Final Year BSc Practical – Electrochemical Determination of Glucose

Dr. Toby Jenkins, October 2004

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Introduction

This practical is intended to allow you to make an electrochemical sensor for glucose. Glucose sensing is the extremely important commercially, since diabetics need to keep careful control of their blood glucose to prevent either *hypoglycaemia* – (low blood sugar, normally following over injection of insulin), which can lead rapidly to coma and death, or *hyperglycaemia* (too high blood glucose levels), which can lead to long term chronic effects such as kidney damage, blindness, impotence and gangrene.

In healthy humans, blood glucose should be in a range between 4 mmol dm^{-3} up to about 7 mmol dm^{-3}. Our blood is quite sweet! Diabetics may need to test their blood glucose levels up to twelve times per day in order to try to keep their blood glucose in this range.

The early sensors tested for ketones in urine, which are indicative of raised blood glucose levels. Unfortunately the delay in metabolism of glucose to (toxic) ketone metabolites prevented close monitoring of blood glucose. The second generation 'true' glucose sensors appeared in the early 1980's. These were colorimetric assay sticks, which changed colour depending on the concentration of a drop of blood placed on them. Unfortunately they need considerable skill to use and were not very accurate.

Electrochemical Glucose Sensing

The big advance in direct measurement of blood glucose came in the late 1980's, when the enzyme glucose oxidase (GOD) became more widely available, thanks to advances in molecular biology. As the name suggests, glucose oxidase oxides glucose to gluconic acid, in doing so liberating hydrogen peroxide, which can then be detected electrochemically.

$$GOD + Glucose \rightleftharpoons GOD^{-} + Gluconic\ acid$$

$$GOD^{-} + H^{+} + O_2 \rightarrow H_2O_2 + GOD$$

Electrochemical detection of H_2O_2:

$$H_2O_2 + 2H^+ + 2e^- \rightleftharpoons 2H_2O$$

So the hydrogen peroxide reduction current is proportional to the concentration of hydrogen peroxide: $2e^-$ are released per mole of hydrogen peroxide reduced. Providing very slow scan rates are used, measured reduction current is proportional to $[H_2O_2]$ and hence [glucose], provided all else in excess.

Remember, current, measured in amps, is simply the rate of flow of charge (electrons):

$$i = \frac{dq}{dt}$$

So, the SI units of current are coulombs per second (Cs^{-1}).

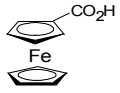
The major problem with using the hydrogen peroxide reduction current as an indirect measure of glucose concentration is that oxygen concentration can vary in different samples, thus providing a large error in measurements.

Next Generation Sensors: Ferrocene mediated systems

The solution to the problem of variable oxygen concentration, and the errors that come from this, was to realise that oxygen's only role is as an electron shuttle (via hydrogen peroxide), removing electrons from the reduced form of GOD, and taking them to the electrode. This problem could be by-passed by providing an artificial electron shuttle, which transports electrons more effectively than O_2 / H_2O_2.

The mediator would need to be a molecule that can easily be reduced, but also easily re-oxidised back to its original form. Transition metal complexes obviously fit this description, especially iron based systems, since oxidation states II and III in Fe are both easily accessed. However, the mediator would need to be sufficiently bio-compatible with GOD that it a. would not denature the GOD, and b. could access the free electron within the reduced GOD.

It was found that ferrocene, and ferrocene compounds fitted these requirements. Ferrocene is not very water soluble, but ferrocene carboxylic acid is more soluble:



Ferrocene carboxylic acid (abbreviated to Fc)

The glucose detection scheme could now proceed as follows:

$$GOD + Glucose \rightleftharpoons GOD^- + Gluconic\ acid$$

$$GOD^- + Fc^{3+} \rightarrow Fc^{2+} + GOD$$

$$Fc^{2+} \rightarrow Fc^{3+} + e^-$$

It can now be seen that 1 mole of charge flows per mole of glucose oxidised to gluconic acid.

Electrochemical response

Ferrocene and ferrocene carboxylic acid like most iron centred inorganic complexes undergoes reversible 1 electron oxidation / reduction. However, in the above scheme, it can be seen that the oxidation of Fc^{2+} to Fc^{3+} is essentially non-reversible, since as soon as Fe^{3+} is formed at the electrode it will react with GOD^-, and thus be chemically reduced to Fe^{2+}. This is because the chemical reduction is facile. The result is that by the time the applied voltage at the electrode is reversed, the Fc^{3+} within the depletion layer will already have been reduced.

The effect of this can be seen by looking at the CV's of Fc^{2+} with and without the associated GOD^-, glucose etc.

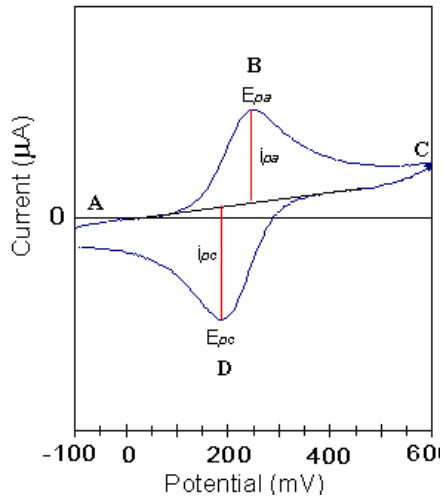
Important: It is very important that you now re-acquaint yourself with the electrochemistry you learnt in Year 2, CH20016 lectures. Read again lectures 11, 12 and 13.

Notes on: http://staff.bath.ac.uk/chsataj

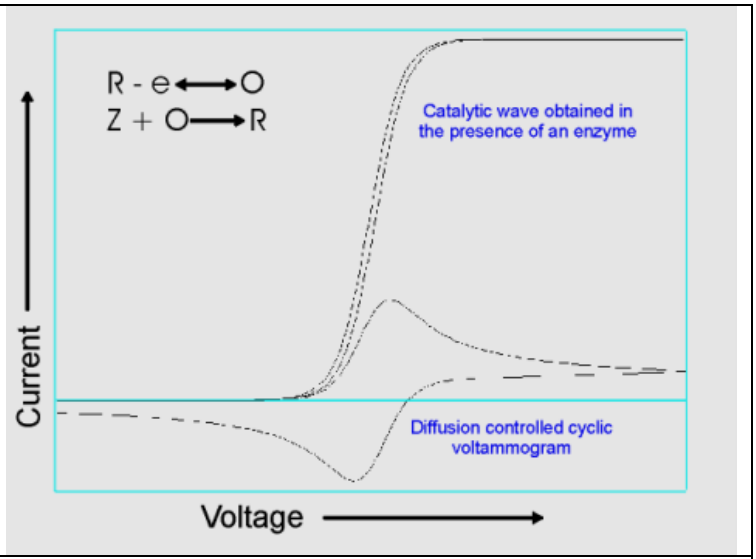
Check out:
http://www-biol.paisley.ac.uk/marco/Enzyme_Electrode/Chapter1/electrochemistry_is_fun.htm

Cyclic voltammetry

Application of a linear voltage sweep, and measurement of current.



Classical, fully reversible CV

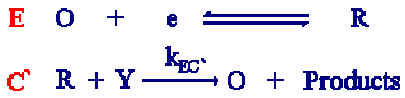


Electron transfer coupled to a catalytic electron transfer reaction

It is vital that you understand the shape of the classical CV before proceeding further

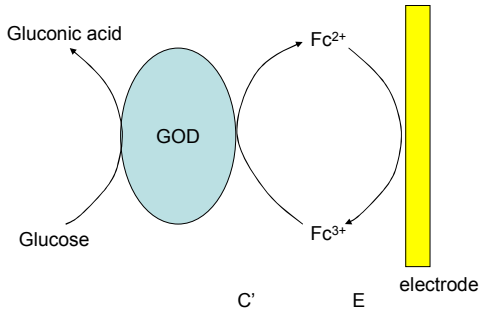
The EC' Mechanism

This stands for **E**lectron transfer – **C**atalysis In this case the C' refers to a catalytic chemical reaction step which drives the electron transfer reaction, thus increasing the rate of electron transfer, which is observed as an increase in peak current (oxidation or reduction, depending on the system under study).



In this situation, one of the products of the second step, the chemical reaction, is the original starting material O. So the system is autocatalytic: the faster the chemical reaction, the more starting material O is made, which is reduced to form R in the electron transfer step, which forms further O.

The EC' mechanism in the context of glucose biosensor:



$GOD + Glucose \rightleftharpoons GOD^- + Gluconic\ acid$

$GOD^- + Fc^{3+} \rightarrow Fc^{2+} + GOD$ C'

$Fc^{2+} \rightarrow Fc^{3+} + e^-$ E

Experimental Procedure

Preparation of buffers, glucose stock solutions, ferrocene carboxylic acid solution.

1. Prepare two solutions in volumetric flasks:

   100 ml 0.1 mol dm^{-3} sodium phosphate **monobasic** (monohydrate) (Calbiochem reagent)
   100 ml 0.1 mol dm^{-3} sodium phosphate **dibasic** (heptahydrate) (Calbiochem reagent)

2. First, calibrate the pH meter with buffer standards, provided.
3. Mix 39 ml of the monobasic sodium phosphate with 61 ml of the dibasic sodium phosphate in a 200 ml volumetric flask. Add *ca*. 80 ml of water and mix. Then check the pH. It should be = 7.0. If it is not, add more of the monbasic or dibasic sodium phosphate. Then make up to just under 200 ml. Finally add solid sodium sulphate, to make a solution of 0.1 mol dm^{-3} in the phosphate buffer solution. Add any further water required to make up to exactly 200 ml and re-check the pH.
4. Make up a solution of 5 mM ferrocene carboxylic acid in ethanol in a plastic eppendorf tube. Sonicate to mix.
5. Prepare a stock solution of 20 ml of 0.1 mol dm^{-3} glucose in the pre-made up phosphate buffer. To this solution, add sufficient ferrocene carboxylic acid solution (in ethanol) to make a final concentration of ferrocene carboxylic acid of 100 μmol dm^{-3}.

Electrochemistry

It is important to prepare the electrochemical cell, and especially to polish the gold counter electrode prior to the experiment. You are provided with an electrochemical cell, a Ag|AgCl reference electrode, a gold wire (or Pt) counter electrode and a gold working electrode. They should be situated such that the reference electrode is close to the working electrode.

Add 5 ml of pure phosphate buffer to the cell, place in the electrodes and connect to the computer controlled potentiostat with the cables provided.

Switch on the PC which runs the potentiostat and open the software which drives the potentiostat.

You should choose *Cyclic Voltammetry* in the software options.

Initial Voltage: 0.1 V
First vertex voltage: 0.55V
Second vertex: 0.1 V
Final voltage: 0.1 V

Measure the electrode at a variety of sweep rates, between 2 mV s^{-1} and 100 mV s^{-1}. If your cell and electrode are clean, you should only see a flat line, which slopes up at more positive voltages. If you are happy with this base line, make a final measurement a 2 mV s^{-1}. Otherwise, re-polish your electrode, and clean the cell.

The next stage is to measure the electrochemical response of the ferrocene carboxylic acid. Pipette sufficient stock solution of ferrocene carboxylic acid into your buffer electrolyte within the cell to give a final ferrocene carboxylic acid concentration of 100 μm dm^{-3}.

Measure the cyclic voltammogram of this solution at 100 mV s-^{1}, 50 mV s^{-1}, 10 mV s^{-1} and 2 mV s^{-1}. Plot the peak current vs. square route of the scan rate (in V s^{-1}). Thus, can you say whether the

oxidation / reduction of ferrocene carboxylic acid is reversible, quasi-reversible or non-reversible? You need to note this in your final report. Save all your CVs.

The glucose oxidase (GOD) is supplied as a liquid. It must be kept cold (in ice) with the lid on. The activity of enzymes such as GOD are quoted in IU (International Units).

> International Unit: IU: That amount of an enzyme that will catalyze the transformation of 1 micromole of substrate per minute under standard conditions of temperature, optimal pH, and optimal substrate concentration

Since we want our response to be dependent on glucose concentration, not GOD, we will use an excess of GOD. Pipette 400 IU of GOD into the phosphate buffer containing the ferrocene carboxylic acid. Mix, and make another CV measurement. Save this, and compare with your CVs of pure ferrocene carboxylic acid in phosphate buffer. Do you see any difference?

Scan rate dependence on adding glucose

Next, pipette in enough glucose stock solution into your cell to make a final glucose concentration in the cell of 50 mmol dm^{-3}. Make measurements at 2 mV s^{-1}, 10 mV s^{-1}, 50 mV s^{-1}, 100 mV s^{-1}, 1 V s^{-1} and 2 V s^{-1}. How has the shape of the CV changed, both as a result of adding glucose, and as a function of scan rate? You must discuss these results in your final report and produce a figure with the responses overlaid. Don't forget to label the legend and axis.

Glucose Concentration Dependence of CV

This part of the experiment needs to be repeated to give three separate measurements for each glucose concentration.

The buffer from the previous experiments should be put down the sink and the cell plus electrodes rinsed in high purity water. Now make up a fresh cell, with phosphate buffer, 100 μmol dm^{-3} ferrocene carboxylic acid and 400 IU of GOD. Do not add glucose yet. Record the CV at 2 mV s^{-1} and save the response. Then pipette in enough glucose stock solution to give 100 μm total glucose concentration. Mix the solution briefly, then record a CV at 2 mV s^{-1}. You must now increase your total glucose concentration sequentially by pippeting in stock glucose solution, remembering that you need to record the cumulative total concentration each time. Increase glucose concentration up to 10 mmol dm^{-3}, recording at least 7 separate concentrations and record the CV without added glucose.

Each CV should be saved with a different name. Finally, either in Excel, or in the Echem software overlay all the CVs for the different glucose concentrations. Record the limiting current for each system (the flattened out oxidation current at 0.50 V). This figure should appear in your report. Now repeat this experiment twice more.

At the end of this experiment you should have three sets of limiting currents / glucose concentration. Measure both the average and calculate the standard error in Excel. Plot a concentration of glucose vs. limiting current graph, with error bars showing the standard error in the limiting current. Can you fit a straight line through all or any part of the graph (minimum 3 data points)? If so, then do so. This is your calibration plot, and will be used for calculating unknown quantities of glucose. This plot must also appear in your final *Chem Commun* style report.

You now have (should have) a sensor for glucose.

Glucose determination in unknown liquids

Ideally at this point we would measure glucose concentration in blood. Unfortunately this is a messy and potentially slightly hazardous procedure. Instead we will measure glucose concentration in some drinks. It is now up to you to design an experiment for determining the glucose content in four different drinks. These will be labelled. Be sure to record which drinks (code) you measured.

The drinks are all widely available soft and fizzy drinks including:

Lemonade
Ribena
Cola
Diet Cola
Ribena low sugar

Don't be fooled by the colour – some we deliberately re-coloured!

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It is your responsibility to make sure it gets to me by the deadline. No excuses accepted.

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References

Sharon Marx-Tibbon, Eugenii Katz, and Itamar Willner Chiral Recognition in Mediated Electron Transfer in Redox Proteins *J. Am. Chem. SOC. 1995,117,* 9925-9926 9925

Chemical Communications

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The experimental evidence necessary to support a communication should be supplied for the referees and eventual publication as *electronic supplementary information*.

In certain circumstances where additional space is required to present the chemistry clearly to the community a third page may be allowed. Authors wishing to submit a three page communication **must**:

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