Cliffel, D. E.; Bonds, A. B.; Swain, G. M. *Diamond Relat. Mater.* 2004, *13*, 2009-2015.
[77] Park, J.; Quaiserova-Mocko, V.; Peckova, K.; Galligan, J. J.; Fink, G. D.; Swain, G. M. *Diamond Rel. Mater.* 2006, *15*, 761-772.
[78] Patel, B. A. *The Analyst* 2008, *133*, 516-524.
[79] Patel, B. A.; Bian, X.; Quaiserova-Mocko, V.; Galligan, J. J.; Swain, G. M. *The Analyst* 2007, *132*, 41-47.
[80] Halpern, J. M.; Xie, S.; Sutton, G. P.; Higashikubo, B. T.; Chestek, C. A.; Lu, H.; Chiel, H. J.; Martin, H. B. *Diamond and Related Materials* 2006, *15*, 183-187.