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Application Note

1 Introduction

Glucose is an important analyte. In food industry the glucose levels of beverages determine the taste or are essential for the fermentation process to create alcoholic beverages. The amount of glucose in onions and potatoes determine the vegetables' suitability for different products.
In human body fluids glucose is present as energy source. The level of glucose is regulated by the pancreas via the production of insulin. In the body of a person diagnosed with diabetes this control mechanism does not work anymore properly. As a result the level of glucose needs to be monitored multiple times per day.

In this paper we are using the Sensit Smart and Z&P glucose sensing electrodes to demonstrate how easy it is to make the necessary measurements for a calibration curve with a mobile device.

1.1 Sensit Smart

The Sensit Smart is the world smallest ready-to-go potentiostat available on the market. Its design is based on the EmStat Pico, an OEM potentiostat module developed with the combined knowledge of Analog Devices and PalmSens. You can find more information about the EmStat Pico on our website.

The Sensit Smart can be directly inserted in a smartphone or tablet and controlled via the Android app PStouch. A USB-C to micro USB converter included in your Sensit Smart delivered together with your Sensit Smart. This means all Android devices can be connected to the EmStat Pico conveniently. You can use the USB-C Female to USB-A cable to connect the Sensit Smart to a classic USB port on your PC and control the Sensit Smart via our PC software PSTrace too.

The Sensit Smart has a connector for Screen Printed Electrodes (SPEs) build in. If you want to connect via cables to your electrodes, you can use the included screw terminal adapter to connect your cables.

The Sensit Smart supports common electrochemical techniques, including Cyclic Voltammetry, Square Wave Voltammetry and Impedance Spectroscopy (FRA/EIS). You can find a list of all supported techniques on our website.

In this application we will use the USB-C to micro USB converter and connect the Sensit Smart directly to a Galaxy Tab A by Samsung. As technique we will use Chronoamperometry.

Figure 1.1 The Sensit Smart with a Galaxy Tab A and the USB-C to micro USB converter

1.2 Zimmer & Peacock Glucose Electrodes

In this application note Glucose Sensors (A-AC-PAC-203-G) by Z&P have been used. These SPEs are printed on alumina ceramics. They have a platinum working electrode and the counter as well as the reference electrode are made from a mixture of silver and silver chloride. The electrode is modified with an enzyme, which consumes glucose, a stabilizer and a redox mediator. The redox mediator transports the electrons from the enzyme's active centre to the working electrode. The formulation used for this modification allows the electrodes to be stored for a long time, makes them suitable for continuous and discrete measurements. The precise composition of the formulation is not provided by Z&P. You can find out more about the Glucose Sensors (A-AC-PAC-203-G) by Z&P on their website.

Figure 1.2 Glucose Sensors (A-AC-PAC-203-G) by Z&P

Even though the exact composition of the Z&P Glucose Sensors is not public knowledge, some principles about electrochemical glucose sensors are.
Usually the enzyme glucose oxidase is immobilized on the electrode.
Sometimes the properties of the electrode and the enzyme allow it to just adsorb the enzyme and it will stick.
An enzyme can be trapped in a polymer matrix on the electrodes surface. There are multiple ways to apply such a polymer to the electrode. A very common way is drop casting. The polymer is mixed with the enzyme solution and a droplet is applied to the electrode. The solvent evaporates and the dried droplet is the enzyme trapped in the polymer matrix. Another option is to precipitate the polymer via electrodeposition.
Covalent bonding is another common way to immobilize an enzyme. A bond is formed between an organic layer on the electrode and a functional group of the enzyme.
These are just some examples of immobilization methods and in the literature more can be found.

The immobilized glucose oxidase needs to be still capable of oxidizing glucose. During this reaction the electrons are removed from the glucose oxidase and stored in the active centre of the enzyme. These electrons cannot be extracted from the enzyme directly. Oxygen serves as an electron acceptor in nature. It is reduced to hydrogen peroxide by the glucose oxidase. The hydrogen peroxide can be detected electrochemically.
Another option is to replace the oxygen with another suitable redox mediator. These are reversible electrochemical active species, which can commute between the electrode and the enzyme's active centre. Artificial redox mediators have the advantage that the detection does not depend on oxygen anymore.

Conducting polymers modified with redox mediators can combine the mediation and immobilization techniques.

2 Preparation

In this chapter we are going to talk about the steps you should perform before performing the measurement.

2.1 Installation of PStouch

Connect you Android device to the internet. A W-LAN connection is preferred over the mobile internet. Open the Google Play Store and search for “PStouch”. Please be aware that Photoshop touch is often abbreviated PS touch and might show up as first hit. Please, scroll down until you see PStouch by PalmSens.

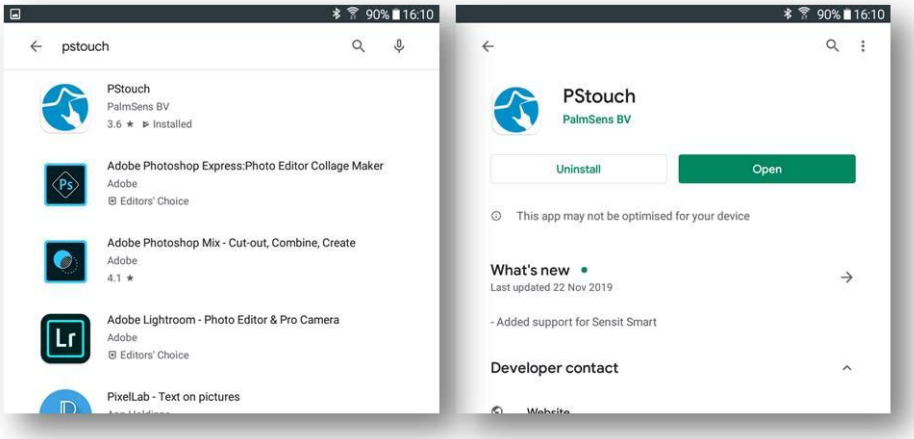

Figure 2.1 Google Play Store search results for pstouch (left) and PStpuch site in the Google Play store

Download and install PStouch.
Open PStouch. The first time you open PStouch you will have to wait for a few files to be copied. This is only happening when you run PSrouch for the first time and after updates. Then you will be asked a few questions to set the settings properly. Also a few tips will pop up. These are helpful advices we would like to give you, but feel free to close them when you feel informed.
PStouch can be used now.

2.2 Solutions

2.2.1 0.5 M Glucose solution

Dissolve 90 mg of D-glucose per 10 mL of demineralized water. The solution should be prepared the day before the experiment, so the equilibrium between α- and ß-glucose is established when the experiment starts.

2.2.2 Phosphate Buffer

Make a phosphate buffer containing 0.05 M K_2HPO_4 and 0.1 M KCl. Adjust the pH to 5.5 by adding 6 M HCl.

2.2.3 Calibration solutions

For the calibration curve 5 different concentrations are used. As 0 glucose concentration the pure buffer is used. The other concentrations are 5 mM, 10 mM, 15 mM and 20 mM glucose.
The 5 mM glucose solution is made by diluting 200 µL of 0.5 M glucose solution with the buffer solution to 20 mL.
The 10 mM, 15 mM and 20 mM are made analog to the 5 mM solution by diluting 400 µL, 600 µL and 800 µL with the buffer solution to a total volume of 20 mL.

As an alternative you can also purchase glucose calibration solutions from Zimmer and Peacock.

3 Measurement

First insert the glucose sensor into the Sensit Smart. To avoid any contamination of the glucose sensor wear gloves and don't touch the sensor stripe with bare hands. Take the electrode out of the tube and hold it on the edges. If you cannot apply enough force or do not have enough grip that way, you can carefully place you finger between the silver contact pads and the electrode at the other end. Avoid touching the electrodes even with gloves
Insert the sensor with the silver pads first into the Sensit Smart. Above the sensor slit are three grooves. These indicate the location of the contacts inside the Sensit Smart. The silver pads and the grooves need to be aligned, so all the electrode contacts are connected to the Sensit Smart's contacts.

Figure 3.1 Front of the Sensit Smart with highlighted grooves

Connect the Sensit Smart to you Android device. If your Android device has a micro USB port, you use the USB-C to micro USB convertor delivered together with the Sensit Smart We used a Galaxy Tab A, which has a micro USB port. After connecting the Sensit Smart the blue Led should indicate that the Sensit Smart is powered If the blue LED does not illuminate, it is possible that your Android device does not act as a USB host and cannot power peripheral devices.

Start PStouch. It will ask for permission to connect to Sensit Smart. Press OK. The white, triangular *Run* button should appear and at the bottom left of the PStouch window you should see that the device is connected.
Go to the *Method* tab. Choose from the list of techniques *Chronoamperometry*. Slide the blue circles to set the current ranges. We can use all the current ranges. This means one circle is at 100 nA and the other on 5 mA.
Open the *Chronoamperometry settings* and set the following values:

t equilibration 5 s
E dc 0.65 V
t interval 1 s
t run 1200 s
None of the following check boxes should be checked.

Lay the Android device with the attached Sensit Smart on a flat surface and apply 100 µL buffer solution to the glucose sensor. Make sure all 3 electrodes are covered by the droplet.
Start the measurement by pressing the white triangle shaped button.

Figure 3.2 Sensit Smart and glucose sensor during the measurement

Wait until the current is (almost) constant. This can take 5 minutes.
To change the solution just take a paper tissue and suck up the solution on the electrode and apply 100 µL of the 5 mM glucose solution.
Wait until the current is (almost) constant and change as previously described the solution to 10 mM glucose.
Repeat these steps until you have measured all solutions. After a stable current with the 20 mM glucose solution is reached stop the measurement by pressing the white square shaped stop button.
Save the data by pressing on the menu button in the top right corner and choose *Save data*. Choose a file name and directory. You can create a new folder, if necessary. Tap *Save* to save the data.

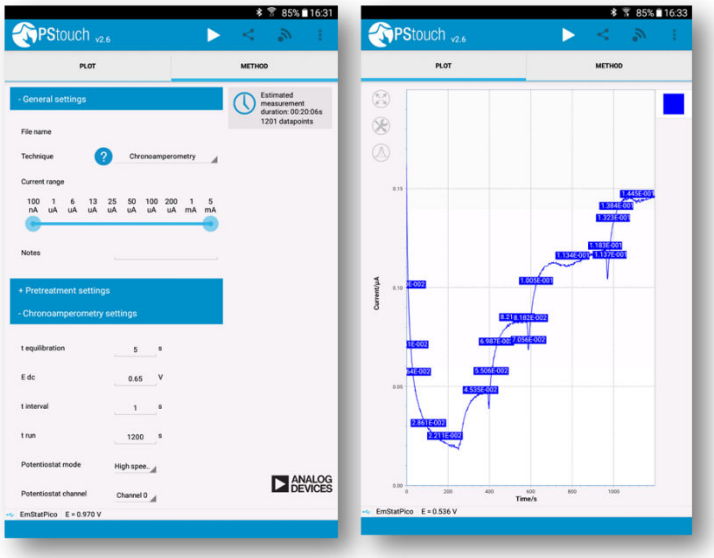

Figure 3.3 Screenshots of the PStouch method editor (left) and the measurement for the calibration curve (right)

4 Calibration Curve

Extract from the curve the constant current values for each solution.
Make a plot of current versus concentration.
Make linear fit to get your calibration curve.

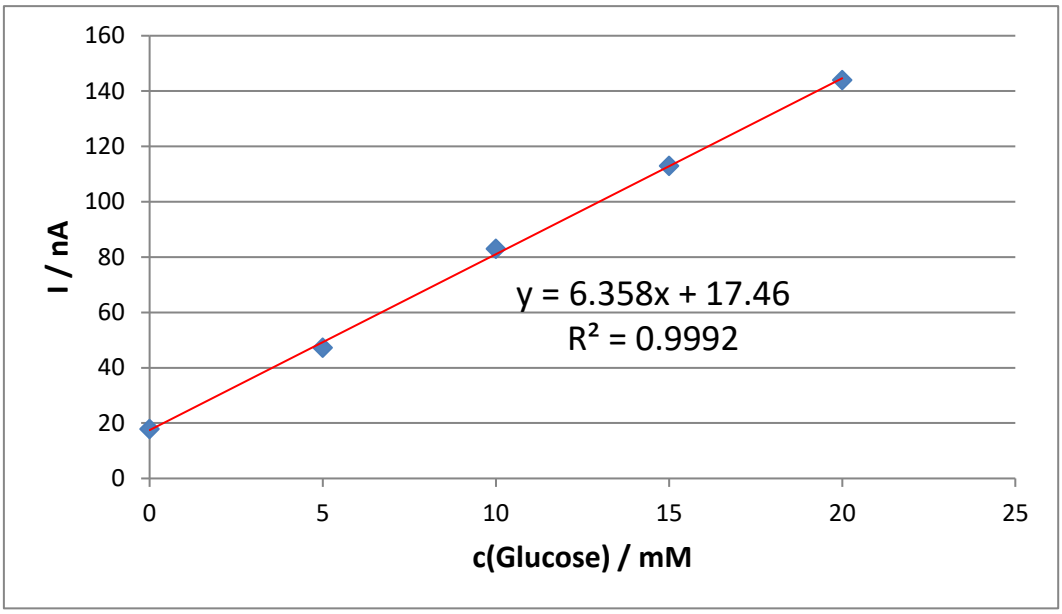

Figure 4.1 Calibration of a glucose measurement with a Glucose Sensors (A-AC-PAC-203-G) by Z&P

Different temperatures, ionic strength, pH, interfering species, etc. influence the enzymes activity and can lead to different calibration curves. Even under the same conditions different batches of biosensors can show differences in their behavior. So before you make your linear fit, you check, if you are still in the linear range of the sensor. This means the current values plotted against glucose concentration should show a linear behavior.

If the points do not show a linear behavior this is usually caused by a saturation of the sensor. The curve will look like it is approaching a final limit in the higher concentration. As a result values at higher concentrations show too low currents for the linear relationship. If this is the case, you can still make a calibration curve by ignoring the higher concentration values. However, be aware that the concentrations, which were taken out of the curve, are too high for quantification. If your sample is in this non-linear concentration range, you will need to dilute it until it is in the linear range.

5 Measuring Samples

If you want to measure a sample, it should have properties close to your measuring conditions. To achieve that the sample can be diluted with the buffer solution which was used to make the calibration curve. Since the sugar content of many soft drinks is very high, the sample often needs to be diluted anyway to bring the concentration down to linear concentration range.
You can first rinse your electrode with some buffer solution and carefully dry it with a paper tissue. Apply 90 µL of buffer solution and wait for a stable current. Add 10 µL of you sample solution. To mix the buffer and sample pump the solution a few times with your pipette. When a constant current is reached, you can use the calibration curve to calculate the glucose content of the sample. Do not forget that your sample was diluted 10 times.