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Carbon Fiber-based Microelectrodes and Microbiosensors

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1. Introduction

The chemically relatively inert carbon fiber (CF) has outstanding mechanical and electrical properties and provides an excellent base electrode for electrophysiological, electrochemical and biosensor applications on a micrometer or perhaps even on a submicrometer scale. CF microelectrodes have been used to record neuronal action potentials (spikes) since 1979 (Armstrong-James & Millar, 1979). The CFs are graphite monofilaments about 7 μm in diameter. In microelectrodes, they have outstanding extracellular recording qualities similar to those of the best tungsten electrodes.

The CF microelectrodes have been demonstrated to be very suitable for *in vivo* electrochemical detection of catecholamines (Ponchon et al., 1979) and other oxidizable biological species including nitric oxide (NO) (Malinski & Taha, 1992). Since the early times in CF applications for biorecording, a great variety of enzyme-modified CF microbiosensors has been introduced for the *in situ* determination of glucose, acetylcholine, choline, lactate, glutamate and other important compounds. The immobilization of DNA molecules (Millan & Mikkelsen, 1993) or carbon nanotubes (CNTs) (Zhang et al., 2007) onto CF microelectrodes has opened up new avenues in electrochemical detection of biologically significant species.

A basic CF microelectrode is an elementary carbon filament built in a mechanically supportive and electrically insulating borosilicate glass or plastic sheathing. The uninsulated carbon tip protruding from the sheathing by 10 to 100 μm provides a conductive surface for picking up spikes from the near vicinity of neurons and/or surface for electron transfer in microbiosensor applications. In the latter case, the carbon tip is covered with biological sensing elements such as an enzyme, receptor protein, antibody or nucleic acid immobilized in a conducting polymer matrix.

This chapter will discuss the fabrication of single- and multibarrel CF microelectrodes, the covalent modifications of the carbon surface as well as the applications of CF microelectrodes in recording spikes from neurons, electrochemical or biosensor signals from the nervous or other tissues. Attention will primarily be focused on microelectrodes containing CFs that have diameters no greater than 30 μm . A novel use of CF microelectrodes as oxygen detectors usable *in vitro* and *in vivo* will also be described for the first time.

Source: Intelligent and Biosensors, Book edited by: Vernon S. Somerset,
ISBN 978-953-7619-58-9, pp. 386, January 2010, INTECH, Croatia, downloaded from SCIYO.COM

2. Construction of CF microelectrodes

During the fabrication of CF microelectrodes, individual carbon fibers are inserted into borosilicate glass capillary tubing and single or multibarrel electrode blanks can easily be assembled. Because of the great tensile strength of the carbon fibers, they do not break when blanks are pulled to microelectrodes. After the pulling, the microelectrode is left with several centimeters of carbon fiber protruding from the glass tip. The simplest way to trim the end of the carbon fiber to the correct tip length (10-30 μm) is to cut off the excess with microscissors under a microscope. This is a difficult operation even for an experienced worker with steady hands, and the glass tip can easily be damaged. Another method of trimming the carbon fiber is electrochemical etching in dilute chromic acid or saline by applying a few tenths of a mA of alternating current. A third technique is spark etching, which allows the best control of tip length and shape for selective extracellular unit recording or electrochemical measurements (Budai & Molnár, 2001).

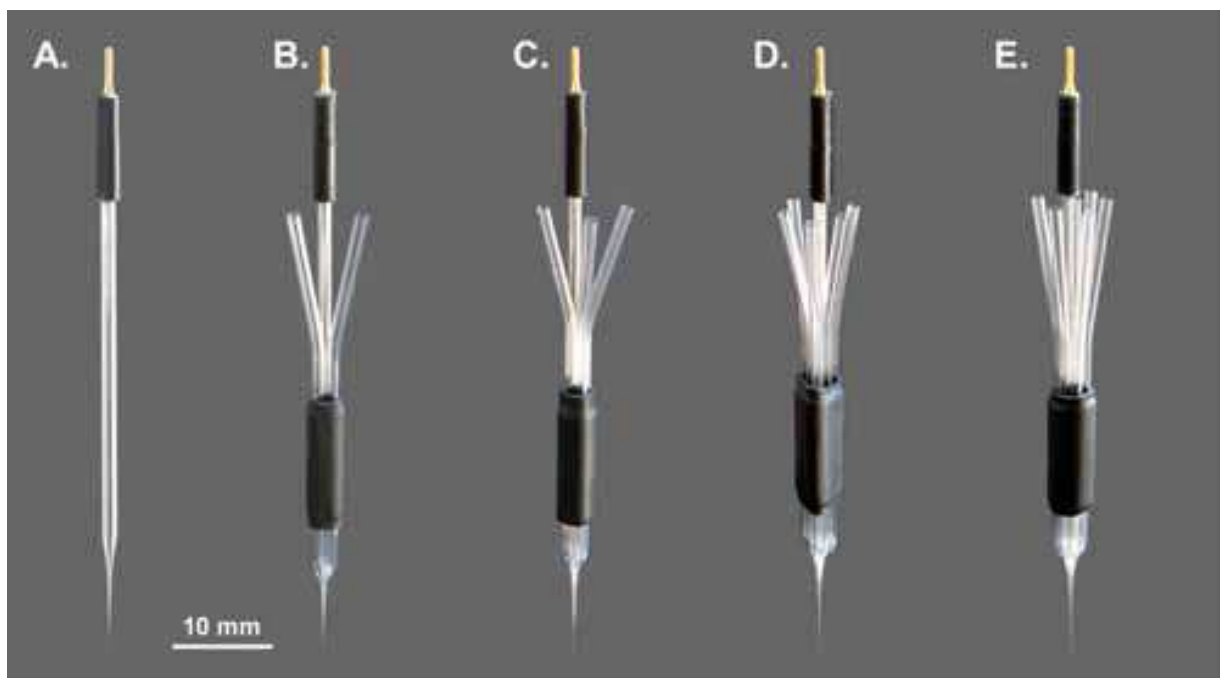


Fig. 1. View of CF microelectrodes. The base, single-barrel type (A) recording microelectrode can be completed with a varying number of micropipette barrels (B-E) used for drug delivery by means of iontophoresis or pressure or for reference/auxiliary electrodes. Tip ultrastructures are shown in Fig. 2. Courtesy of Kation Scientific.

CF microelectrodes are fabricated using 1.5 mm diam. borosilicate glass capillary tubings. Single-barrel, recording only, CF electrodes are made from standard borosilicate glass capillaries with no internal glass filament (Fig. 1A). Multi-barrel, recording and iontophoresis combination electrodes (Fig. 1, B-E) are constructed from the appropriate number of thin-wall glass tubings glued together before pulling. The CF containing recording barrel has no inner filament, whereas the iontophoresis barrels are made from glass tubings with a solid inner glass filament fused to the inner wall, which accelerates the filling of the barrels. A 10 cm long individual CF with a diameter of about 7 μm is glued to a piece of tin-plated copper wire with conductive paint or silver-filled epoxy glue. One end of the wire has previously been soldered into a gold plated male connector pin. Beginning at

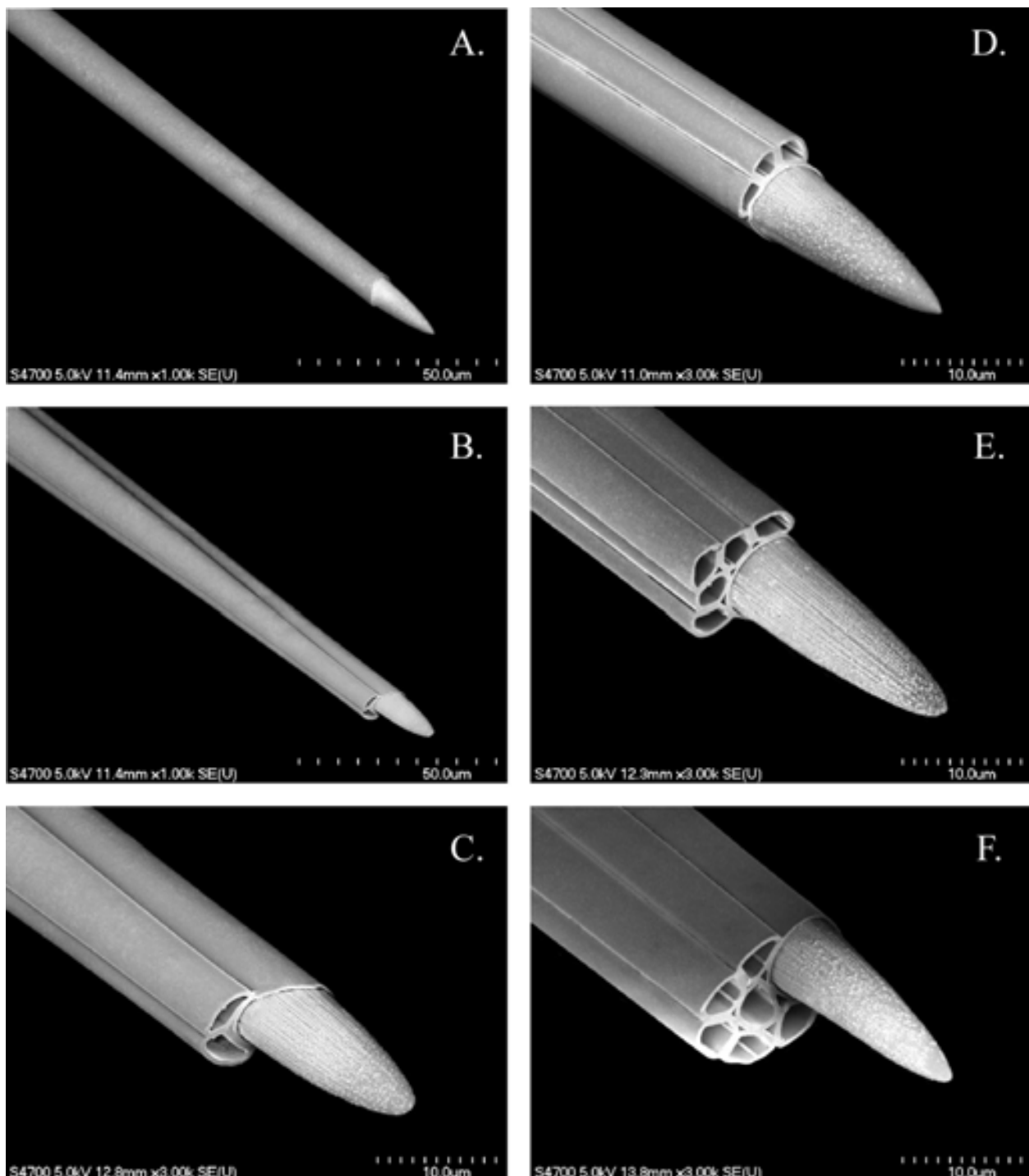


Fig. 2. Scanning electron micrographs of tips of CF microelectrodes shown in Fig. 1. Single-barrel microelectrodes are consisted of a conical carbon tip protruding from the borosilicate glass insulation (A). A varying number of micropipettes can be attached to the recording carbon fiber containing barrel (B-F) for delivering drugs by microiontophoresis or pressure. Filling of the drug barrels is facilitated by inner glass microfilaments fused to the inner wall of the microcapillaries (for example, see panel F). Courtesy of Kation Scientific.

its free end, the carbon fiber is sucked into the glass capillary tubing by gentle vacuum. The connector pin is then fixed onto the end of the glass tubing by heat-shrinkable plastic tubing.

For single-barrel electrodes, this assembly is ready to be pulled. For multi-barreled arrays, the appropriate number of inner filament-containing capillary tubes are attached to the recording barrel with two-component epoxy glue at both ends of the arrays. The glued portions of the arrays are covered with heat-shrinkable plastic tubings at both ends to provide further stability for the assembly and suitable locations for keeping the multi-barrel blanks in place during pulling and later in the electrode holder. The two ends of the electrode blank are then held by the chucks of a vertical electrode puller and the heating coil is used to soften the glass gently in the central portion of the assembly. As the glass is beginning to soften, the lower chuck is slowly rotated by one-half to two-thirds of a full circle while the electrode blank is pulled slowly by gravity only. This rotation and pulling cause the lengths of tubing to fuse together. The combination of the current supply to the heating coil and the degree and timing of the pull may be varied to produce pipettes of different lengths and diameters. Due to the very high tensile strength of the carbon fiber, it does not break during the pulling procedure. The excess fiber protruding from the tip of the glass assembly is shortened with fine scissors to about 5 mm. The exposed carbon fiber is finally trimmed by spark etching under a light microscope. Sparks are generated by a high voltage of about 800 V using a piece of polished gold wire as counter electrode. Finally, the free ends of the glass tubings in the multi-barrel electrode are heated up and bent out radially from the center to facilitate access and to reduce cross-contamination between barrels during filling. The finished single- and multi-barrel CF microelectrodes are shown in Fig. 1. Scanning electron micrographs of tips of the same electrode types are shown in Fig. 2. Using etching in chromic acid, longer tips ending in submicrometer size can also be achieved (Fig. 3). This type of carbon tip is recommended for enzyme-modified CF microbiosensors. See also Fig. 9.

3. Modifications of CF microelectrode surfaces

Modifications of carbon surfaces are of great importance in electrochemistry and material science. Most of the procedures used for modifying the carbon surface involve oxidation leading to the formation of carboxylic, quinonic, ketonic or hydroxylic groups that can be covalently coupled with molecules of further interest.

3.1 Unmodified CF microelectrodes

Unmodified or bare carbon tips are used in extracellular spike recordings (Fig. 4) or in simple electrochemical measurements of electroactive biomolecules by voltammetry or amperometry. However, oxidation of the carbon surface may occur when these electrodes are manufactured using electrochemical, flame or spark etching (Strand & Venton, 2008). Similarly to more complex biosensor CF microelectrodes, carbon surfaces of microelectrodes made for voltammetry or amperometry are covered with Nafion, a sulphonated polymer which repels anions but is selectively permeable to cations.

3.2 Nafion coating

Nafion film coating has been widely used for surface modification of CF microelectrodes for sensitive and selective determination of biological species such as dopamine in the presence of ascorbic acid. However, Nafion coating may significantly increase the electrode response time. Nafion coating is usually formed by simple dipping the electrode several times in 5% Nafion solution in aliphatic alcohol followed by drying at room temperature or in an oven at 170 °C (Gerhardt et al., 1984).

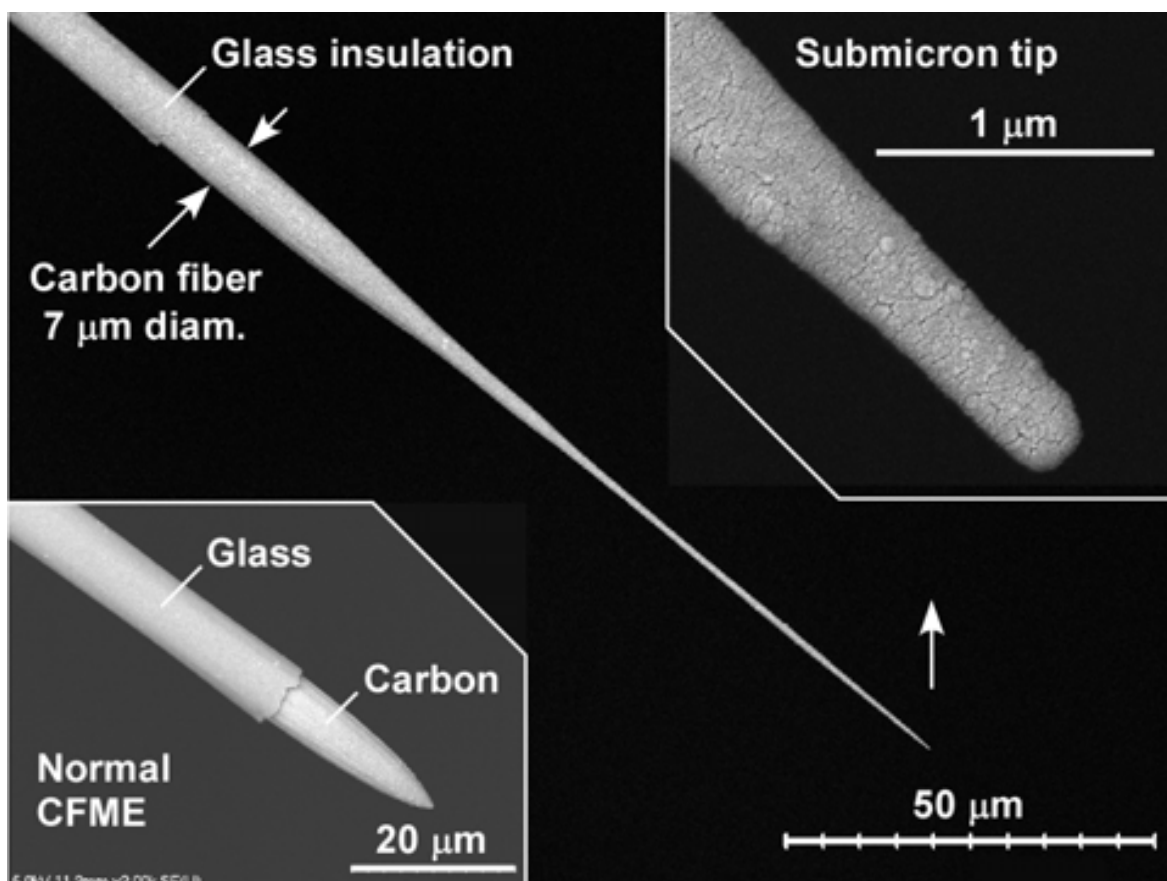


Fig. 3. Scanning electron micrograph of a single-barrel CF microelectrode with tip etched to a submicron size. Courtesy of Kation Scientific.

3.3 Covalent modifications of carbon electrodes using electrochemical methods

Using electrochemical procedures, primary and secondary amines may be covalently coupled to CF surfaces via oxidation of the amine in anhydrous ethanol or acetonitrile electrolyte solutions. One-electron reduction of aryl diazonium salts at carbon electrodes leads to grafting of aryl groups to the surface. Oxidation of arylacetates in acetonitrile may result in a monolayers of freely rotating naphthyl and anthryl groups. Formation of a covalently attached layer of alkyl groups has been described when high positive potential was applied to carbon electrodes in anhydrous solution of primary aliphatic alcohol. The robust linkage between the carbon surface and the modifier makes the initial covalently attached monolayer very suitable for functionalization of CF microelectrodes. For reviews, see (Downard, 2000; Pinson & Podvorica, 2005).

Recent research has shown that CF microelectrodes modified electrochemically with 4-sulfobenzene showed increased sensitivity and selectivity for catecholamines. The sulfonate group can provide a cation exchange site similar to Nafion but with a thinner grafted layer that is covalently attached to the electrode surface (Hermans et al., 2006).

Electrically conductive polymer layers of pyrrole or thiophene derivatives with immobilized anionic or cationic groups have recently been electrodeposited onto 7 μm carbon fibers (Sarac et al., 2008; Sarac et al., 2009). Insulating poly(oxyphenylene) polymer layers may also be deposited by electrochemical means onto CF microelectrodes (El-Deen et al., 2006; Budai et al., 2007).

3.4 Carbon nanotubes-modified CF microelectrodes

The carbon nanotube (CNT) with attractive physicochemical property has become a material of great interest for the neuro-electronic or biosensor interface. By growing CNTs on CF microelectrodes, the nanostructure of CNTs inherently increases the effective interfacial area between microelectrode and neuron (Yeh et al., 2009). Cyclic voltammetry results indicate that the prepared multi-walled CNT-modified CF microelectrodes possess a marked electrocatalytic activity toward ascorbic acid oxidation and can be used for its selective measurement in the presence of other kinds of electroactive species coexisting in rat brain (Zhang et al., 2007). Application of single-walled CNTs on a CF microdisk electrode dramatically increased the sensitivity of CF microelectrode for nitric oxide (NO) as the detection limit proved to be about 10 times lower for NO than that of the bare carbon surface (Du et al., 2008).

4. *In vivo* applications

4.1 Extracellular neuronal spike recording and microiontophoresis

Bare or Nafion-treated carbon tips of 7 μm carbon fibers protruding from the borosilicate glass insulation of CF microelectrodes provide excellent tools for extracellular recordings (Fig. 4). Electric current flows in the tissue around the neurons during action potentials can be detected by means of extracellular microelectrodes as extracellular 'spikes'. Extracellular spike potentials recorded from the mammalian central nervous system have a duration of between 0.2 and 20 ms. Their amplitudes are typically a few hundred microvolts depending on the type of neuron and the quality of the recording system. The greatest advantage of extracellular recording is that the activity of neurons can be recorded without having to impale and damage them. For this reason, most *in vivo* neuronal spike detection is done with extracellular recording. Signals picked up by extracellular electrodes need to be amplified to be able to be processed in more conventional electronic devices such as oscilloscopes or computers. The usual degree of amplitude amplification in extracellular amplifiers is around 10,000. The main difficulty with extracellular recording is the electrical "noise" which may result from external interference from electrical sources in the vicinity of the recording set-up and from the intrinsic properties of the substances making up the electrode and electrical circuit (thermal noise) used to amplify electrode signals.

In extracellular recordings and in terms of noise, CF microelectrodes are superior to tungsten microelectrodes used for the same purpose. This is due to the roughly 10 times less electrical resistance of CF microelectrodes as compared to that of tungstens. If the measured noise voltages are squared and the mean square is computed, the fluctuations of both signs contribute positively. The square root of this mean (RMS) is the usual way of expressing the magnitude of noise voltages. The RMS of the thermal (Johnson) noise is $(4kTR\Delta f)^{1/2}$, where k is the Boltzmann constant ($1.38 \times 10^{-23} \text{ JK}^{-1}$), T is the absolute temperature in Kelvin, R is the resistance of the microelectrode in ohm, and Δf is the noise bandwidth in Hz. If Δf is 5 KHz, this formula gives 5.6 μV RMS for thermal noise in the case of carbon fiber electrodes with a resistance of 0.4 $\text{M}\Omega$ when the temperature is 20 $^{\circ}\text{C}$ = 293 $^{\circ}\text{K}$. In a good-quality recording system, CF microelectrodes used *in vivo* exhibit a total peak-to-peak noise level of about 25 μV which corresponds to about 6 μV RMS (Budai, 2004).

Microiontophoresis is the technique whereby ions and charged molecules can be ejected in very small amounts from solutions contained in glass micropipettes. Microiontophoresis is most often used for: (1) deposition of dyes and neural transport tracers for histological

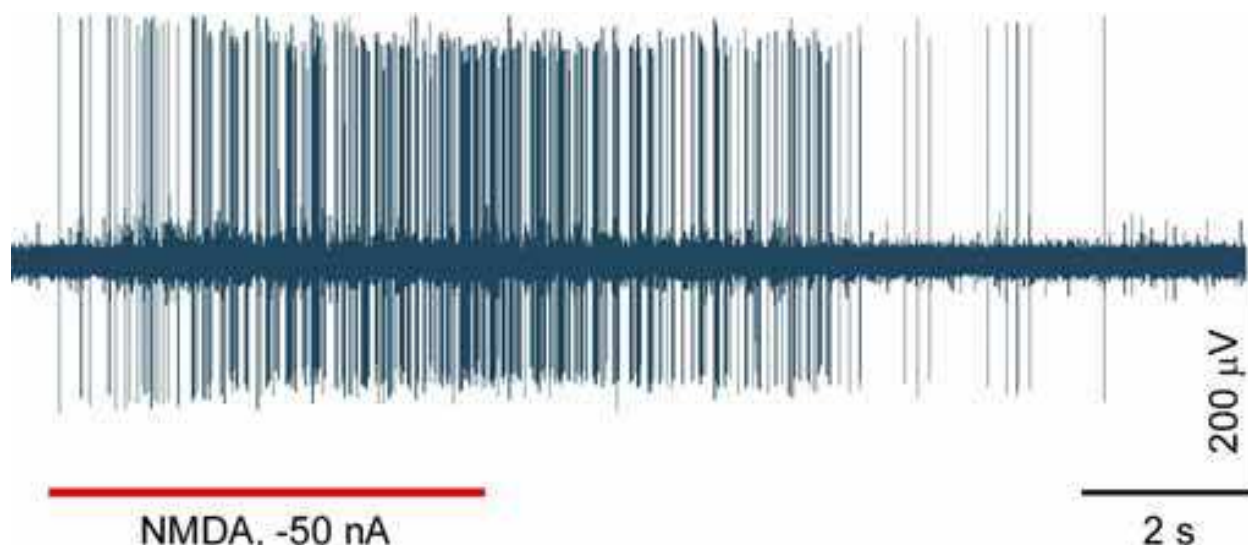


Fig. 4. Extracellularly recorded spikes from a hippocampal CA1 neuron stimulated by iontophoretic application of N-methyl-D-aspartate, NMDA. Spike recordings and microiontophoresis were performed using a Kation Scientific-made six-barreled CF microelectrode (see Figs. 1D and 2E). Courtesy of Dr. Viktor Szegedi, University of Szeged, Hungary.

examination or (2) for administration of neuroactive compounds (*e.g.* neurotransmitters, modulators, drugs or hormones) by microiontophoresis to examine their effects on firing parameters of single neurons *in vivo* (Fig. 4). Microiontophoretic ejection is accomplished by applying a voltage across the micropipette and causing it to become polarized. If a voltage is applied to a solution, ions and charged molecules will migrate toward and away from the source of the imposed electrical field depending upon the sign of their net charge. If the pipette is positioned close to a neuron, drugs may be ejected and their pharmacological effects inferred by resulting changes in the rate or pattern of firing. Typically, this neuropharmacological technique is used to determine the effects of various substances upon firing parameters of neurons. A chief advantage of the microiontophoretic method is that it is possible to examine the effects of drugs upon single neurons without affecting the whole of the nervous system such as may occur when drugs are administered systemically.

4.2 *In vivo* voltammetry

In voltammetry, voltage is applied to a working electrode and information about an electroactive analyte is obtained by measuring the current as the potential is varied. In a classical, three-electrode voltammetric system the redox reaction (electron transfer) of interest takes place at the surface of the working electrode at applied potentials and measured relative to the reference electrode which is kept at a constant potential and through which no currents flow. The current circuit is completed by an auxiliary (counter or ground) electrode to which sufficient potential is applied to balance the current produced at the working electrode. For picoampere currents produced by ultramicro working electrodes, voltammetry can be performed successfully using two-electrode configurations without significant disturbance of the reference potential.

In vivo voltammetry involves the electrochemical detection of oxidisable substances in the central nervous system. The technique now benefits from ultraminiature and more sensitive

and selective working electrodes than ever before, allowing high temporal and spatial resolutions. In *in vivo* voltammetry, the commonly used reference electrodes are the sodium-saturated calomel (SSCE), Ag/AgCl pellet (Kruk et al., 1998) or a piece of chlorided silver wire while the indifferent auxiliary electrode can be made of platinum, chromalloy (Kawagoe et al., 1993), stainless steel or it is simply a brass screw attached to the skull. When CF containing multi-barrel microelectrodes are used to record electrochemical signals, many times along with neuronal spikes in a 'time-shared' manner, the Ag/AgCl reference electrode (chlorided silver wire) is placed in one of the electrolyte filled barrels (Armstrong-James et al., 1980; Armstrong-James et al., 1981).

Small diameter (5-30 μm) carbon fibers have been used for working electrodes since 1979 when Pujol and colleagues first used them to measure oxidation of neurotransmitters including dopamine, norepinephrine and serotonin. (Ponchon et al., 1979). Ever since, a great number of surface treatments, chemical modifications and film coatings have been developed to improve the sensitivity, selectivity and temporal resolution of CF working microelectrodes used in electrochemistry *in vitro* or *in vivo*. Due to their physicochemical properties and biocompatible nature, CF microelectrodes can be used for working electrode in all forms of voltammetry used *in vivo* including chronoamperometry, linear potential scanning (e.g. cyclic voltammetry, Fig. 5) or pulsed voltammetry techniques as wells as in constant potential amperometry (Fig. 6).

The extracellular fluid in the central nervous system contains a variety of electroactive organic species that oxidize at similar potential on the carbon surface of a CF working microelectrode. These include ascorbic acid (AA); neurotransmitter catecholamines such as dopamine (DA) and norepinephrine (NE), as well as their metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC), 3-methoxytyramine (3MT), and homovanillic acid (HVA); the 5-hydroxyindole neuromediator 5-hydroxytryptamine (serotonin, 5-HT) and its metabolite 5-hydroxyindoleacetic acid (5HIAA); and the purine metabolite uric acid (UA).

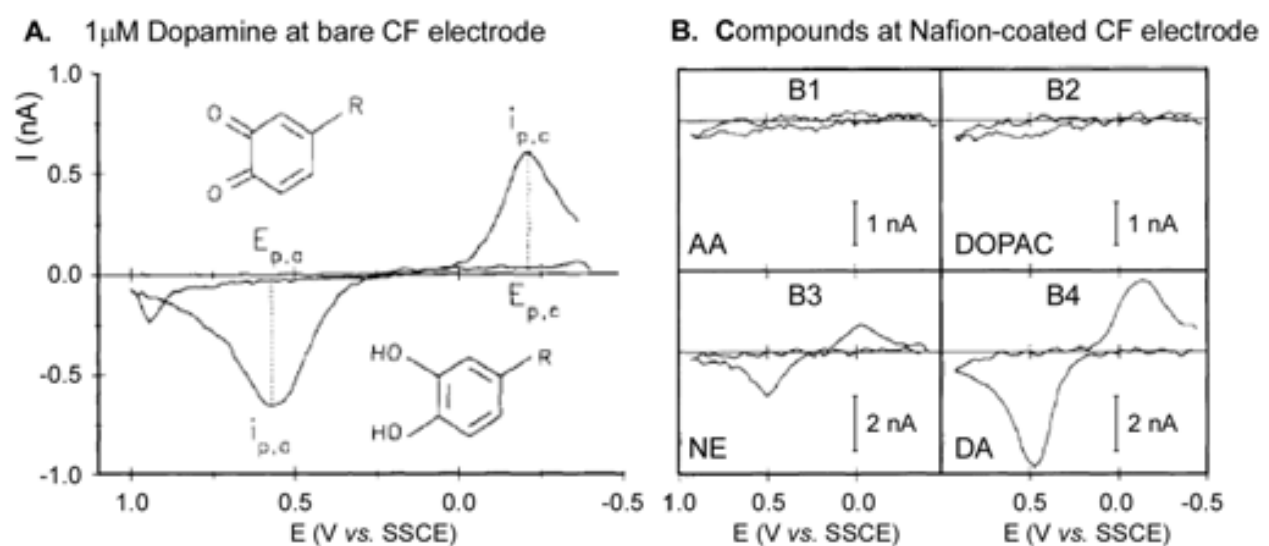


Fig. 5. Cyclic voltammograms of electroactive biological compounds. Compounds in panel B are: B1, ascorbic acid (AA), 200 μM ; B2, DOPAC, 200 μM ; B3, norepinephrin (NE), 10 μM and B4, dopamine (DA), 10 μM . Adapted with permission from Kawagoe *et al.*, 1993.

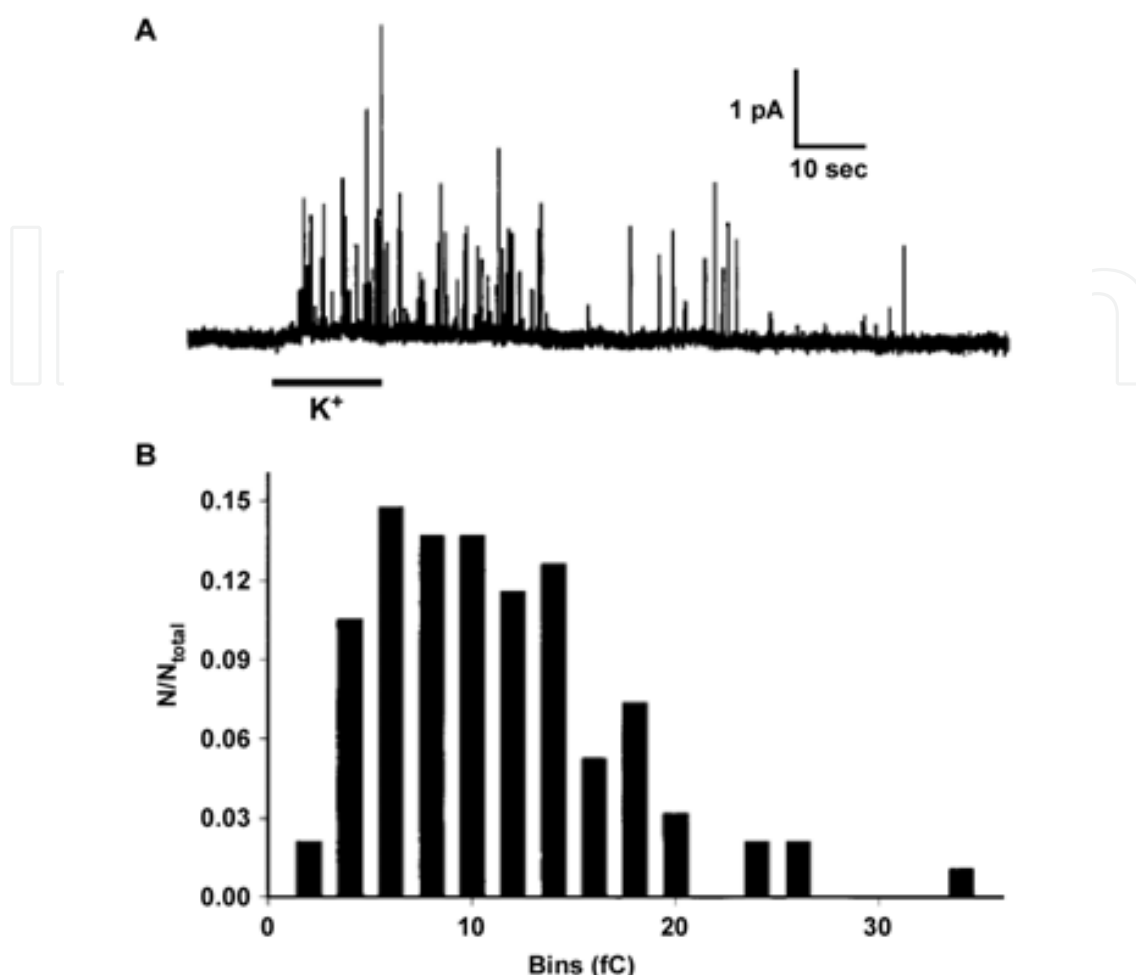


Fig. 6. Exocytotic current spikes from a dopaminergic amacrine cell stimulated with potassium (K^+). Recording was taken by constant potential amperometry using a $5\ \mu\text{m}$ diameter CF microelectrode (A). Panel B shows charge distribution from the spikes in panel A. Reprinted with permission from Hochstetler *et al.*, 2000.

One way of increasing the selectivity of CF working electrodes between these species is using discriminative measurement techniques including chrono- or constant potential amperometry, linear sweep-, cyclic-, fast cyclic- staircase-, differential pulse- or differential normal pulse voltammetry. For review, see (O'Neill *et al.*, 1998). The other possibility is to apply a coating like Nafion to repel unwanted species or to chemically change the carbon surface by modifying the carbon surface with 4-sulfobenzene (Hermans *et al.*, 2006) or using flame etching (Strand & Venton, 2008).

4.3 Monitoring nitric oxide (NO)

Nitric oxide (NO) is a gaseous signaling molecule known to influence a great variety of physiological and pathological events in all vertebrate species including humans. NO has a lifetime of a few seconds, diffuses freely across cell membranes and quite easily reacts with other biological components such as superoxide, oxygen or thiols. Electrochemical detection of NO (mostly amperometry) is the most suitable technique sensitive enough to measure concentrations of NO in biological tissues in real time without significant interference

caused potentially by other species such as nitrite, nitrate, dopamine, ascorbate and L-arginine. For review, see (Barbosa et al., 2008). The first amperometric NO electrode based on a classical Clark electrode design and consisted of a fine platinum wire as the working electrode and a separate silver wire used for reference electrode (Shibuki, 1990).

Oxidation of NO at solid electrodes, which is usually used to measure NO, takes place via an electrochemical reaction (electron transfer to the electrode):



followed by a chemical reaction:



Carbon fiber NO microelectrodes can be made on the micro- and nanoscale allowing extreme spatial and temporal resolutions and they cause minimal damage when inserted in a living tissue. Early CF NO microelectrodes were covered a variety of porphyrins and Nafion to increase selectivity by repelling interfering species (Malinski & Taha, 1992). Later, to circumvent some of the problems with porphyrin coatings (Lantoine et al., 1995; Nagase et al., 1997) phthalocyanins were used instead to modify CF microelectrode surfaces (Vilakazi & Nyokong, 2001). A great variety of different CF microelectrode coatings for NO sensors have also been reported. For review, see (Davies & Zhang, 2008).

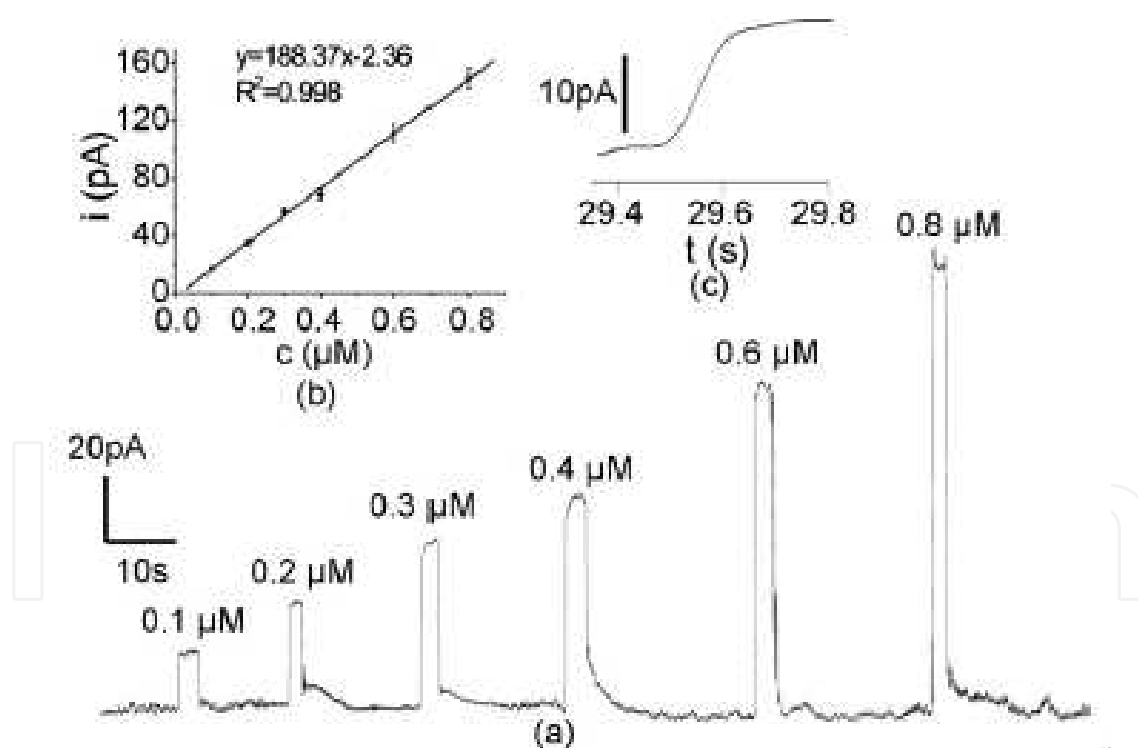


Fig. 7. (a) Typical amperometric response of a carbon microdisk electrode modified with carbon nanotubes to increasing concentrations of NO using a calibration system. (b) The calibration curve based on data shown in panel a. (c) An amperometric curve illustrating the sensor's response time to NO. Reprinted with permission from Du *et al.*, 2008.

As a new approach, combination CF-based NO sensors with micron or submicron size tip diameters have been developed by Zhang and co-workers. These sensors combine a CF

working electrode with an integrated Ag/AgCl reference electrode and both coated with a proprietary NO-selective membrane. The electrode is operated as the platinum wire-based Clark-type NO sensor (Zhang et al., 2001; Zhang et al., 2002).

Surface variations among types of carbon fibers made by different companies seem to influence the characteristics of their NO sensitivity and selectivity when coated with Nafion and *o*-phenylenediamine. The 30 μm Textron CF showed high selectivity for NO against ascorbate, nitrite and dopamine than fibers from other makers. This may be due to the strong adhesion or more uniform coating of Nafion to the smooth surface of the Textron fiber (Santos et al., 2008).

A novel NO microsensor has recently been reported using a single-walled carbon nanotubes (SWNTs) attached to the surface of a CF microdisk (cross-cut area of a 7 μm carbon fiber) and covered with a Nafion membrane (Du et al., 2008). Application of carbon nanotubes dramatically increased the sensitivity of CF microelectrode as the detection limit proved to be about 10 times lower for NO (4.3 nM) than that of the bare carbon surface and lower than most electrochemical sensors reported before (Fig. 7). The Nafion layer provided a good barrier to some of the interferents without decreasing the response speed to NO. The microsensor has been successfully applied to the measurement of NO release from single isolated endothelium cells.

4.4 A novel use of CF microelectrodes; sensing tissue oxygen levels

In its simplest form, a polarographic oxygen sensor consists of two electrodes, between which a negative polarization voltage is applied. Oxygen is chemically reduced at the cathode according to the following reaction:



Typically, a Ag/AgCl reference electrode is used for the anode, providing the following half-reaction:



The amount of current flowing through the sensor is proportional to the concentration of oxygen at the surface of the cathode. Polarographic oxygen sensors of various designs have been used for over 60 years to measure changes in oxygen tension within the brain.

The CF microelectrodes can be converted into amperometric oxygen sensors by applying a negative polarization voltage between the carbon tip (cathode) and an external Ag/AgCl reference electrode. Polarization between the anode and cathode is set to the center of the plateau region in the current voltage curve. The center of the plateau region is empirically determined for each electrode, and is consistently found to be between -0.8 and -0.95 V (Fig. 8). Within this region, the sensor current is determined by the diffusion of oxygen at the cathode and is relatively insensitive to small changes in the applied voltage. Measurements with the novel sensor are highly similar to the spontaneous and stimulus-induced oxygen responses obtained with a conventional Clark-style oxygen electrode (Allen, 2008). However, the ultraminiature barrel size and extended carbon tip make CF microelectrodes superior for combined transcranial magnetic stimulation and electrophysiology investigations (Allen et al., 2007). Multi-barreled configuration of the CF microelectrode represents a further advantage as the built-in microcapillaries can be used for recording neuronal extracellular spikes and/or applying various drugs of interest by iontophoresis or pressure.

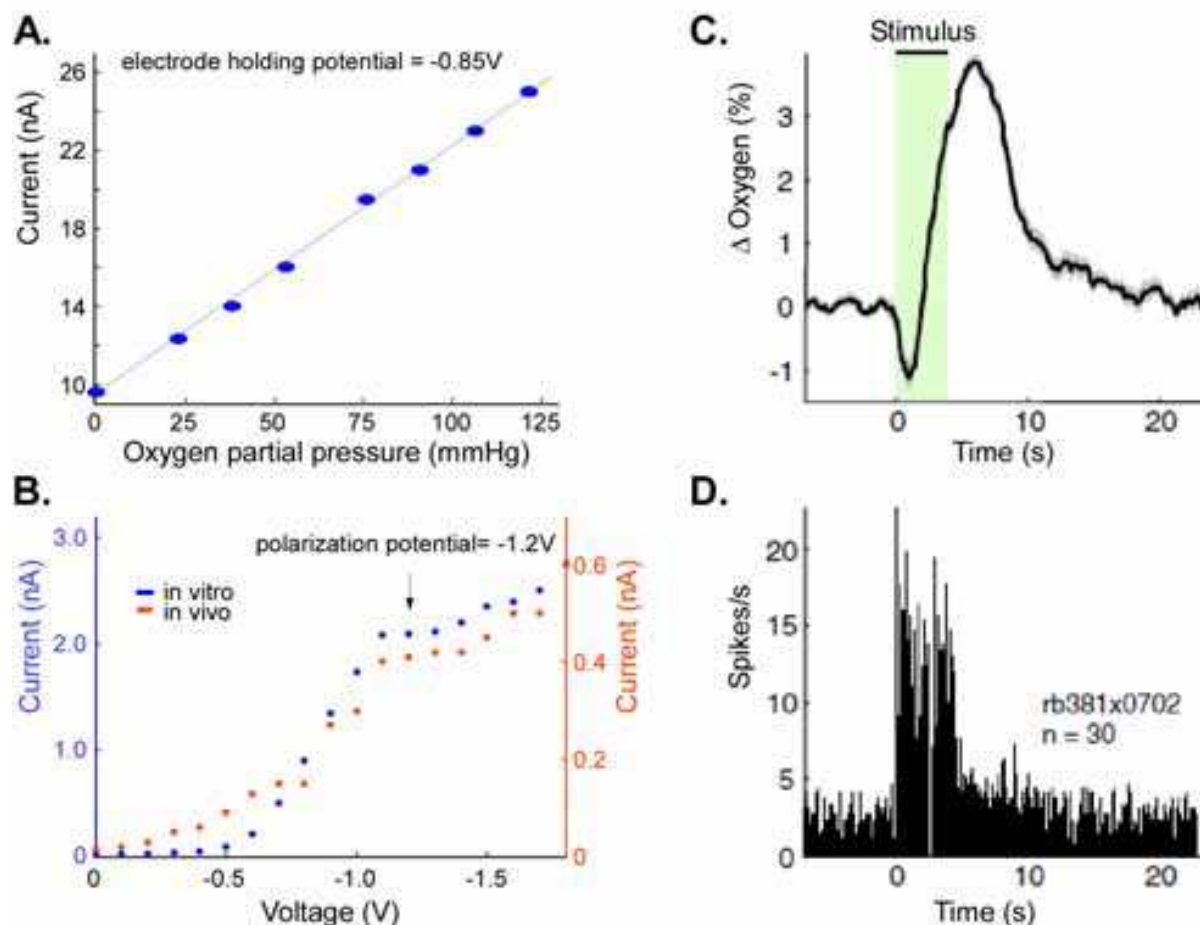


Fig. 8. Responses of CF microelectrodes to oxygen *in vitro* (A, B) and *in vivo* (C, D). A typical calibration curve for the CF oxygen sensor is shown in panel A. Note CF current plateau nearly identical in the *in vitro* and *in vivo* conditions (B) Visually evoked oxygen responses (C) and co-localized multi-unit neural activity (D) recorded from the lateral geniculate nucleus. Recordings were taken using a Kation Scientific-made seven-barreled CF microelectrode (see Figs. 1E and 2F). Reprinted with permission from the dissertation of E.A. Allen (Allen, 2008).

4.5 Enzyme-based CF biosensors

The enzyme-linked microelectrode is the fundamental component of amperometric biosensors. Physicochemical change produced by specific interactions between a target analyte (substrate) and the biorecognition element (enzyme) is detected and measured by a transducer. The transducer converts the biochemical signal into an electrical signal which can be further processed using more conventional electronic devices.

The enzyme is selected based upon the type of reaction being used to determine the analyte of interest. The most frequently used redox enzymes are: alcohol dehydrogenase, aldehyde dehydrogenase, glucose oxidase, glutaminase, horse radish peroxidase, catalase, xanthine oxidase, choline oxidase, urease, bilirubin oxidase and lactate oxidase. In addition to redox enzymes, hydrolytic enzymes like lipases or esterase are also used along with the redox enzymes for added specificity or to sense a substrate where no oxidoreductase is known. For review, see (Sarma et al., 2009).

The supporting electrode material is selected based upon the electrical conductivity and hardness of the material and is conventionally made of solid supports, such as gold, platinum or carbon. To establish electrical communication between redox proteins and electrode surface, the insulating effect of the immobilized protein layer must be transformed into a charge transport matrix. Immobilization of enzymes in conducting polymers or functionalized polymers, application of composite materials, composites of metal complexes, carbon nanotubes or other nanomaterials are used to overcome this obstacle.

Carbon fibers of 7 to 30 μm in diameter are widely used for supporting base microelectrodes in fabricating enzyme-based electrochemical sensors. Dehydrogenase enzymes have been immobilized onto CF microelectrode via avidin-biotin technology and a covalently linked hydrophilic tether to detect neurotransmitters including glutamate (Pantano & Kuhr, 1993; Hayes & Kuhr, 1999). CF microelectrodes have been coated with cross-linked redox polymer hydrogel containing glutamate oxidase (Figs. 9 and 10), horse radish peroxidase and ascorbate oxidase to monitor glutamate and ascorbate levels in extracellular space (Kulagina et al., 1999; Oldenzien & Westerink, 2005; Oldenzien et al., 2006).

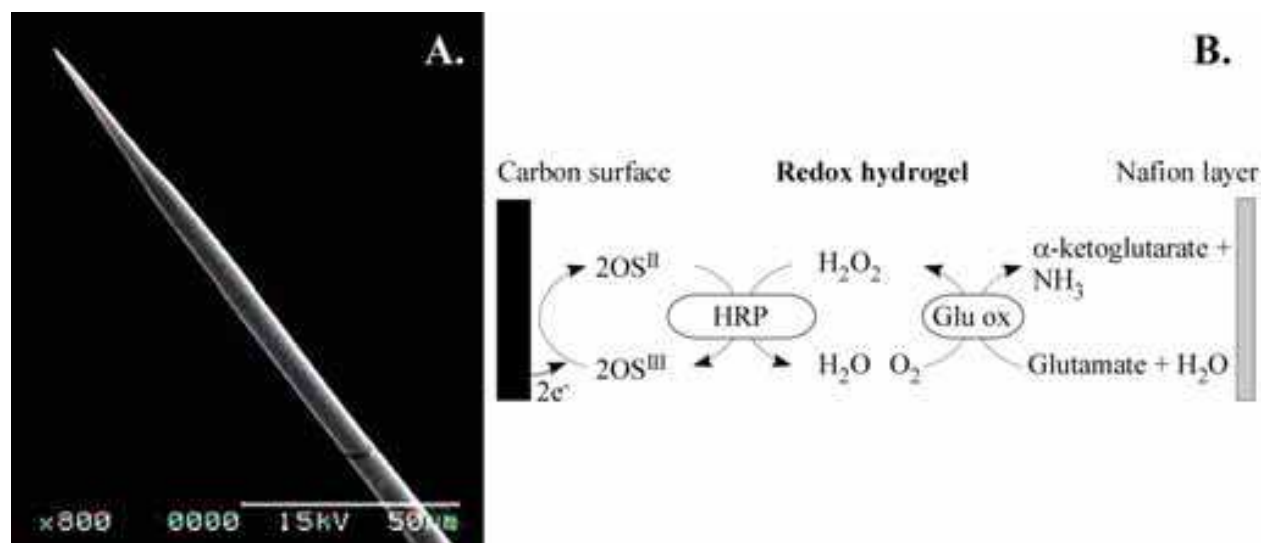


Fig. 9. (A) Scanning electron micrograph of a CF glutamate microbiosensor. Note the 7 μm carbon fiber ends in submicron tip and protrudes from the glass insulation by about 100 μm (courtesy of Kation Scientific). (B) Schematic of electrochemical detection of glutamate using osmium and oxidase-peroxidase enzymes containing redox hydrogel. Abbreviations: HRP, horse-radish peroxidase; Glu-ox, glutamate oxidase; OS, osmium.

Acetylcholine and choline microbiosensors were developed using acetylcholinesterase and choline oxidase immobilized onto CF microelectrodes (Navera et al., 1991; Tamiya & Karube, 1992; Karube et al., 1993; Garguilo & Michael, 1994; Garguilo & Michael, 1996; Cui et al., 2001; Schuvailo et al., 2005). Lactate oxidase or superoxide dismutases were immobilized on CF microelectrodes to detect lactate in rat brain (Shram et al., 1998) or superoxide anions (Tian et al., 2005), respectively. In the latter case, a third-generation biosensor was implemented by electro-deposition of gold nanoparticles on the 10 μm CF microelectrode and then modification of the gold nanoparticles by cysteine followed by immobilization of superoxide dismutase. For review on electrical contacting of redox proteins by nanotechnological means, see (Willner et al., 2006; Willner et al., 2007).

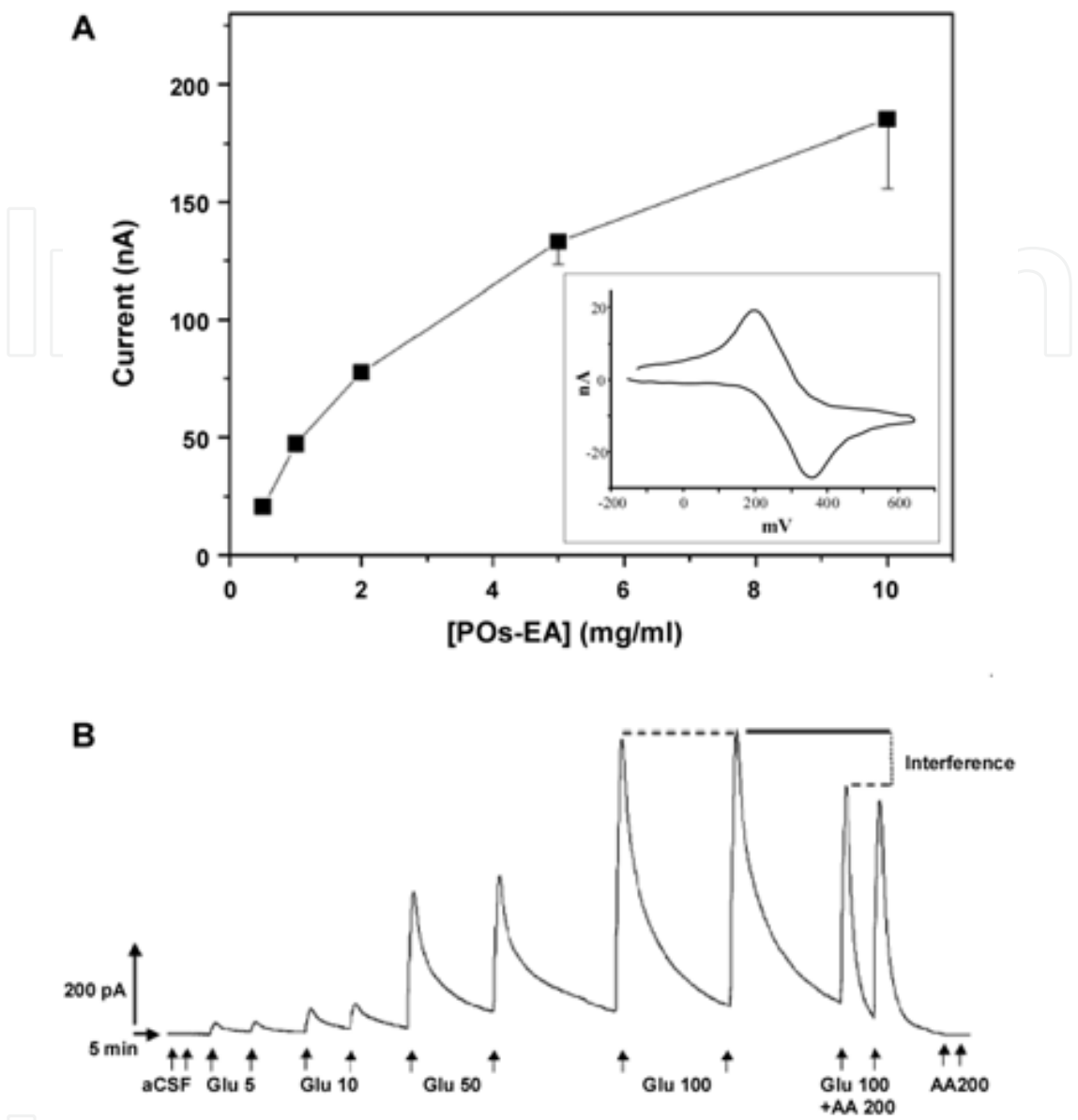


Fig. 10. Performance of a CF microelectrode-based glutamate microsensor. (A) Correlation between the concentration of the glutamate oxidase containing redox polymer (Pos-EA) and current quantified by cyclic voltammetry. A typical example of the cyclic voltammogram is shown in the inset. (B) Amperometric calibration of the glutamate microsensor. Concentration is shown in μM . Abbreviations: aCSF, artificial cerebrospinal fluid; Glu, glutamate; AA, ascorbic acid. Reprinted with permission from Oldenziel and Westerink, 2005.

Carbon fiber-based glucose microbiosensors were made by immobilization of glucose oxidase on the carbon surface using various methods (Nakayama & Matsuda, 1992; Karube et al., 1993; Furbee et al., 1994; Netchiporouk et al., 1996; Wipf et al., 2000; Cui et al., 2001). Recently, single-walled carbon nanotubes and platinum nanoparticles (Hrapovic et al., 2004), osmium redox polymer/enzyme composite film (Fei et al., 2005) or electrochemical electrometallization and electropolymerisation of phenylene diamine film with covalently bound enzymes (Schuvailo et al., 2006) were introduced to improve selectivity and

sensitivity of CF glucose biosensors. An implantable version of these glucose biosensors has also been reported (Ahmad et al., 2007).

4.6 DNA biosensors on CF microelectrodes

A tremendous progress has been made in the field of DNA biosensors since Mikkelsen and colleagues first reported an electrochemical DNA hybridisation biosensor (Millan & Mikkelsen, 1993; Millan et al., 1994). There have been two main approaches to the electrochemical transduction of DNA hybridisation, which can be broadly referred to as labelled methods and label-free methods. Labelled methods use redox active molecules (e.g. Co(Phen)_3^{3+} , methylene blue or AQMS) that bind to DNA and give different electrochemical signals depending on whether the DNA is double- or single-stranded. Label-free methods rely on either changes to the electrical characteristics of the DNA-modified interface upon hybridisation or on the natural electroactivity of DNA. For tutorial review, see (Odenthal & Gooding, 2007). A further advantage of DNA deposition on biosensing surfaces is the ability to greatly increase the effective surface area. In comparison with Nafion film coatings, the DNA sensing layer also displays some affinity toward cationic species and repelling ability toward anionic species. Equally important that the DNA layer exhibits adsorption, insertion and interchelating abilities with many bioactive species.

DNA can directly be deposited on the carbon surface of highly oriented pyrolytic graphite, carbon fiber or carbon disk microelectrodes under controlled DC potentials. Using a labelled method, the covalently bound DNA deposition resulted in a 500 to 1000-fold increase in the effective surface area and similarly enlarged voltammetric response to Co(Phen)_3^{3+} (Lin et al., 2005).

In a label-free method, double-stranded DNA was attached to the carbon surface via gold nanoparticles and was applied to determine dopamine, serotonin and ascorbic acid using cyclic or differential pulse voltammetry (Lu et al., 2004). Simultaneous differential pulse voltammetry determination of dopamine and serotonin could be achieved in the presence of 1 mM ascorbic acid.

An overoxidized microporous polypyrrole film could serve as template for directing DNA immobilization on the surface of CF microelectrodes. The formed DNA-polypyrrole biocomposite layer exhibited more effective rejection of anionic ascorbate or uric acid and more preferential collection of the cationic dopamine and epinephrine than pure polypyrrole or DNA coatings. The electrochemical signal from ascorbic acid could be totally suppressed at concentrations lower than 20 mM (Fig. 11). The selectivity factors of dopamine/ascorbate and epinephrine/ascorbate were 5000 and 2000, respectively. Unfortunately, responses of dopamine and epinephrine superposed one another with no adequate separation (Jiang & Lin, 2005).

5. Conclusion

In this chapter, the fabrication, main properties and a great deal of applications of CF microelectrodes have extensively been reviewed. It has been shown that, taking advantages of the unique and biocompatible physicochemical properties of carbon fibers, even submicron CF microelectrodes can be made for *in vivo* applications. This allows an unprecedented spatial and temporal resolution in understanding basic signalling mechanisms of dopamine and other neurotransmitters. The ever increasing sensitivity and selectivity of CF microelectrodes will facilitate detection of even lower concentrations of

analytes and the number of detectable and biologically important species will probably increase.

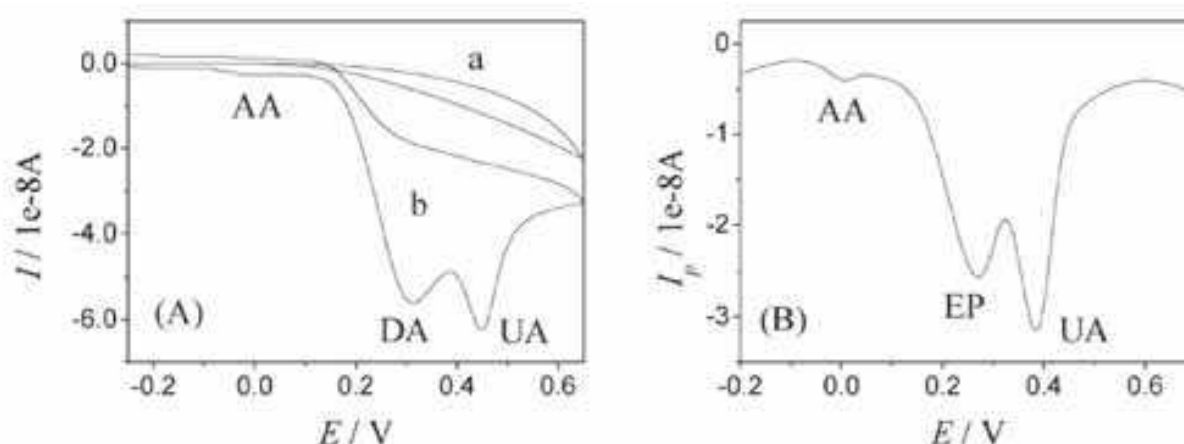


Fig. 11. (A) Cyclic voltammograms of 0.5 mM uric acid (UA), 0.1 mM dopamine (DA) and 25 mM ascorbic acid (AA) at (a) bare CF microelectrode and (b) DNA-polypyrrole covered CF microelectrode. (B) Square wave voltammogram of 0.5 mM uric acid, 0.1 mM epinephrine (EP) and 25 mM ascorbic acid using a DNA-polypyrrole CF microelectrode. Reprinted with permission from Jiang & Lin, 2005.

Despite the relative chemical inertness of CFs, their surface can be functionalized using vigorous oxidation methods. The strong bond between the carbon surface and the modifiers (carboxylic, quinonic, ketonic, hydroxylic, aryl, naphthyl, anthryl or alkyl groups, primary and secondary amines) makes the initial covalently attached layer very suitable for further functionalization of CF microelectrodes. Electrically conducting polymer matrices deposited onto the carbon surface provide means to electrically connect redox enzymes with the electrodes. Non-conducting polymer layers allow an ultrathin electrical insulation on portions of CF microelectrodes where needed.

Reports on attaching DNA or carbon nanotubes (or functionalized CNTs) onto CF microelectrodes signals the coming of a new and even more intriguing era of sensing biomolecules of interest *in situ* and in real time. Both procedures greatly extend not only the sensing surfaces of CF microelectrodes but the number of detectable organic compounds, too.

6. Acknowledgement

This work was supported by the National Office for Research and Technology, Hungary (grant no.: GVOP 3.3.1-0141/3.0) and Kation Scientific, Minneapolis, MN USA. Scanning electron microscopy is gratefully thanked to Dr. Zsolt Tóth, University of Szeged, Hungary.

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Intelligent and Biosensors

Edited by Vernon S. Somerset

ISBN 978-953-7619-58-9

Hard cover, 386 pages

Publisher InTech

Published online 01, January, 2010

Published in print edition January, 2010

The use of intelligent sensors have revolutionized the way in which we gather data from the world around us, how we extract useful information from that data, and the manner in which we use the newly obtained information for various operations and decision making. This book is an attempt to highlight the current research in the field of Intelligent and Biosensors, thereby describing state-of-the-art techniques in the field and emerging new technologies, also showcasing some examples and applications.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Dénes Budai (2010). Carbon Fiber-based Microelectrodes and Microbiosensors, Intelligent and Biosensors, Vernon S. Somerset (Ed.), ISBN: 978-953-7619-58-9, InTech, Available from:
<http://www.intechopen.com/books/intelligent-and-biosensors/carbon-fiber-based-microelectrodes-and-microbiosensors>

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