Supporting Information

Facile preparation of nanostructured copper-coated carbon microelectrodes for amperometric sensing of carbohydrates

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Experimental

Chemicals and reagents

The standards of the tested carbohydrates (xylose, fructose, galactose, glucose, sucrose, maltose, lactose, raffinose, dextran) were obtained from Sigma-Aldrich. De-ionized water, sodium hydroxide obtained from Fluka (Fluka AG, Buchs, Switzerland), and gradient-grade acetonitrile (LabScan, Dublin, Ireland) were used to prepare the mobile phase.

Carbon fiber microelectrode fabrication

The procedure for microelectrode fabrication is as follows: the carbon fiber is glued using conductive silver epoxy (EC101, Polytec, Germany) onto a copper wire and the junction is then cured at 150 °C for 10 min. The fiber with copper contact attached is fitted into the glass capillary, about 10 mm of the fiber is left protruding from its contracted end. Both ends of the capillary are sealed using epoxy resin (CHS Epoxy 1200, Sindat Pilsen, Czech Republic). Prior to use, the protruding fiber is cut to a length of about 5 mm by lancet, and the fiber end of the electrode is briefly sonicated in dichloromethane.

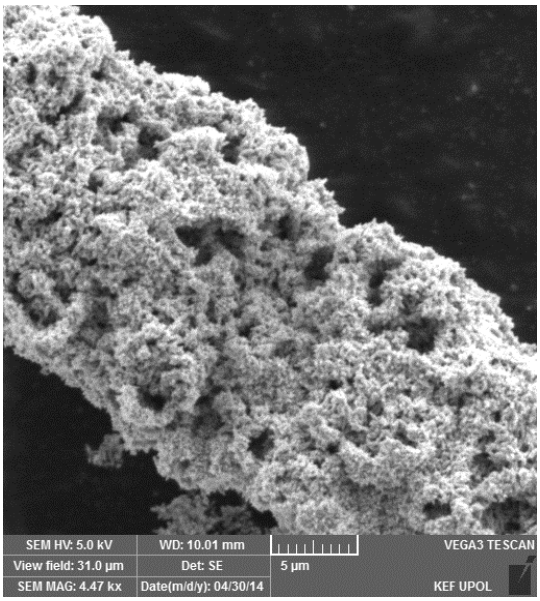

Fig. S1: SEM image of copper-coated carbon fiber; E_{dep} = 20 V, t = 20 min.

Modification of carbon fiber microelectrode with copper

3 cm of copper wire (99.99%, 0.5 mm diameter, Alfa Aesar, Karlsruhe, Germany) was immersed into ultrapure water (Millipore) contained in 25 ml quartz beaker. CFME was

placed 1 cm apart from copper wire. Copper wire was attached to the positive pole of a regulated power supply while CFME was connected to the negative pole. The voltage of the power supply was set to the desired level for the desired period of time. Optimum coverage was obtained with the following deposition parameters: $E_{dep} = 15$ V, $t = 20$ min. Higher voltages gave a thicker coating, providing higher carbohydrate sensitivity but diminished operational stability (Fig. S1).

Amperometric detector layout

For flow measurements (FIA and HPLC), copper-modified CFME with a fiber length of 5 mm was partially introduced (~4 mm) into the outlet of the PEEK tubing. Both the capillary and the CFME (by means of its glass body) were mounted to a (~30 × 30 mm) part of a printed circuit board and fixed firmly to avoid any accidental positional changes. A three-electrode arrangement was used: the auxiliary large surface electrode was made using the copper foil of the printed circuit board, and an Ag/AgCl reference mini electrode (L-Chem, Czech Republic) was placed in close proximity to the end of the capillary (Fig. S2). The interconnection of the electrodes was mediated by the perfusion solution (0.01 or 0.025 M NaOH). The conversion of the copper layer into copper oxide layer was performed in-situ prior to FIA/HPLC experiments using the anodisation at 700 mV for five minutes.

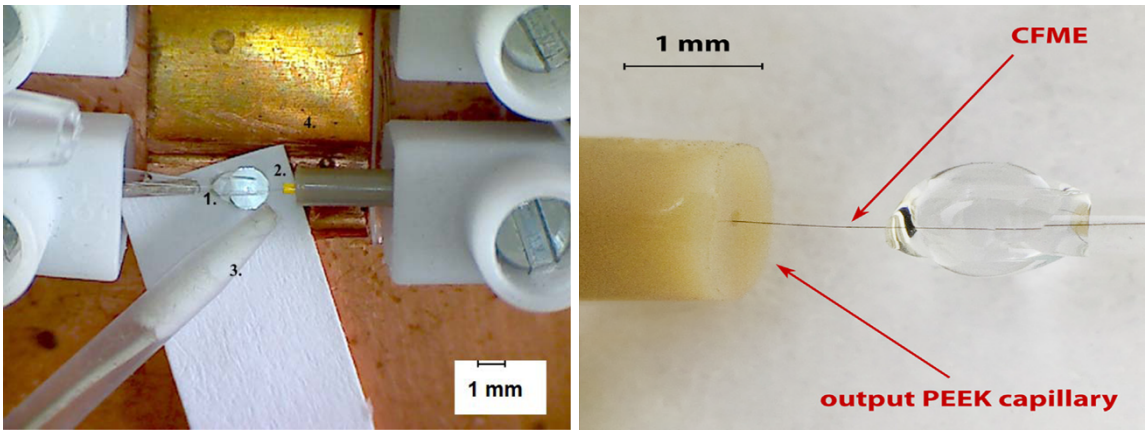

Fig. S2: Photograph of electrode system layout (a piece of white paper was inserted below the carbon fiber electrode to make the fiber-capillary connection visible); (1) CFME, (2) output FIA/HPLC PEEK capillary of diameter 75 µm, (3) reference electrode, (4) copper foil auxiliary electrode.

Scanning electron microscopy and EDX spectroscopy

Scanning electron microscopy (SEM) images were obtained with a Vega 3 (Tescan, Brno, Czech Republic, SE image resolution 2 nm at 30 kV). The images were collected with a high voltage of 10 kV at a working distance ranging from 5 mm to 25 mm. The sample material (i.e. carbon fiber) was immobilized onto conductive carbon discs after cutting the fiber from the CFME. EDX spectra were collected using the Quantax EasyEDS module (Bruker) integrated into the SEM instrument. The analysis of EDX spectra was performed using EasySEM software with the One-Touch EDX tool.

Amperometry/FIA/HPLC instrumentation and analytical conditions, response stability testing

Amperometric measurements were performed using Nanoampere electrochemical workstation (L-Chem, Czech Republic) in stirred solutions.

The HPLC system consisted of an ESA isocratic pump (Model 582), (ESA Inc., Chelmsford, MA, USA) with a pulse damper, a Rheodyne manual injector (Rheodyne, Cotati, CA, USA) equipped with a 10 μL loop. For FIA experiments, the ESA pump was replaced with a Pye-Unicam Philips isocratic pump (Model PU4015).

Electrochemical detection was performed using an Coulochem III ESA coulometric detector (ESA Inc., Chelmsford, MA, USA), equipped with a CFME cell. A Clarity chromatographic station (DataApex, Prague, Czech Republic) was used for chromatogram recording. The samples were introduced into the system using a glass 25 μL syringe (Hamilton, Reno, NV, USA). HPLC separations were performed using a Hypercarb porous graphitic column, 100 × 2.1 mm i.d. (Thermo Fisher Scientific, Waltham, MA, USA). All fittings, injection loop, connecting ferules and tubings were PEEK™. The final mobile phase consisted of 25 mM sodium hydroxide/acetonitrile (97/3, *v*/*v*). The mobile phase was vacuum-filtered through a 0.2 μm porous filter (Supelco, Bellefonte, PA, USA) and degassed by helium sparkling before use. The flow rate was 0.25 ml min^{-1}.The temperature of the HPLC column including connecting tubing and solvent reservoir was maintained at 45 °C using a Techlab K2 thermostat (Techlab GmbH, Braunschweig, Germany). The analytical potential for chromatographic detection was maintained at +500 mV or +700 mV for FIA measurements (both vs. Ag/AgCl), respectively.

The set-up detector gain ranged from 10 nA V^{-1} to 100 nA V^{-1}. Calibration curves expressed as peak area vs. concentration were plotted and basic parameters of the method were calculated (Table 1, see main text). LODs and LOQs for the studied compounds measured under final experimental conditions were obtained from the equations LOD=3.3σ/*b* and LOQ=10 σ/*b*, where σ was the standard deviation of the mean value for 6 signals using the blank and *b* represented the slope of the corresponding calibration curve.

The response stability of the nanostructured copper coated CFME was tested for lactose in FIA mode (using the conditions used for HPLC experiment, i.e. 0.025 M NaOH mobile phase and a potential of 500 mV) in a following manner: after setting-up the experiment the electrode was subjected to the working potential, the background current was allowed to decay for 5 minutes and then five consecutive injections of lactose solution were carried out. The electrode was kept at working potential and the procedure was repeated after 0.5, 1, 2 and 3 h. Then, the electrode was disconnected from the potentiostat and left overnight immersed in the mobile phase. Next day, the working potential was set-up again and the test was continued at 12, 13 and 14th hour. The R.S.D. values were 2.34, 1.75, 6.29, 4.27, 1.61, 7.25, 5.63, 6.35 and 4.16 % for individual runs at 0, 0.5, 1, 2, 3, 12, 13, and 14th hour, respectively (Fig. S3).

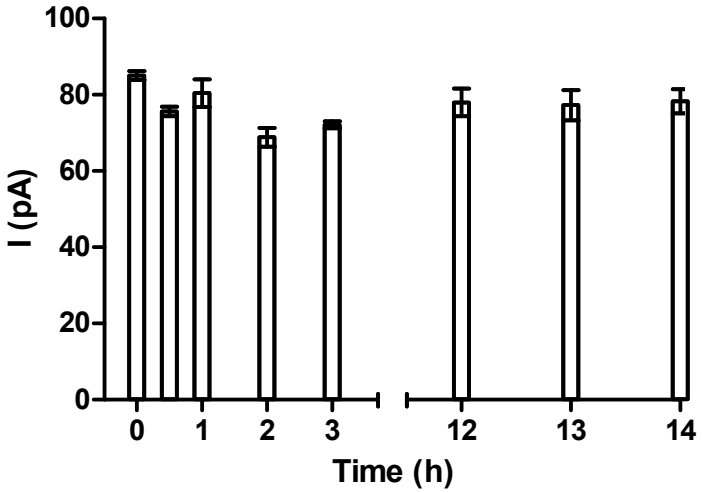

Fig. S3: Response stability for repeated injections of 1.5×10^{-6} mol L^{-1} lactose, 10 µL each, 0.025 M NaOH mobile phase, flow rate = 0.5 mL min^{-1}, E = 500 mV.