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INSTRUCTION MANUAL

CF10, CF30

*Carbon fiber microelectrodes
for use with MicroC™ potentiostat*

Serial No. _____

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CONTENTS

ABOUT THIS MANUAL	1
INTRODUCTION	1
Features.....	1
Parts List.....	2
Unpacking.....	2
INSTRUMENT DESCRIPTION	2
Selectivity.....	3
Sensitivity	4
OPERATING INSTRUCTIONS.....	4
Electrochemical Pretreating Electrodes	4
Other Pretreatment Options	5
Activating the Electrode in a Base Solution	5
WARRANTY	7
Claims and Returns	7
Repairs.....	7

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ABOUT THIS MANUAL

The following symbols are used in this guide:



This symbol indicates a CAUTION. Cautions warn against actions that can cause damage to equipment. Please read these carefully.



This symbol indicates a WARNING. Warnings alert you to actions that can cause personal injury or pose a physical threat. Please read these carefully.

NOTES and TIPS contain helpful information.

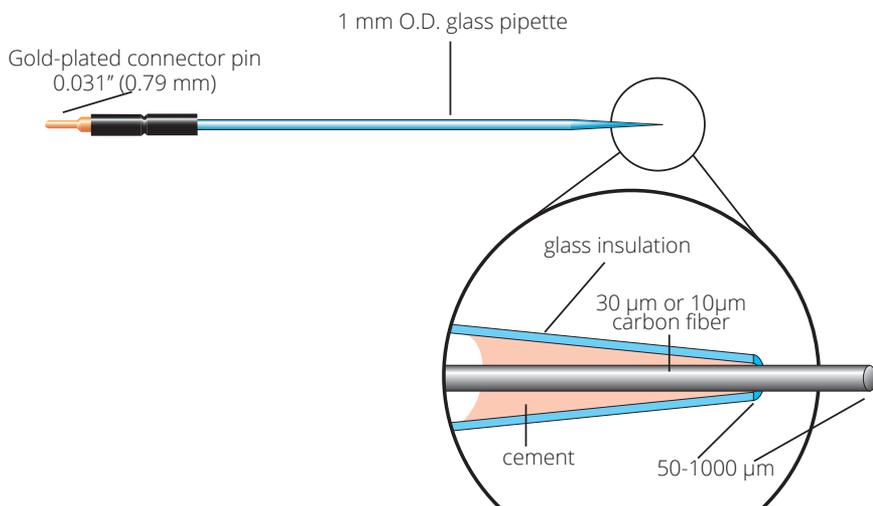


Fig. 1—Carbon Fiber microelectrodes

INTRODUCTION

WPI's ultra-sensitive carbon microelectrodes can be used, with WPI's low-noise carbon fiber potentiostat/picoammeter, **MicroC**, for the electrochemical detection of oxidizable biological compounds such as epinephrine (EP), norepinephrine (NE), serotonin (5-HT) and dopamine (DA).

Features

WPI's CF microelectrodes, made from carbon fiber monofilaments of 10 μm or 30 μm in diameter, respond with an excellent linearity to the oxidizable compounds and have a detection limit as low as 0.2nM. The shorter carbon fiber electrodes are useful in the *in vivo* amperometric and (fast) scan voltammetric measurements (FSV) for two reasons.

- In the *in vivo* studies, the spatial resolution of a measurement is important (especially in the brain research where the signal from a single neuron or small group of neurons needs to be isolated).
- In FSV the length of carbon fiber electrodes has to be restricted in order to limit the large charging background current which may stimulate biological tissue and

cause saturation of an instrument. Longer carbon fiber electrodes, on the other hand, provide higher sensitivity and larger signal to noise ratio. They are suitable for *in vitro* amperometric and differential pulse voltammetry (DPV) in which the voltage scan rates are much lower.

The CFN electrodes are Nafion-coated. The Nafion-coated carbon fiber electrodes have a ratio of up to 200:1 in sensitivity to cationic compounds versus ascorbic acid. The perfluorosulfonated polymer membrane formed on the carbon surface strongly rejects the passage of anionic metabolites like ascorbic acid and uric acid and is highly selective for cationic species such as DA, NE, 5-HT, all of which are protonated at physiological pH.

Parts List

After unpacking, verify that there is no visible damage to the sensor. Verify that all items are included:

- (1) CF Carbon Fiber Microelectrode
- (1) Instruction Manual

Unpacking

Upon receipt of this sensor, make a thorough inspection of the contents and check for possible damage. Missing cartons or obvious damage to cartons should be noted on the delivery receipt before signing. Concealed damage should be reported at once to the carrier and an inspection requested. Please read the section entitled "Claims and Returns" on page 7 of this manual. Please contact WPI Customer Service if any parts are missing at 941.371.1003 or customerservice@wpiinc.com.

Returns: Do not return any goods to WPI without obtaining prior approval (RMA # required) and instructions from WPI's Returns Department. Goods returned (unauthorized) by collect freight may be refused. If a return shipment is necessary, use the original container, if possible. If the original container is not available, use a suitable substitute that is rigid and of adequate size. Wrap the instrument in paper or plastic surrounded with at least 100mm (four inches) of shock absorbing material. For further details, please read the section entitled "Claims and Returns" on page 7 of this manual.

INSTRUMENT DESCRIPTION

Type	Diameter (µm)	Length (µm)	Impedance* (MΩ)	IBG (pA)	Detection Limit (nM)
CF30-50	30	50	0.50-2.85	5-60	10-150
CF30-100	30	100	0.35-1.90	30-120	5-50
CF30-500	30	500	0.08-0.95	50-180	0.2-10
CF30-1000	30	1000	0.02-0.45	80-280	0.2-5
CF10-100	10	100	0.50-2.75	20-70	20-250
CF10-250	10	250	0.35-2.20	30-110	5-100
CF10-500	10	500	0.15-0.95	50-180	2-50
CFN30-xx	30		Nafion-coated Carbon Fiber Electrode		
CFN10-xx	10		Nafion-coated Carbon Fiber Electrode		

* Electrode impedance is measured using WPI's **OmegaTip-Z**.

Notes

- Impedance serves as a useful index to assess active carbon area. Lower impedance and larger background current yields a lower detection limit. A small increase in impedance reading may be observed from time to time, which is thought to be due to the formation of the electrochemical graphite oxide film on the carbon fiber.
- The detection limit is defined as the concentration of compound needed to evoke 1pA of redox current response. It was determined using dopamine as the oxidizable agent and uncoated electrodes (see “Selectivity” on page 3).
- Electrode impedance was measured on WPI’s **OmegaTip-Z** meter. CFNXX-XX are Nafion-coated carbon fiber electrodes which have 20–30% smaller impedance values than comparable uncoated electrodes.
- The background currents were measured using WPI’s **MicroC** with an electrode poise voltage of +0.65V in pH7.4 salt solution. For a non-activated carbon electrode, the background current will continuously decrease. The listed values are taken at least five minutes after the electrodes are soaked in saline. A stable and small background reading can usually be obtained for all the electrodes after they are polarized for 10–15 minutes. For instructions on activating WPI’s carbon fiber electrodes, see “Activating the Electrode in a Base Solution” on page 5.

Selectivity

In the electrochemical study of cationic primary neurotransmitters such as DA, NE, and 5-HT, the discrimination against the interference of such chemicals as ascorbic acid is important in the brain tissue research. The selectivity is achieved typically by coating carbon electrodes with Nafion. The polymer membrane formed on the electrode surface responds minimally to ascorbic acid or uric acid, strongly rejects passage of anionic metabolites and is highly selective for only the cationic species such as DA, NE and 5-HT, all of which are protonated in the physiological pH.

Fig. 2 shows the time course of the current responses, measured using the **MicroC** potentiostat, of a Nafion-coated fiber electrode **CF30-500** to 10mM ascorbic acid and 10mM dopamine.

Dopamine Concentration/Response Curve

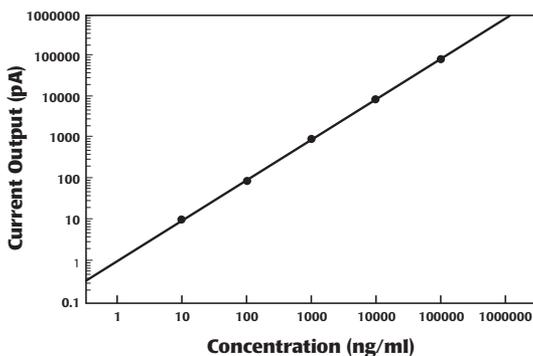


Fig. 2—Excellent linearity in the response of the carbon fiber electrode (CF30-500) to dopamine recorded with a MicroC. (Courtesy of Dr. D. Yeomans and Dr. X.-T. Wang, University of Illinois at Chicago.)

In A ratio of 200:1 in sensitivity to the cationic compounds versus ascorbic acid can be easily achieved by electro-coating a carbon electrode in a 1–5% Nafion solution. Fig. 3 shows the time course of the current response, measured using WPI's **MicroC** potentiostat and a Nafion-coated fiber electrode (**CF30-500**) to a solution containing 10 μ M ascorbic acid and 10 μ M dopamine.

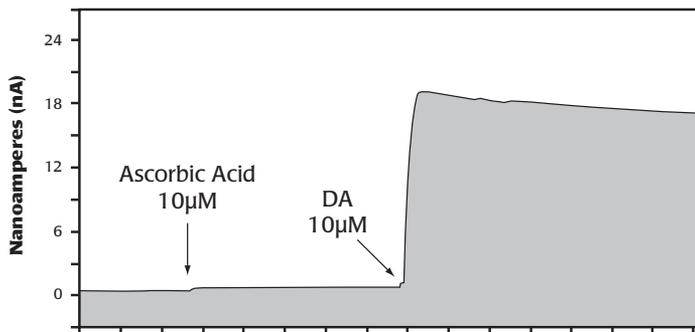


Fig. 3—Selectivity of the electrode

WPI can supply Nafion-coated carbon fiber electrodes. Experimental data indicates that WPI's Nafion-coated carbon electrodes not only show excellent selectivity for cationic compounds, but also have increased sensitivity to them (up to 50% greater) in comparison with the uncoated electrodes. This is in accordance with an observed decrease in electrode impedance following coating with a Nafion surface membrane.



TECH TIP: Users of WPI's uncoated fiber electrodes may choose to coat electrodes before an experiment. To deposit a Nafion membrane, apply a poise voltage and dip the electrode into a Nafion solution three times, each time for 3-5 seconds. Then, air-dry the electrode for 10-15 minutes before the experiment.

Sensitivity

WPI's carbon fiber electrodes series CFX-XX are highly sensitive. Using uncoated fiber electrodes, oxidizable compounds such as dopamine with a concentration as low as 2nM can be detected. Detection of 0.20nM of dopamine can be achieved using a Nafion-coated fiber electrode (**CF30-1000**).

OPERATING INSTRUCTIONS

Electrochemical Pretreating Electrodes

Since catecholamines, indolamines and ascorbic acid all have labile hydroxyl groups, they exhibit oxidation reactions at similar applied potentials. To detect one compound in the presence of others, various protocols have been used to electrochemically pretreat carbon fiber electrodes. Although experimental results proved their validity the precise underlying mechanisms of these pretreatment, like the surface chemistry of carbon fiber in general, are still unknown.

You can use electrochemical pretreatment to activate carbon electrodes to improve their response. The sensitivity, selectivity and stability (repeatability) of carbon electrodes can be significantly improved through electrochemical activation.

Numerous techniques have been proposed for such activation/pretreatment, although the precise working mechanism(s) of these treatments are mostly unknown. To treat the carbon fiber electrodes on WPI's potentiostat, **Micro-C**:

1. Turn the Select knob to V-ext and I-Range to 2 μ A.
2. Apply the required waveform (from an external source).

TIP: If you prefer, use the **Micro-C** V-internal to apply a static DC potential to the electrode. Set the appropriate voltage by adjusting the V-int control potentiometer screw. If you use this method, the Select knob should be set to V-int. This can be monitored with the Display in the V mode.

Other Pretreatment Options

These pre-treatment procedures usually involve application of triangular voltage waveform from 0 to a positive voltage for seconds. The exact waveform and duration of treatment determines the carbon electrode selectivity. The treated electrodes usually possess their selectivity for several hours, which makes the treated electrodes ideal for the acute implanted recordings.

NOTE: Since the working mechanisms of these electrochemical treatment procedures are unclear, it is recommended that users follow strictly the proposed protocols without deviations.

- **Selectivity for Catecholamines**—Treat the carbon fiber electrodes in PBS (phosphate-buffered-saline) using a 70Hz triangle wave (from 0 to +3.0V) for 20 seconds. Then, hold the electrode in the PBS at +1.5V for 20 seconds (Gonon, et al., *Anal. Chem.*, (1981),53 1386-1389). This procedure separates the ascorbic acid response peak at -80mV from the catechols peak at +70mV (vs. Ag/AgCl) electrode.
- **Selectivity for 5-hydroxyindoles** (such as serotonin or melatonin)—Treat the electrode with a 0 to +3.0V triangular wave at 70Hz for 20 seconds, followed by 0 to +2.5V at 20Hz for another 20 seconds. Finally, apply a 0 to +1.0V at 7Hz for 20 seconds (Cespuglio, et al, *Brain Res.*, 223, 287 (1981)).
- **Simultaneous detection of compounds**—Electrochemical treatment of the carbon fiber electrodes can not only achieve a high sensitivity ratio for catecholamines or indolamines against readily oxidizable “interfering” compounds such as ascorbic acid, certain procedures have been shown to resolve the oxidizing potentials of these three categories of compounds and make the simultaneous detection of the them possible. A three step procedure proposed by Cahill and Wightman (1995), as mentioned above, creates carbon fiber electrodes having good sensitivity for catecholamines and ascorbic acid at 180 and 50mV versus sodium-saturated calomel electrode (SSCE), respectively. A complex five-step procedure (Crespi, et al., *Neurosci. Lett.*, 52, 159 (1984).) is used to detect the catecholamines, 5-hydroxyindoles, and ascorbic acid simultaneously.

Activating the Electrode in a Base Solution

Since the first reported use of graphite paste electrodes for detection of electroactive substances in brain tissue, an activation step has been shown to be imperative to obtain a reproducible response from carbon electrodes. There are a few techniques proposed to obtain reproducible response at carbon electrodes. One of the most useful procedure is electrochemical treatment of glass carbon and pyrolytic carbon film electrodes in a base solution.

Optimum activation conditions used a pH of 13 while applying a potential of +1.2V for a duration of 5 minutes (Anjo, D.M. et al., *Anal. Chem.*, 61, 2603-2608, 1989). This pre-treatment has been shown to minimize electrode absorption and capacitance and enhance the reversibility of its response to oxidizable compound such as catecholamines.

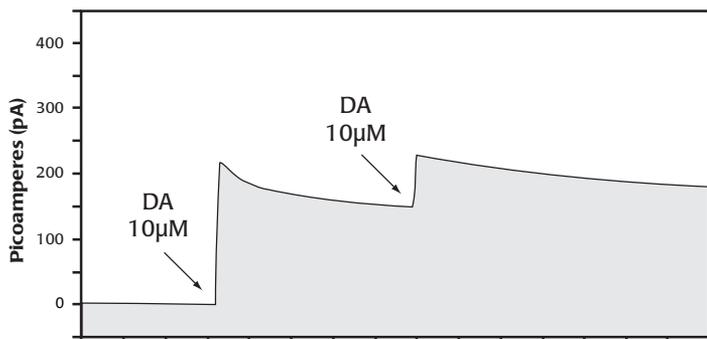


Fig. 4—Selectivity of the electrode

Carbon electrodes are commonly sealed with epoxy cement, heat-shrink Teflon tubing or polypropylene insulation, which cannot withstand the strong base solution. Also, both the absorption and capacitance of electrodes reach plateaus when they are activated in solutions with pH ranging from 10 to 13. Considering these factors, WPI recommends using a medium base solution (pH 9.5 NaCl solution) for electrochemical activation of carbon fiber electrodes. Fig. 4 and Fig. 5 illustrate the responses of a WPI carbon fiber electrodes similar to **CF10-500** (10 μ m diameter, 1mm in length) to applications of 10 μ M dopamine before (Fig. 4) and after (Fig. 5) the electrochemical activation of the electrode. The activation procedure not only improved the response reversibility of the electrode but also increased its sensitivity by nearly 10 times.

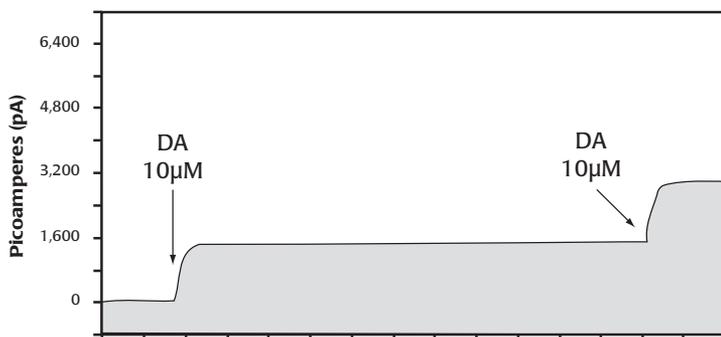


Fig. 5—Current response after activation

WPI recommends activating all carbon fiber electrodes in a pH 9.5 salt solution (for example, NaCl 150mM), at 1.2V (relative to an Ag/AgCl electrode) for 5-10 minutes before use, reuse or calibration.

TIP: This can be conveniently accomplished using the V-act position on **Micro-C** potentiostat.

WARRANTY

WPI (World Precision Instruments, Inc.) warrants to the original purchaser that this equipment, including its components and parts, shall be free from defects in material and workmanship for a period of 30 days* from the date of receipt. WPI's obligation under this warranty shall be limited to repair or replacement, at WPI's option, of the equipment or defective components or parts upon receipt thereof f.o.b. WPI, Sarasota, Florida U.S.A. Return of a repaired instrument shall be f.o.b. Sarasota.

The above warranty is contingent upon normal usage and does not cover products which have been modified without WPI's approval or which have been subjected to unusual physical or electrical stress or on which the original identification marks have been removed or altered. The above warranty will not apply if adjustment, repair or parts replacement is required because of accident, neglect, misuse, failure of electric power, air conditioning, humidity control, or causes other than normal and ordinary usage.

To the extent that any of its equipment is furnished by a manufacturer other than WPI, the foregoing warranty shall be applicable only to the extent of the warranty furnished by such other manufacturer. This warranty will not apply to appearance terms, such as knobs, handles, dials or the like.

WPI makes no warranty of any kind, express or implied or statutory, including without limitation any warranties of merchantability and/or fitness for a particular purpose. WPI shall not be liable for any damages, whether direct, indirect, special or consequential arising from a failure of this product to operate in the manner desired by the user. WPI shall not be liable for any damage to data or property that may be caused directly or indirectly by use of this product.

Claims and Returns

Inspect all shipments upon receipt. Missing cartons or obvious damage to cartons should be noted on the delivery receipt before signing. Concealed loss or damage should be reported at once to the carrier and an inspection requested. All claims for shortage or damage must be made within ten (10) days after receipt of shipment. Claims for lost shipments must be made within thirty (30) days of receipt of invoice or other notification of shipment. Please save damaged or pilfered cartons until claim is settled. In some instances, photographic documentation may be required. Some items are time-sensitive; WPI assumes no extended warranty or any liability for use beyond the date specified on the container

Do not return any goods to us without obtaining prior approval and instructions from our Returns Department. Goods returned (unauthorized) by collect freight may be refused. Goods accepted for restocking will be exchanged or credited to your WPI account. Goods returned which were ordered by customers in error are subject to a 25% restocking charge. Equipment which was built as a special order cannot be returned.

Repairs

Contact our Customer Service Department for assistance in the repair of apparatus. Do not return goods until instructions have been received. Returned items must be securely packed to prevent further damage in transit. The Customer is responsible for paying shipping expenses, including adequate insurance on all items returned for repairs. Identification of the item(s) by model number, name, as well as complete description of the difficulties experienced should be written on the repair purchase order and on a tag attached to the item.

** Electrodes, batteries and other consumable parts are warranted for 30 days only from the date on which the customer receives these items.*

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