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INSTRUMENTS AND TECHNIQUES

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A simple way to build a grinder for carbon-fibre electrodes for amperometry or voltammetry

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Abstract Carbon-fibre electrodes are used widely for studying exocytosis by amperometry. Currently, there are two major methods for insulating fibres so as to leave the tip as the only conductive surface: encapsulation with plastic or glass. The latter offers advantages such as better insulation and a known electro-active surface. In addition, such electrodes are suitable for in vivo electrochemistry because they can penetrate brain tissues. However, the construction of glass-encapsulated electrodes requires a grinder to polish the electrode surface with precision. This apparatus is expensive because it needs a very stable motor, a diamond surface and a micromanipulator. We describe the construction of a cheap precision grinder using a computer drive and an old microscope.

Keywords Electrochemistry · Exocytosis · Catecholamines · Chromaffin · Secretion

Introduction

Carbon-fibre electrodes have contributed to the increase of our knowledge of exocytotic phenomena [14] and to the clarification of many aspects of CNS physiology and pharmacology [10, 13]. To reduce capacitative and basal currents in such electrodes, it is important to restrict the surface of the electrode to the area over which the measurements are to be performed. The reduction of the detective surface to obtain disk electrodes is especially important for single-cell recordings because the electric noise is proportional to the background current, which increases with the exposed carbon surface. Several methods have been used to make circular/elliptical disk electrodes. Plastic insulation is a simple method: a carbon fibre is inserted into a polyethylene tube [2–4] or micropipette tip [6, 7] that is subsequently pulled by heat and cut with a scalpel. A second method uses a paint electrodeposition [11] and the third option consists of polishing a glass-encapsulated electrode sealed previously with epoxy- [5] or electrodepositing resin [2, 4].

Cutting plastic-encapsulated or paint-insulated fibres in the usual way (scalpel) produces a much less defined and rather rough surface than those bevelling glass-insulated fibres: compare Fig. 6 in [7] (plastic-insulated) with Fig. 7 in http://www.biophysics.org/education/wightman.pdf (glass-insulated). With a bevelled surface, it is possible to place the polished surface evenly onto an isolated cell. These characteristics are very important for calibrating electrodes with known concentrations of amines (noradrenaline and serotonin) by flow injection analysis. In addition, rigid electrodes are mandatory for penetrating the brain to perform in vivo studies using amperometry or cyclic voltammetry.

One important disadvantage of glass-encapsulated electrode is the cost of commercially available grinders. Hence, whilst electrode pullers are usually available in many laboratories, grinders are seldom found. The cost of the currently available grinders (WPI, Sarasota, Fla., USA; Sutter Instruments, Novato, Calif., USA; Narishige, Tokyo, Japan) is relatively high for many laboratories (~$3,500). Other methods using custom-made bevelling devices have been described previously. These methods include vibration [8], "jet-stream" [9], or dry bevelling [1]. Here, we present a simple method for constructing a grinder that produces excellent polished electrode surfaces. We needed to purchase only the diamond lapping film (€ 50).

A 5 1/4″ floppy disk drive from a PC provides a stable and vibration-free device that spins at 300 rpm. Although 3 1/2″ disk drives are also suitable, the 5 1/4″

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drive is more stable and provides a bigger rotor surface to accommodate the polishing dish. The rotor is usually located on the lower side of the drive. Once connected to a computer power supply, the spin will start by connecting the pin No. 16 of the signal cable with any odd-numbered pin (ground). A floppy disk must be inserted into the rotor to spin continuously.

A general view of the apparatus is shown in Fig. 1; photos of the grinder are shown in Fig. 2. The polishing plate is built by glueing a 3-μm diamond lapping film (Ref 48014-30, WPI) in a 10-cm Ø Petri dish. The Petri dish can be glued or affixed to the rotor using double-side adhesive tape that allows the removal of the plate when desired. Most micromanipulators could be used to hold the electrode, although we built one using an old Nikon teaching microscope. After removing the base and the stage from the stand, we sawed off the upper part in order to use just the focussing system. The focussing wheel for the condenser will also help to situate the electrode tip. The electrode holder was adjusted at 45° angle to produce an elliptical electrode surface.

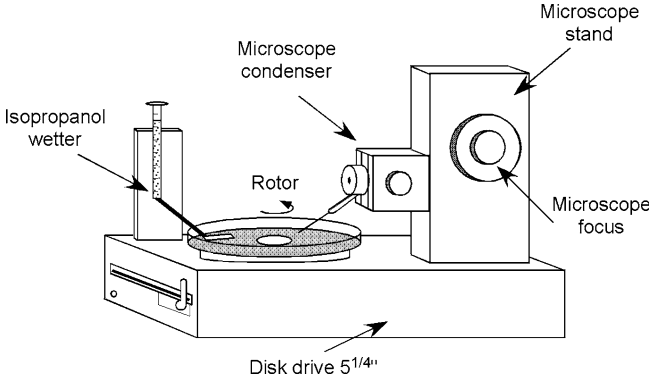

Fig. 1 General view of the grinder. To hold the electrode, the arm of an old student microscope is screwed to the disk drive. A lapping film stuck onto a Petri dish, which is glued onto the rotor of the disk drive rotor, makes the polishing surface. The polishing surface is kept wet with isopropanol, which is distributed evenly from a reservoir via a piece of cotton material. For simplicity, the light illuminator and the magnifying lens are not shown. Full views are shown in Fig. 2

Isopropanol improves carbon electrode performance. It removes most of the grease and debris and also incorporates hydroxyl radicals from the polished surface [5]. For that reason, we added a holder for an isopropanol reservoir, from which it can be distributed across the diamond surface. It consisted of a 1-ml syringe attached to 16-G stainless steel tubing (Abbocath-T, Abbott, Sligo, Ireland) and a bent 22-G needle. The system was completed by attaching four rubber stoppers to the underside of the disk drive to minimize vibration and to assure a firm position on the table. Additionally, a light bulb and a magnified lens can be added to improve the observation of electrode tip (see Fig. 2).

The calibration of the polished electrodes in a flow injection system showed responsiveness similar to that obtained with a Narishige EG-40 grinder (Fig. 3a). Using 5-μm radius carbon-fibre electrodes (Thornel P55, Amoco Greenville, SC, USA), 50 μM noradrenaline evoked an oxidation current under stop-flow conditions of 235 ± 23 pA ($n = 5$), compared with 242 ± 15 pA ($n = 12$) for electrodes bevelled with the Narishige SV-40 grinder. Polished electrodes offer an even surface that can touch the membrane of a chromaffin cell without producing spontaneous secretory spikes. When cells are stimulated with 5 mM $BaCl_2$, they release catecholamines, thus producing typical amperometric traces, which are similar for electrodes polished with either grinder (Fig. 3b,c).

Our new design offers a low-cost electrode grinder for the construction of fine amperometric sensors. These glass-encapsulated electrodes can be repolished several times so as yield a new clean surface every day.

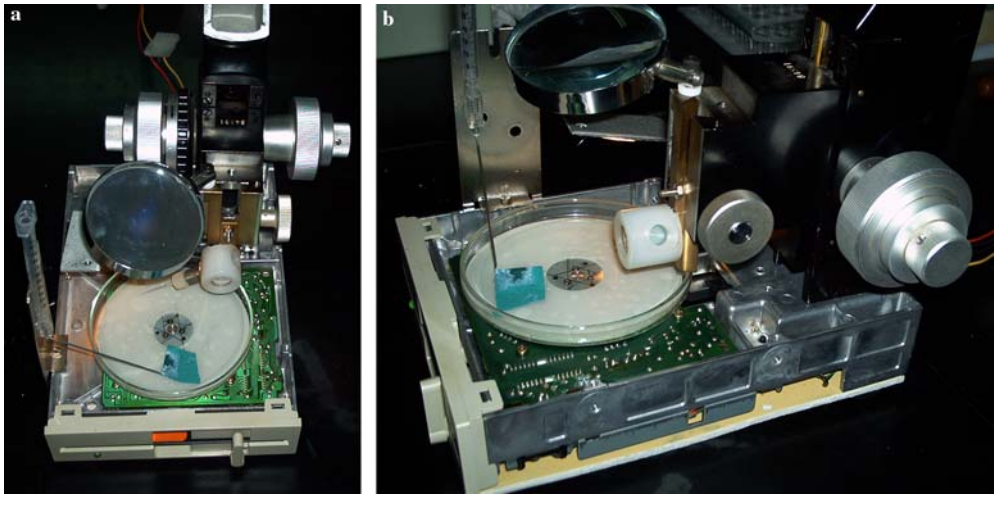

Fig. 2 Views of the grinder from the front (*left*) and side (*right*)

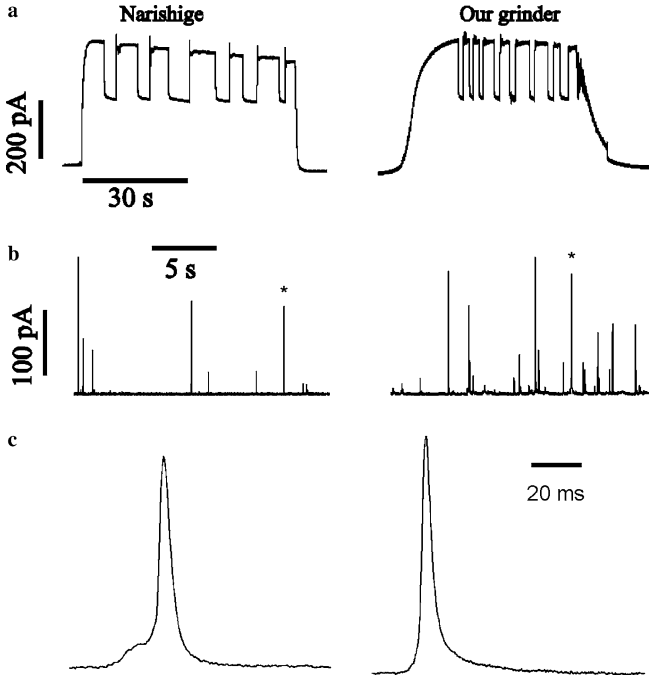

Fig. 3a–c A comparison between electrodes polished with a Narishige EG-40 (*left*) and with the grinder described in the present paper (*right*). *a* Typical traces obtained in a flow injection cell on switching the Krebs-HEPES solution to the same buffer containing 50 μM noradrenaline; the flow is stopped several times to quantify the sensitivity of electrodes when amperometric currents are not affected by the continuous supply of new catecholamine molecules. *b* Typical secretory traces obtained after stimulating mouse chromaffin cells with a Krebs-HEPES solution containing 5 mM $BaCl_2$ for 5 s. The spikes marked with * are enlarged in *c*. Amperometric traces were analysed using our macros for Igor Pro that can be downloaded as freeware from: http://webpages.ull.es/users/rborges [12]

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