Detection of Glucose with a Self-Made Biosensor Based on Glucose Oxidase

PSAPP-001 Electrochemical Experiment

PalmSens
Compact Electrochemical Interfaces

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1 Instructions

The following instructions will guide you to perform the experiment. The theory on which these experiments are based, can easily be checked online at palmsens.com, then search for “Introduction of Glucose with a Self-Made Biosensor”. The material needed for the experiments can be ordered via https://www.palmsens.com/product/educational-kit/#contents

2 Devices and Equipment

- EmStat / EmStat Blue / PalmSens3
- sensor cable
- sensor connector
- maybe a USB cable
- computing unit (PC, Laptop, notebook, tablet PC (Android), smartphone (Android))
- potentiostat software (PStrace, PStouch)
- calculation and plotting software (Excel, Origin, MatLab, Mathematica)
- ItalSens IS-C
- retort stand
- retort clamp
- beaker (electrochemical cell)
- stirrer

3 Chemicals

- Resydrol AY 498w/35WA
- Glucose oxidase (powder)
- 0.1 M phosphate buffer pH 7 (at least 50 mL)
- 1 M glucose solution (has to be prepared one day in advance)

Note: Resydrol is an additive for paints that is the precipitating part during electrodeposition. Often it is not available in common chemical stores. Resydrol AY 498w/35WA can be obtained from Allnex. Ton van Oers from the Dutch distributor Caldic can give you the contact information for your local distributor. For most lab courses a sample of Resydrol will be more than sufficient.

4 Instructions

4.1 Preparing the Glucose Sensor

By immobilizing glucose oxidase (GOx) on an electrode surface a simple glucose biosensor can be created. The commercially available blood sugar test is optimized and has a more robust design, but the basic principle is the same. In this part of the experiment the GOx will be immobilized on the electrode by electrodeposition of a polymer in presence of GOx.

1. Take an ItalSens IS-C electrode and insert it into the sensor connector. You may need to cut excess plastic away in order to fit the electrode into the connector. Connect the sensor holder to the potentiostat and fix it in the retort stand. The electrode should be parallel to the table.
2. Prepare the polymer enzyme solution by mixing 600 µL Resydrol with 1.4 mL phosphate buffer. Be aware that Resydrol is very viscous and the volume transfer should be done very slowly. Dissolve 16 mg of GOx powder in the polymer solution.
3. A droplet of ca. 100 µL is applied to the electrode. Make sure all three electrodes (WE – black circle, CE and RE – the two little arms left and right of the circle) are covered with solution.

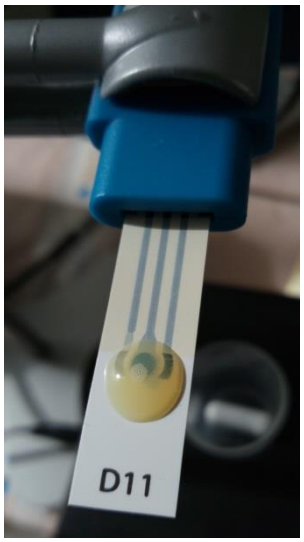

Figure 1: photo of an ItalSens IS-C with an enzyme-polymer solution drop on it

1. A cyclic voltammetry is performed to create a low pH close to the electrode by water splitting. During the more cathodic potentials the lack of polymer in front of the electrode will be removed due to diffusion. If a method for performing a CV was already prepared for you, load the method. If not, choose Cyclic Voltammetry from the drop down menu. Choose the current ranges 1 µA to 100 mA. The fields *Sample* and *Sensor* are for your own notes. Since we do not want a pre-treatment of the electrode set *t condition* and *t deposition* to 0. Set the other parameters to:
    a. *t equilibrium* = 8 s
    b. *E start* = 0 mV
    c. *E vertex1* = 0 mV
    d. *E vertex2* = 1.9 V
    e. *E step* = 0.005 V
    f. *Scan rate* = 0.1 V/s
    g. Number of Cycles = 20
2. Start the measurement. And observe the working electrode. Above 1.4 V a gas evolution should be visible.
3. Rinse the electrode with buffer. We recommend using the electrode as soon as possible. Although the electrode can be stored in a cool and dry environment overnight, the activity of the electrode will decrease.

4.2 Determination of the Michaelis-Menten Constant

After preparing the electrode the K_M and I_{max} (v_{max}) can be determined easily. A chronoamperometry is performed with increasing glucose concentration.

1. Fill the cell with 20 mL buffer, put a magnetic stirring bar in the cell and put the cell on the magnetic stirrer. Insert the prepared GOx modified electrode in the sensor connector. Connect the connector to the potentiostat and immerse the electrode in the buffer solution. Switch on the stirrer and adjust it to a velocity that does not create an air tunnel.
2. A chronoamperometry is performed to detect the hydrogen peroxide that is produced by the enzyme during the glucose oxidation. If a method for performing a chronoamperometry was already prepared for you, load the method. If not choose *Amperometric Detection / Chronoamperometry* from the drop down menu. Choose the current ranges 1 nA to 10 µA. The fields *Sample* and *Sensor* are for your own notes. Since we do not want a pre-treatment of the electrode set *t condition* and *t deposition* to 0. Set the other parameters to:
    a. *t equilibrium* = 8 s
    b. *E dc* = 800 mV
    c. *t interval* = 0.5 s
    d. *t run* = 7200 s

3. Start the measurement and wait for a constant background current. This can take quite a while (20 minutes or more).
4. After a constant current is reached 1 M glucose solution is added. This is done in multiple steps. First add a volume of 1 M glucose solution that will raise the concentration to 2 mM. Wait until a steady current is reached (ca. 90 seconds). Repeat this twice and neglect the volume change inside the cell. After these three steps perform 2 steps that will each increase the concentration by 5 mM. Two final steps follow and each of these two additions increases the glucose concentration by 10 mM. The final glucose concentration of the cell should be 36 mM, assuming the volume change had no impact. When a stable current is reached after the last addition, you can press *Abort* (the orange square) and save the curve (*Curve* menu – *Save …*).
5. Remove the sensor from the solution and rinse it with buffer or water.
6. Calculate the true concentrations of glucose for each step by taking the volume change due to glucose solution addition into consideration. Read the steady current after each addition from the curve. Plot the current versus the Glucose concentration. Does the curve show the expected behavior?
7. Plot 1/I versus 1/c(Glucose) to make a Lineweaver-Burke plot. Determine I_{max} and K_M. Do the values match the estimations you would have made looking at the plot from point 6. What might cause differences between the K_M of your sensor and the values found in literature for free diffusing GOx?

4.3 Determination of an Unknown Glucose Content

There are two basic strategies to perform a quantitative analysis with a linear relationship between analyte concentration and signal, in this case current. One is the use of a calibration curve and the other one is the use of the standard addition. A calibration curve can only be used if the sensor will not change its properties for a long period and the sample matrixes will not influence the sensitivity of the sensor. For this glucose sensor both conditions are not given. Therefore the standard addition will be used.

1. Fill the cell with 20 mL buffer, put a magnetic stirring bar in the cell and put the cell on the magnetic stirrer. Insert the prepared GOx modified electrode in the sensor connector. Connect the connector to the potentiostat and immerse the electrode in the buffer solution. Switch on the stirrer and adjust it to a velocity that does not create a tunnel.
2. A chronoamperometry is performed to detect the hydrogen peroxide that is produced by the enzyme during the glucose oxidation. If a method for performing a chronoamperometry was already prepared for you, load the method. If not choose *Amperometric Detection / Chronoamperometry* from the drop down menu. Choose the current ranges 1 nA to 10 µA. The fields *Sample* and *Sensor* are for your own notes. Since we do not want a pre-treatment of the electrode set *t condition* and *t deposition* to 0. Set the other parameters to:
    a. *t equilibrium* = 8 s
    b. *E dc* = 800 mV
    c. *t interval* = 0.5 s
    d. *t run* = 7200 s
3. Start the measurement and wait for a constant background current. This can take quite a while (20 minutes or more).
4. While waiting for a stable background current, prepare your sample. A solid sample, for instance hard candy, sugar cubes, needs to be dissolved in a known volume of demineralized water or buffer. After the solid sample is dissolved check the volume of the solution and dilute if necessary. Very viscous samples need to be diluted to a viscosity that allows precise pipetting. A good way to do this is to dissolve a defined mass of the syrup in a demineralized water or buffer, just like a solid sample. Carbonated beverages will release gas if sucked into a pipette. This will lead to a wrong volume in the pipette. The carbon dioxide and the carbonate can be removed by boiling the beverages for 15 min, but you need to replace the evaporated water that was released during boiling.
5. If the sample has a form that can be used with a volumetric pipette and a steady base current is reached add 40 µL sample solution to the solution in the cell. When a steady current is reached again add 40 µL 1 M glucose solution. Repeat this step two more times. After the last steady current is reached stop the measurement and save the curve. You should take care that the glucose from the sample and the addition of glucose solution do not produce a glucose

concentration above the K_M of your sensor in the cell. If necessary adjust the sample dilution or volumes of sample and standard solution added to the cell.

6. Analogous to the calculation of K_M and I_{max} extract the current values for each of the additions. Plot the current versus the concentration due to the added glucose solution. Make a linear fit of the measured points. The absolute value of the intersection of the line and the x-axes is the concentration of the sample, so the null of the line shows the concentration that was in the solution before glucose solution was added.

Find values from literature, the producer's website or the packaging. Do these values match your measurement?

In this experiment you detected the amount of glucose using standard addition.

Please note that teachers can request the answers to the question in the instructions, using https://www.palmsens.com/contact/