Analyst

COMMUNICATION

Electronic Supplementary Information (ESI)

Biocatalysis on Plastic Stick Tips: Atypical Electrochemical Enzyme Biosensing on Adjacent Bare Working Electrode Disks

Hannadige Sasimali Madusanka Soysa, Kawin Loha, Wipa Suginta and Albert Schulte*

Corresponding author, email: albert.s@vistec.ac.th

Experimental Section

1.Reagents, materials and solutions

GOx (source: *Aspergillus Niger*, #G1741, 228,253 U/g) was from Sigma Aldrich (St. Louis, MI, USA) and glutaraldehyde (Grade I, 25 % in H_2O) from S.M. Chemical Supplies Co., Ltd. (Bangkok, Thailand). Powdered gelatin from porcine skin (gel strength ≈ 300 g Bloom, Type A) was from Sigma-Aldrich (St. Louis, MI, USA). Silver wire with 1 mm diameter and 99.9% purity was from CHEMPUR Feinchemikalien und Forschungsbedarf GmbH (Karlsruhe, Germany). Anhydrous β-D (+)glucose was from Italmar (Thailand) Co., Ltd. (Bangkok, Thailand). All other chemicals were Sigma-Aldrich analytical grade products. De-ionized water was solvent for aqueous solution. The solution for storage of GOx/PVC sticks was 0.1 M sodium phosphate buffer, pH 7.0, and electrolyte for all biosensor tests was 10 µM KCl.

2.Instrumentation for conductivity readout

Conductometric readout was carried out with a reference 600+ potentiostat from *Gamry Instruments* **(**United States of America) controlled by the latest version of the Gamry software and connected to a 2 cm apart pair of Ag/AgCl wires in the conductance cell. To form the conductance cell, one of the Ag/AgCl wires was connected to the working electrode, and the other was connected to the shorted counter and reference electrode termini of the potentiostat. Measurements were made at +200 mV applied potential at room temperature (25°C) in an unstirred 3 mL volume of 10 µM KCl. The deposition of AgCl on the lower half of the two trimmed silver wires was achieved through anodic polarization at 3.5 V in a cell containing 1M HCl/3M KCl, using a platinum coil as the cathode. Simultaneously, two silver wires were immersed in bleach for 20 minutes, yielding performance comparable to electrodeposition. Therefore, for each experimental set, freshly prepared Ag/AgCl wires were utilized by soaking them in bleach for 20 minutes to ensure no potential changes or performance changes on the AgCl wires over time.

3. Instrumentation for IPA readout

IPA readout was carried out with a reference 600+ potentiostat from *Gamry Instruments* **(**United States of America), controlled by the latest version of the Gamry software and connected to a two-electrode system. In the IPA assay 3mm diameter glassy carbon disk (Italsens) electrode was connected to the working electrode and the Pt spiral electrode to the shorted counter and reference electrode termini of the potentiostat. Pulse recording was routinely conducted using a series of high potential pulses, each lasting 0.5 seconds and set at 0.6 V, with a spacing of 99.5 seconds at 0 V between them. This was achieved by using a Gamry-Repeating chronoamperometry setting. IPA assay was performed in 150 rpm stirring, 5 mL beaker type electrochemical cell with 0.1 M sodium phosphate buffer pH 7.0 at room temperature (25°C). In the IPA readout, GOx modified stick is located beneath the electrode, which is positioned using the manipulator. For data analysis, we focused on the last pulse as the indicator for H_2O_2 concentration.

4.GOx immobilized stick fabrication

For the immobilization of GOx, a PVC plastic stick was prepared, as shown in Supplementary Figure S1. A 10 µL gelatin solution (2 mg mL^{-1} in H_2O) was placed on the flat end of the PVC stick and solvent evaporation allowed at room temperature, forming an evenly spread gelatin film on the PVC surface. Next, 136.95 units of GOx (10 µl of 60 mg mL^{-1} of GOx solution) were dropped onto the gelatin-coated PVC stick and air-dried at room temperature. Finally, gelatin/GOx-covered sticks were gently pushed in inverted position through a suitably-sized hole in the parafilm that covered a 10 ml beaker with 1 ml 10 % glutaraldehyde (GA) solution. A 6-hour GA vapor treatment of GOx/ PVC tips triggered chemical GOx/gelatin crosslinking and firm GOx fixation to the plastic carrier. Completed GOx/PVC sticks were stored overnight in phosphate buffer, pH 7.0 at 4°C, prior to use for measurements.

5. Glucose detection in commercial sample.

Reference glucose in the Pepsi and OSRA R.O. was assessed with a commercial glucometer, ACCU-CHEK Active, +Roche Diagnostics (Thailand) Limited (Bangkok, Thailand).

Supplementary Figures

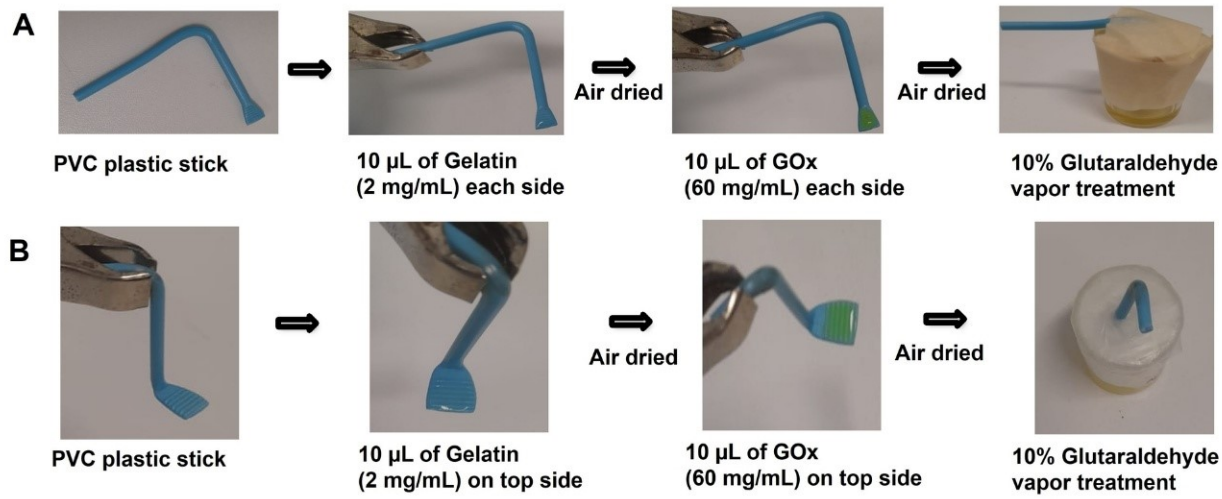

Figure S1: PVC stick preparation. 10 µL gelatin solution (2 mg mL^{-1} in H_2O) was placed on the flattened end of a PVC stick and solvent evaporation was allowed at room temperature, forming an evenly spread gelatin film on the PVC platform surface. Next, 137 units of GOx (10 µl of 60 mg mL^{-1} of GOx solution) were dropped onto the gelatin-coated PVC stick and air-dried at room temperature. Finally, the gelatin/GOx-covered stick was gently pushed in inverted position through a suitably-sized hole in the parafilm covering of a 10 mL beaker containing 1 mL of 10 % glutaraldehyde (GA) solution and left for 6 hours at 4 °C to allow GA vapor to induce GOx to gelatin crosslinking . PVC sticks with immobilized Gox were stored overnight in phosphate buffer, pH 7.0 at 4°C before first test measurements were performed. Differently shaped PVC sticks were used for two conductometric and amperometric biosensing assays, as shown in Figures 1 and 2 of the original publication.

COMMUNICATION

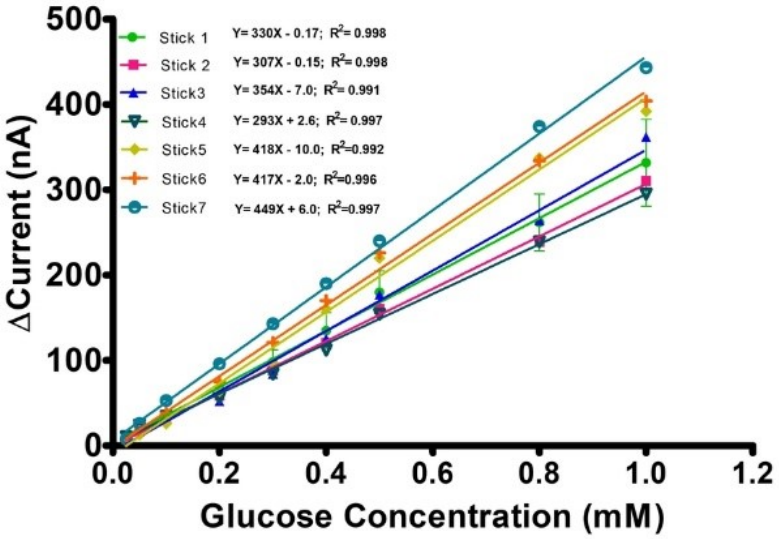

Figure S2: Reliability of the GOx/PVC stick tool/Ag/AgCl conductance cell assembly. Conductometric glucose biosensing was performed with a set of seven GOx-modified PVC sticks that were operated in a dual Ag/AgCl electrode conductance cell as shown in Figure 1 of the original publication. The current vs time traces were constructed with data from calibration trials with the seven GOx sticks, each produced by the same procedure but on seven different days. Conductometric data acquisition used the parameter set that was listed in Figure 1 of the original publication and error bars for data points in the plots represent the standard deviations for triplicate repetitions of the measurements.

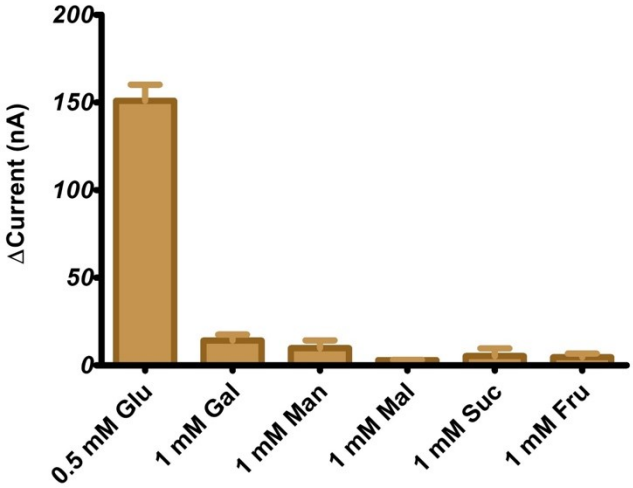

Figure S3: Selectivity of conductometric glucose biosensing with a GOx/PVC stick as biosensor tool. 0.5 mM glucose and 1 mM galactose, mannose, maltose, sucrose and fructose were tested in a conductivity cell to assess the selectivity of the sensor for glucose. Conductometric data acquisition used the parameter set that was listed in Figure 1 of the original publication and error bars for data points in the plots represent the standard deviations for triplicate repetitions of the measurements.

COMMUNICATION

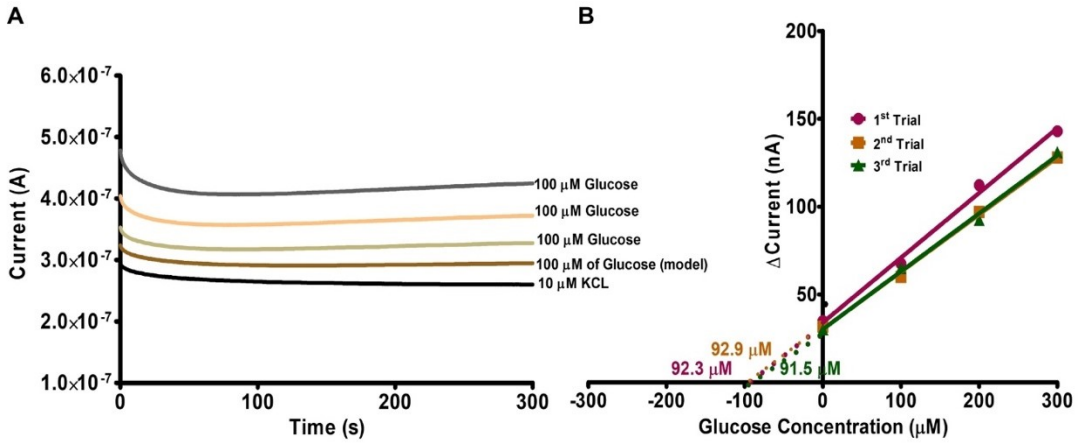

Figure S4: Conductometric glucose biosensing with a GOx/PVC stick operated in a dual Ag/AgCl electrode conductance cell: A model sample analysis. (A) Original current traces obtained from conductivity measurement trials. The black line shows the control current for 10 µM KCl in the presence of a GOx-modified PVC stick. The brown trace shows the current trace related to the conductivity changes after adding 6 µl of a 50 mM stock (100 µM final glucose concentration in the conductance cell; dilution was thus factor 500x). Other traces were obtained with 1, 2 and 3 further raises of 100 µM glucose ('the standard additions'), respectively. (B) The standard addition plots and the extractions of sample glucose concentrations for triplicate repetitions of the trial in (A) through regression line progression towards the x-axes; values of 92.3, 92.9 and 91.5 µM, instead of the theoretical value of 100 µM, were obtained. Conductometric data acquisition used the parameter set listed in Figure 1 of the original publication.

COMMUNICATION

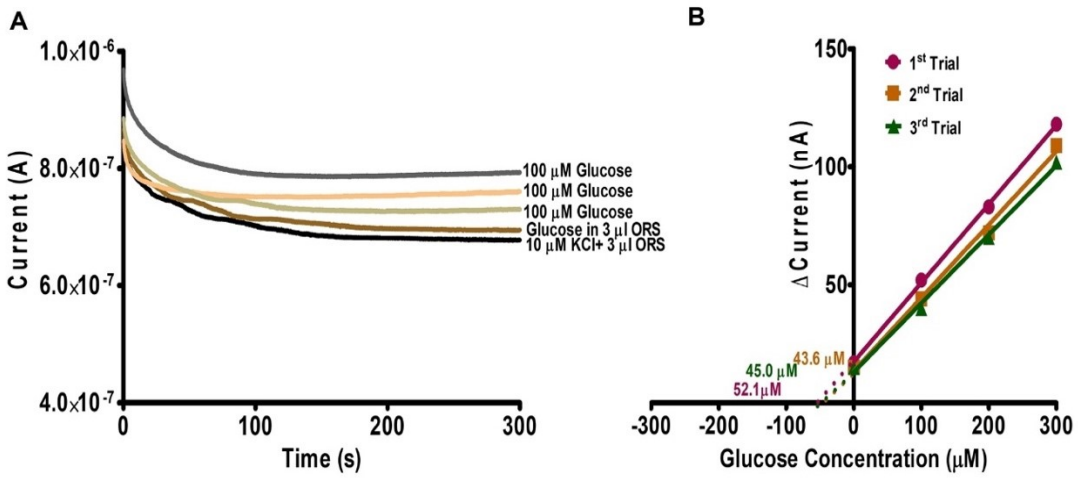

Figure S5: Conductometric glucose biosensing with a GOx/PVC stick operated in a dual Ag/AgCl electrode conductance cell: An oral rehydration solution (ORS) analysis. The inspected ORS sample contained 14.7 mg of the formulation in 1 ml DI water, creating a 50 mM 'ORS glucose' stock solution. (A) Original current traces obtained from conductivity measurements for an oral rehydration solution (ORS) after 1000x dilution (50 µM final concentration in the conductance cell). The black line shows the control current for the 10 µM KCl + ORS sample in the presence of an unmodified PVC stick. The brown trace indicates the final measurement of conductivity changes after replacing the unmodified by a GOx-modified PVC stick, and repeating data acquisition. The other three traces show the current following further standard additions of 100 µM, 200 µM or 300 µM glucose, respectively. B) Three separate standard addition plots using the same stick as was used for Figure S4A. Conductometric data acquisition used the parameter set that was listed in Figure 1 of the original publication.

COMMUNICATION

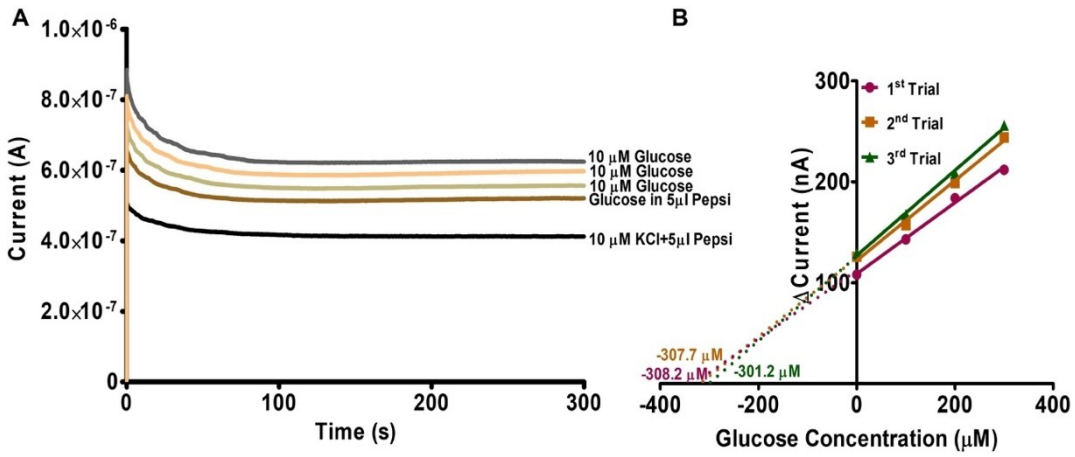

Figure S6: Conductometric glucose biosensing with a GOx/PVC stick operated in a dual Ag/AgCl electrode conductance cell: A Pepsi analysis. (A) Original current traces obtained from conductivity measurements. The black line shows the control current for 5 µl Pepsi + 3mL of 10 µM KCl with an unmodified PVC stick. The brown trace is the current trace after adding a GOx-modified stick to the Pepsi in 10 µM KCl (Pepsi dilution was here 600x). The other three traces indicate 3 additions of 100 µM, 200 µM and 300 µM glucose respectively. B) 3 standard addition plots using the same stick as was used in Figure S4A. Conductometric data acquisition used the parameter set that was listed in Figure 1 of the original publication.

COMMUNICATION

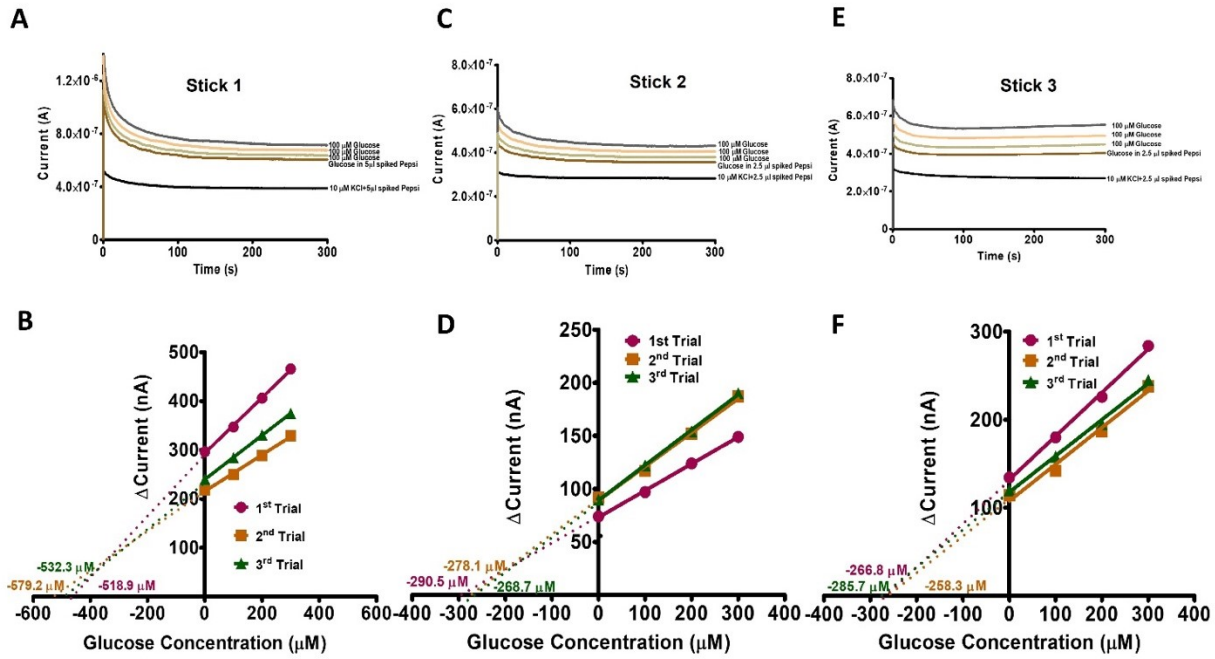

Figure S7: Conductometric glucose biosensing with a GOx/PVC stick operated in a dual Ag/AgCl electrode conductance cell: Analysis of a Pepsi sample with deliberate glucose supplementation. Measurements involved three different GOx/PVC sticks and different dilution factors, which were 600x dilution for (A) and 1200x for (C, E)**.** Shown in A, C and E are representative examples of original current traces for the conductivity measurements on spiked Pepsi samples. The black lines in the plots are traces of the control current for Pepsi plus glucose supplementation in 10 µM KCl, measured in the presence of unmodified PVC sticks with no GOx. The brown traces are the current traces related to conductivity changes after adding a GOx-modified stick to the 10 µM KCl electrolyte with Pepsi and added glucose, in between the two Ag/AgCl electrodes. The other three current traces in A, C and E originate from measurements after the three standard additions of 100 µM, 200 µM and 300 µM glucose, respectively. Shown in B, D and F are the standard addition plots for data of the three measurements with the 3 different sticks.

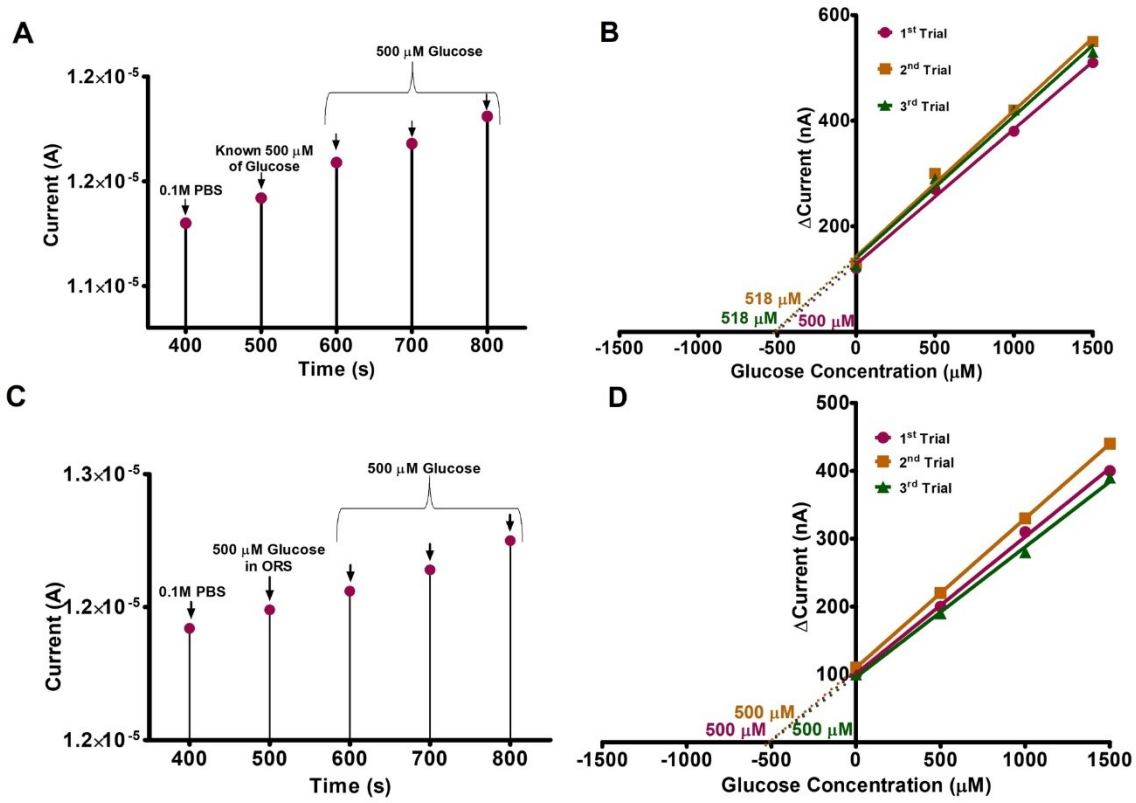

Figure S8: Model and real sample glucose testing with the amperometric GOx/PVC stick glucose assay with IPA readout. (A) One of three IPA recordings of a triplicate repetition of a recovery trial for 500 µM glucose in model sample. During IPA recording, the electrode was held for 99.5 s at 0 V resting potential and for 0.5 s at 0.6 V H_2O_2 detection potential. Red dots are the currents at the end of the 500 ms steps to peroxide detection potential. (B) The three standard addition plots for the triplicate quantification of 500 µM glucose in a model sample. (C) One of three IPA recordings of triplicate repetition of a recovery trial for 500 µM glucose in the electrolyte of the electrochemical cell. The stock solution for the trials was prepared with 146.7 mg of the ORS powder in 1 ml DI water, creating a 500 mM 'ORS glucose' concentration. Worked with in the trials was a 1000x dilution. During IPA recording, the electrode was held for 99.5 s at 0 V resting potential and for 0.5 s at 0.6 V H_2O_2 detection potential. Red dots are the currents at the end of the 500 ms steps to peroxide detection potential. (D) The three standard addition plots for the triplicate quantification of the glucose in the commercial rehydration salt sample.