SUPPORTING INFORMATION

A PAPER-BASED ANALYTICAL DEVICE FOR ELECTROCHEMICAL FLOW INJECTION ANALYSIS OF GLUCOSE IN URINE

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Table of Contents

Experimental Design S-2

Materials S-3

Amplifier S-5

Data logging S-5

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EXPERIMENTAL DESIGN

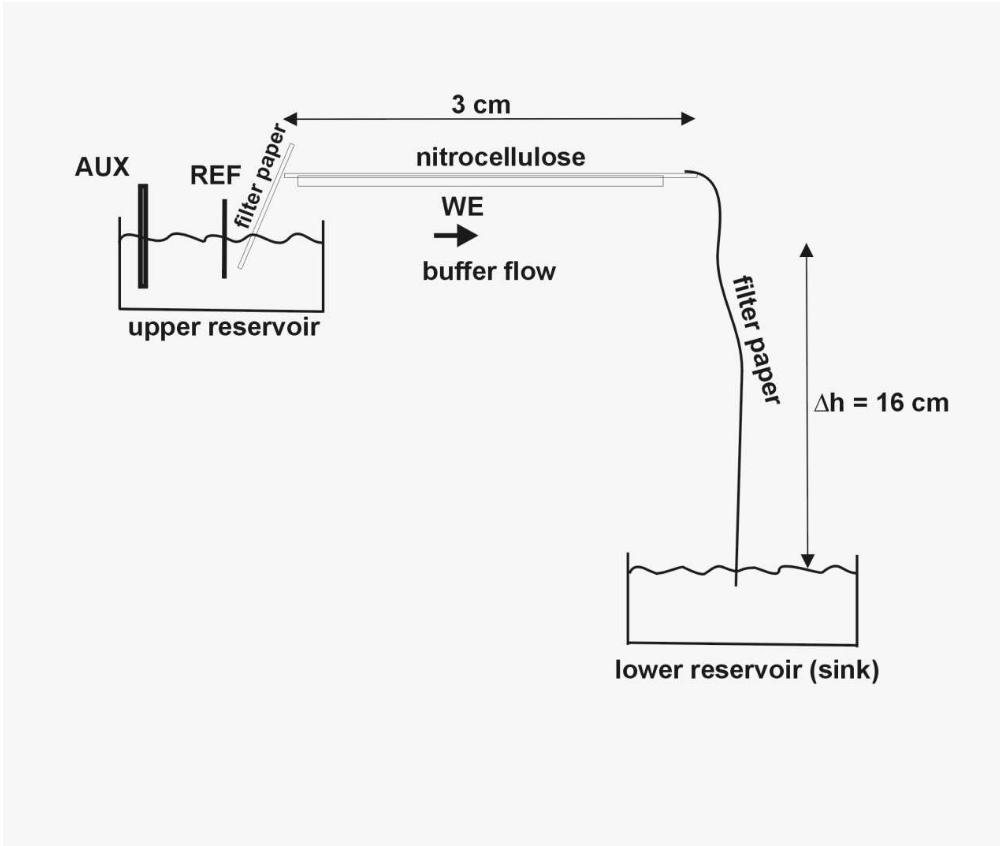

Figure S-1. Sketch of a side view of the experimental set up (larger version of Figure 1**A**). The working electrode (WE) of platinum deposited on glass (or glassy carbon) was covered with nitrocellulose (8mm x 30mm; thickness 140 μm). After wetting the porous membrane (nitrocellulose or filter paper) automatically flattened and adhered to the surface and made conformal contact with it. In the upper reservoir the auxiliary (AUX) and the reference (REF) electrodes were positioned. The working electrode was operated at +0.7 V vs a Ag/AgCl reference (REF) electrode. Both the reference (a fine silver wire) and the counter electrode (auxiliary, AUX; made of stainless steel) were located in the upper reservoir where they were in contact with the buffer, ca. 1 cm below the WE. The nitrocellulose on the WE is connected with both buffer reservoirs by 8-mm wide strips of polyester–cellulose blend paper (VWR®Spec-Wipe). The lower reservoir (sink) was tap water with acetic acid (final conc. 0.1-1%) added to inhibit microbial growth.

Materials. The incubation buffer (pH 7.4) contains 0.14 M sodium chloride and 100 mM phosphate in water. Glucose oxidase (G2133-10 kU) was purchased from Sigma-Aldrich, and a stock of 1 mg (168 units)/ml was prepared in incubation buffer. According to the information leaflet one unit will oxidize 1 µmol beta-D-glucose per min at pH5.1 and 35C. A volume of 0.9 µl (approx. 0.15 unit) was typically spotted onto the nitrocellulose. Based on Faraday's law, the number of moles (n) in the sample is given by eq. (1)

$$n = Q \bullet (F \bullet z)^{-1} \qquad (1)$$

where Q is the total charge transferred at the electrode in coulombs (obtained by integrating the current through the working electrode over time), F = 96,485 C mol^{-1} (the Faraday constant), and z is the number of electrons transferred per molecule. At 100% conversion and assuming that all glucose oxidase remained on the nitrocellulose, a maximum turnover of 0.15 µmol/min (see above) and taking a stoichiometry of two electrons per molecule[1,2] (z = 2), this would lead to a charge of 0.03 C/min = 0.00051 C/sec =0.51 mA. Our highest H_2O_2 oxidation currents were an order of magnitude below this value, indicating that we never used the maximum turnover (Vmax) of the enzyme. We do not know the actual glucose concentration on the nitrocellulose, but after dilution the enzyme will not be active at its Vmax for most samples, as the reported Km is 33-130 mM. Unisart CN 140 nitrocellulose was kindly provided as a test sample by Sartorius Stedim, Nieuwegein, the Netherlands. According to the specifications the nitrocellulose can bind 30 µg IG/cm^2 . The glucose oxidase was spotted on approximately 4 mm^2, corresponding to binding of approximately 1 µg protein. Therefore, the assumption of 100% binding of less than 1 µg glucose oxidase (see above) seems possible. Samples were introduced onto the nitrocellulose using a low-cost 0.3 mL disposable insulin syringe (Terumo MYJECTOR 29G x ½'') and Intramedic PE10 polyethylene tubing (Becton Dickinson; I.D. 0.28 mm). This syringe was used multiple times and was cleaned by tap water between sequential sample introductions by gently pressing and relieving of the piston. The glassy carbon plate (Carbone Lorraine), 2 mm thick, used for supporting the platinum of the working electrode, was purchased from Mersen Benelux B.V. (Schiedam, the Netherlands). It was cut using a diamond saw. The platinum was sputtered by using an Argon plasma. Other (even cheaper) materials, such as glass, could be used for support. We advise to roughen the glass surface for better attachment of the platinum. We had good experience with the roughed part (destined for writing) of Menzel-Gläser microscope slides, using the glass with the grooves in the direction of the flow.

The liquid flow could also be generated by evaporation at the sink-end. We prefer the closed system shown here using siphoning to generate a flow, because the flow rate is more constant and independent of the humidity of the environment. The design we developed may, we believe, have useful additional specific characteristics. For example, during electrochemical analysis of sample, gas bubbles in the fluid (either from electrolysis, or carried in adventitiously) can easily escape from the thin porous paper film. Moreover, the constant flow of buffer along the paper channels reduces the variation of the baseline that would have been observed using active pumping.

Amplifier. The basis is essentially the same as that published previously[3]. Now, a modern mass-produced low-cost operational amplifier (National Semiconductor LMC6464) containing four amplifiers (designated A-D) has been used (Figure S-2). It was selected for low current consumption. Four AA batteries in series, which could be used continuously for several months, supplied the power. From the voltage difference and the resistor across the B-amplifier, the current can be calculated using Ohm's law. A low-current positive voltage regulator (Torex, XC6201P302PR), and a low-current charge pump voltage inverter (Texas Instruments TPS60400DBVT) creating stable +3V and -3V voltages were used according to the manufacturers' instructions.

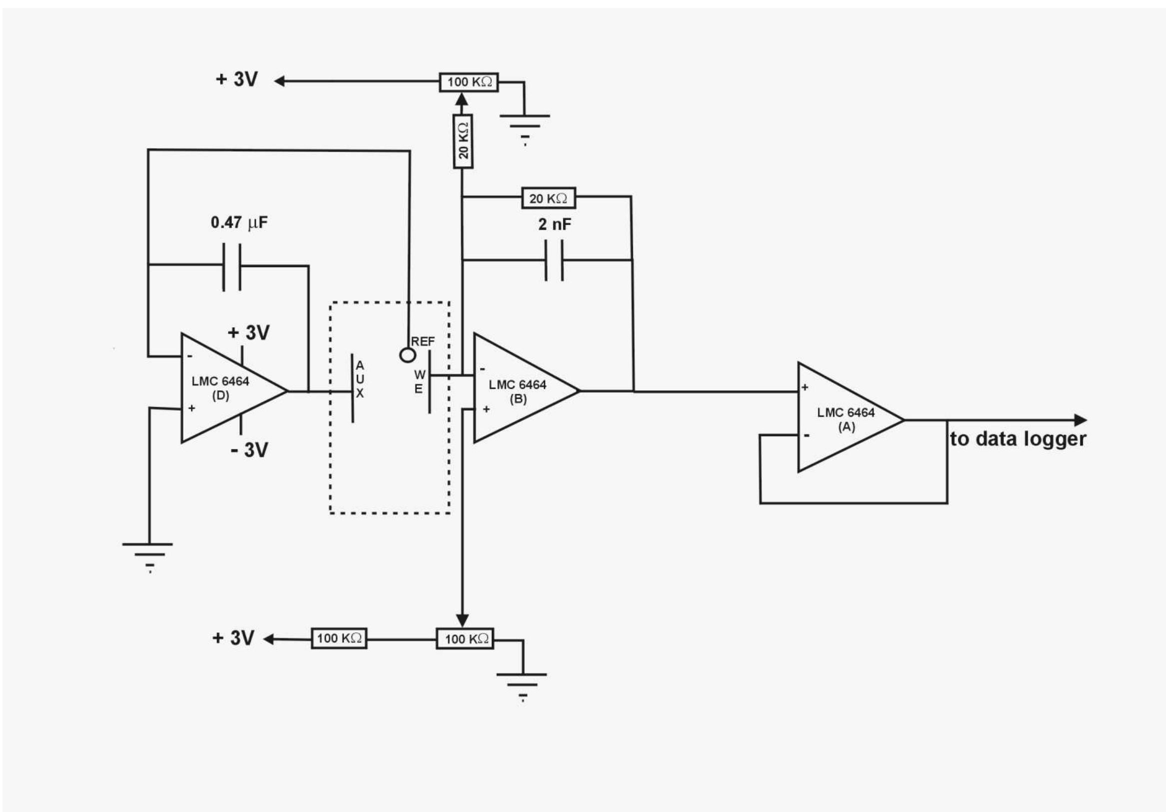

Figure S-2. Basic schematics of the electronics.

Data logging. In the laboratory we collected data using Chromeleon software for chromatography (Dionex). Outside the laboratory we used a Voltcraft VC820 multimeter (Conrad), which was read out manually for peak height measurements or connected to free data analysis software for computerized data logging (Datalyse; author Carl Hemmingsen), installed on a 16 Gb solid state netbook running under Windows XP.

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