

# Non-enzymatic Electrochemical Determination of Glucose Concentration

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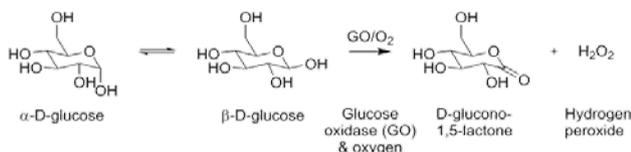
**Abstract** There are a variety of applications for electrochemistry (including synthetic, physical and analytical chemistry), and here we present an experimental protocol for the non-enzymatic electrochemical quantitation of glucose in liquids that can be used in teaching laboratories. This offers an interesting experiential learning experience that is contextualized through a real world application where comparable technology the students employ touches the lives of humans across the world on a daily basis.

**Keywords:** undergraduate, postgraduate, analytical chemistry, physical chemistry, electrochemistry, experiential learning

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## 1. Introduction

Electrochemistry is an interesting area of chemistry that has a variety of applications in synthetic, physical and analytical chemistry [1,2], and therefore offers opportunities for inclusion in undergraduate and postgraduate teaching to help students understand a multitude of different concepts [3-10]. Various pedagogical approaches are used for teaching chemistry [11-17]; laboratory classes are a potent tool for experiential learning in courses that include chemistry, particularly physical and analytical chemistry that can be perceived as difficult [18,19], and real world contextualization facilitates and enhances teaching and learning of chemistry [20-26].



**Scheme 1.** Enzymatic conversion of β-D-glucose to D-glucono-1,5-lactone

The detection of glucose in liquids is an interesting real world application of electrochemistry, of importance for the food and medical industries. The World Health Organization (WHO) predicts that diabetes will be the seventh deadliest disease in the world by 2030 [27], which presents significant burdens to healthcare systems that can potentially be mitigated by diagnosis, management and treatment. This requires effective and regular monitoring of glucose in blood, often (albeit not exclusively)

employing electrochemical sensors that rely on the reaction of α-/β-glucose with Glucose Oxidase in the presence of oxygen, yielding D-glucono-1,5-lactone and hydrogen peroxide, as depicted in **Scheme 1** [28-33].

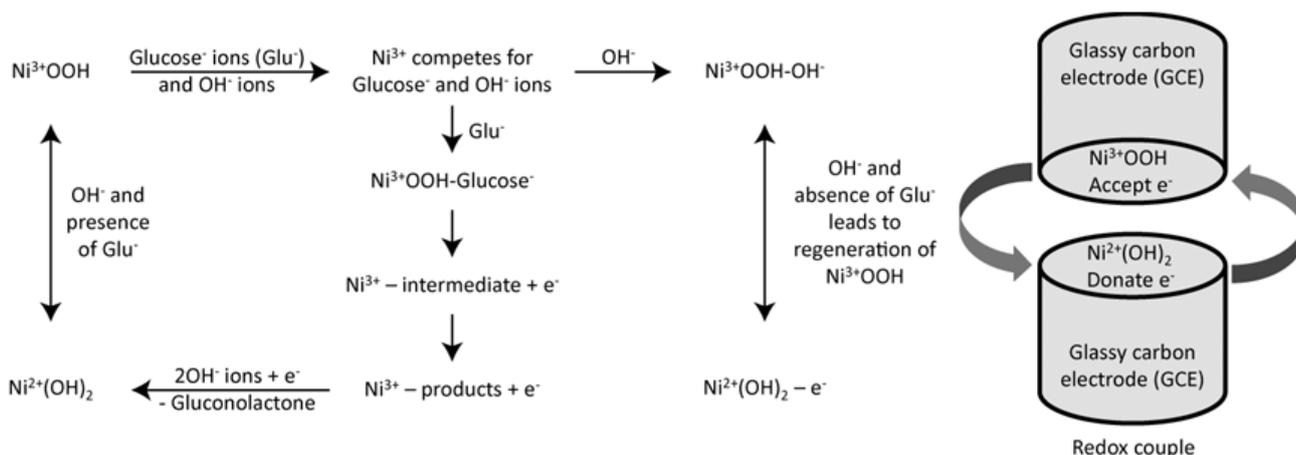
A variety of electrode materials have been explored for their application to catalytic oxidation of carbohydrates showing good sensitivity to glucose (well within the range of blood and urine sugar levels) [28-33], offering opportunities for the development of teaching laboratory activities [34-39] in various disciplinary environments (e.g. chemistry, biochemistry, biology, engineering, forensic science, liberal arts and materials science).

Here we present an example of an experimental protocol for the non-enzymatic electrochemical quantitation of glucose in liquids that is an adaption of an investigation of the electrocatalytic ability of the Ni(II)/(III) redox couple towards the electro-oxidation of glucose [33]. The Ni(OH)<sub>2</sub> present on the electrode surface is initially oxidised to the catalytically active nickel oxyhydroxide species (Ni(O)OH) which subsequently irreversibly oxidises glucose [33].

In this experiment, a Ni(OH)<sub>2</sub> coated glassy carbon electrode (GCE) will be prepared by electrodeposition of both nickel metal (Ni<sup>0</sup>) and nickel hydroxide (Ni(OH)<sub>2</sub>) from a Ni(NO<sub>3</sub>)<sub>2</sub> solution, followed by conditioning in KOH, yielding a layer of the β phase of Ni(OH)<sub>2</sub> on the surface of the electrodes as per reactions 1-3 [24].

- Reaction 1)  $\text{NO}_3^- + 7\text{H}_2\text{O} + 8\text{e}^- \rightarrow \text{NH}_4^+ + 10\text{OH}^-$
- Reaction 2)  $\text{Ni}^{2+} + 2\text{OH}^- \rightarrow \text{Ni(OH)}_2$
- Reaction 3)  $\text{Ni}^{2+} + 2\text{e}^- \rightarrow \text{Ni}^0$

Thereafter, the concentration of glucose will be determined from 1M KOH solutions using cyclic voltammetry and amperometry, as per the equations depicted in **Scheme 2**.



**Scheme 2.** Non-enzymatic conversion of  $\beta$ -D-glucose to D-glucono-1,5-lactone

By undertaking this experiment students will gain experience in the following practical techniques:

- Electrochemical modification of surfaces via electrodeposition.
- Electrochemical quantitation of organic molecules in solution.
- Standard analytical techniques.
- Accurate recording of data.
- Analysis and interpretation of data.

## 2. Materials and Methods

### 2.1. Pre-lab Activities

Read the instructions below. Read the references from Vaidyanathan and co-workers [32] and Toghil and co-workers [33]. Watch a video from the Royal Society of Chemistry on preparing standard solutions [40]. Calculate the quantities of reagents you will need to use. Complete a risk assessment for the practical to ensure you handle and dispose of chemicals safely while wearing appropriate personal protective equipment (e.g. lab coat, lab specs, gloves). Plan to complete steps 1-17 before taking a break (step 18), then finish steps 19-22 before writing the report (steps 23-28).

#### 2.1.1. Apparatus

The potentiostat used while collecting the data presented was an Ivium EmSTAT 3+ in conjunction with the potentiostat's software (PSTrace) and a laptop computer to control the potentiostat; the counter electrode (CE) is a carbon rod; the reference electrode (RE) is Ag/AgCl in 1 M KCl; the working electrode (WE) is a glassy carbon electrode (GCE); all supplied by IJ Cambria Scientific Ltd. The beakers, glass pipettes, micropipettes, plastic pipette tips, tape, Parafilm®, hotplate stirrers, and stir bars were supplied by Fisher Scientific Ltd. Optionally, a 3D printer can be used to print custom voltammetry cell lids [41] designed specifically for this experiment (to fit the beakers, accommodate each electrode and allow additions to the solution without removing the lid). Examples of types of 3D printers to produce the lids include fused deposition modeling (FDM), stereolithography (SLA) and digital light projector (DLP) printers; alternatively a computer numerical control (CNC) mill would also function.

### 2.1.2. Chemicals

Glucose, nickel(II) nitrate ( $\text{Ni}(\text{NO}_3)_2$ ), potassium hydroxide, sodium acetate, were of analytical grade, supplied by Sigma-Aldrich and used as supplied.

### 2.1.3. Note for Instructors

The screenshots included herein were also included in the practical script given to students to ensure the correct entries in the software and facilitate their progress and thereby improve student experience.

For the solution with an "unknown" concentration of glucose (X mM, where X is unknown) used in step 19 we have observed that ca. 400 mM works well.

While Parafilm® is a functional lid for the beakers used for the voltammetry cells (see steps 5/6), cutting holes in it for the electrodes using a scalpel makes it potentially hazardous for whoever cuts the holes. A low risk alternative is to employ a 3D printer to print custom voltammetry cell lids [41]; we have observed that an acrylate resin DLP 3D Printer (Anycubic Photon) works well.

### 2.1.4. Note for Students

- Distances are often measured in metres (m) or divisions/multiples thereof (e.g.  $\mu\text{m}$ , mm, cm, km).
- Quantities are measured in moles (mol) or divisions/multiples thereof (e.g. mmol).
- Concentrations of specific species/chemicals are specified as molarity ( $\text{M} = \text{mol.L}^{-1}$ ) or divisions/multiples thereof (e.g.  $\mu\text{M}$ , mM).
- Volumes are measured in litres (L) or divisions/multiples thereof (e.g.  $\mu\text{L}$ , mL).
- Pipettes have limits to their accuracy which is normally displayed on the specific pipette or in the accompanying manual (e.g. 20-100  $\mu\text{L}$ , or 100-1000  $\mu\text{L}$ ).

## 2.2. Experimental details

### 2.2.1. Electrochemical Modification of Surfaces via Electrodeposition

**Step 1.** All electrochemical measurements will be made using a potentiostat (e.g. an Ivium EmSTAT 3+) in conjunction with the potentiostat's software (e.g. PSTrace supplied with Ivium EmSTAT 3+) and a laptop computer

to control the potentiostat. The counter electrode (CE) is a carbon rod. The reference electrode (RE) is Ag/AgCl in 3 M KCl (the standard potential for the Ag/AgCl RE relative to the normal hydrogen electrode (NHE) is  $E = +0.197$  V). The working electrode (WE) is a glassy carbon electrode (GCE).

**Step 2.** Polish the GCE using a polishing kit supplied with the electrodes. To polish add a small amount of ultrapure water to wet the pad, then add a few drops of polishing suspension to the polishing pad, then move the electrode across the polishing pad surface in a Figure 8 motion, for approximately 2 minutes. Wipe the polishing suspension from the electrode (being mindful of the polished surface) and then rinse the electrode well with ultrapure water to ensure they are free of residue.

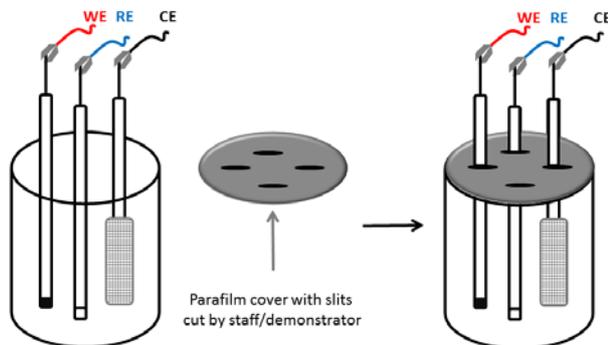
**Step 3.** Prepare 100 mL of standard 1 mM solution of nickel(II) nitrate ( $\text{Ni}(\text{NO}_3)_2$ ) in 0.1 M sodium acetate buffer solution at pH 5 (the pH 5 acetate buffer can be prepared from a 3 M stock solution of sodium acetate, that should be diluted to 0.1 M using deionised/ultrapure water of resistivity of ca.  $18.2 \text{ M}\Omega \text{ cm}^{-1}$  at 298 K).

**Step 4.** Add 10 mL of the standard 1 mM solution of  $\text{Ni}(\text{NO}_3)_2$  to the beaker.

#### Step 5/6.

Either, **step 5:** Cover the beaker with parafilm, securing it with electrical tape if required (see **Scheme 3**), **step 6.** Create holes in parafilm using a scalpel just smaller than the diameter of the electrodes.

Or, (in advance of the practical) **step 5:** employ a 3D printer to print custom voltammetry cell lids [41], and then during the practical **step 6:** place it on the beaker.



**Scheme 3.** Schematic of experimental setup

**Step 7.** Insert a glass pipette attached to a nitrogen line into the solution, and carefully begin to eliminate oxygen from the solution by “degassing” with a controlled, but rapid flow of bubbles for 10 minutes. Ensure the parafilm does not bulge out due to the high pressure (or the printed lid is not dislodged).

**Step 8.** Insert your three electrodes (working, references and counter). Connect these to the potentiostat via the appropriate crocodile clips (using the apparatus described above: red for WE, blue for RE and black for CE).

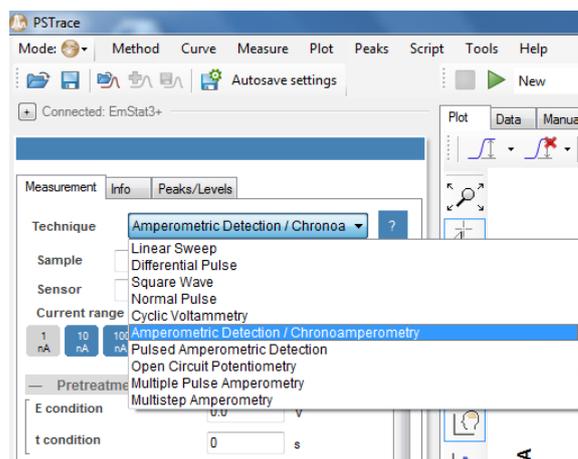
**Step 9.** Switch on the computer and potentiostat, ensure the potentiostat is connected, and click on ‘connect’ (Figure 1).



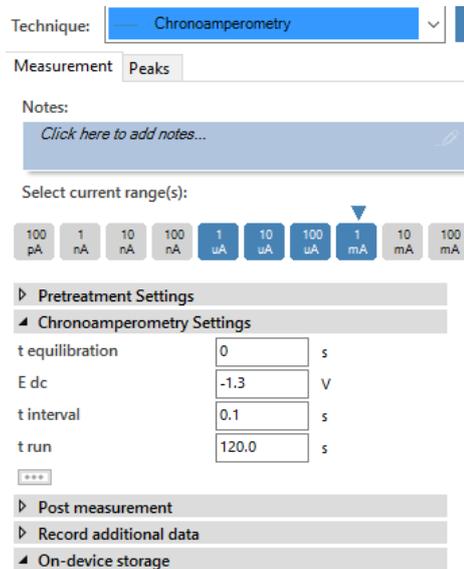
**Figure 1.** Confirmation that the potentiostat is connected

**Step 10.** The freshly polished GC working electrodes will be modified with nickel films to produce a Ni-GC electrode. This will be achieved via the electrodeposition of nickel at  $-1.3$  V vs. Ag/AgCl from a degassed 1 mM  $\text{Ni}(\text{NO}_3)_2$  in 0.1 M acetate buffer deposition solution for 120 s under constant stirring and under a nitrogen atmosphere. Figure 2 and Figure 3 demonstrate software setup and data entries:

- For deposition choose the technique of chronoamperometry (i.e. timed current measurement) as shown in Figure 2.
- Ensure an appropriate current range of  $1 \mu\text{A}$  to 1 mM is selected, set the working electrode potential to  $-1.3$  V ( $E_{\text{dc}}$ ),
- Set a time interval (frequency of measurement) of at most 1 s, and a run time ( $t_{\text{run}}$ ) of 120 s.

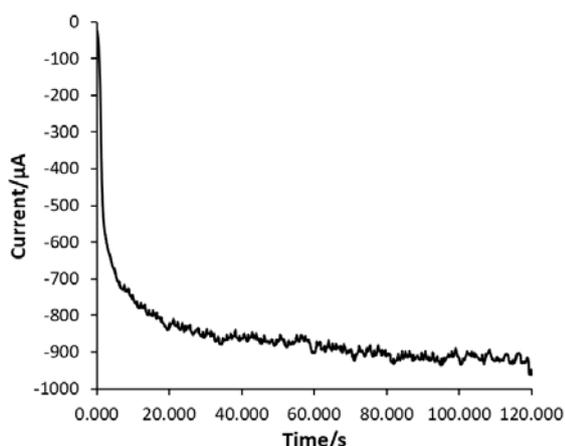


**Figure 2.** Software setup for electrodeposition of nickel



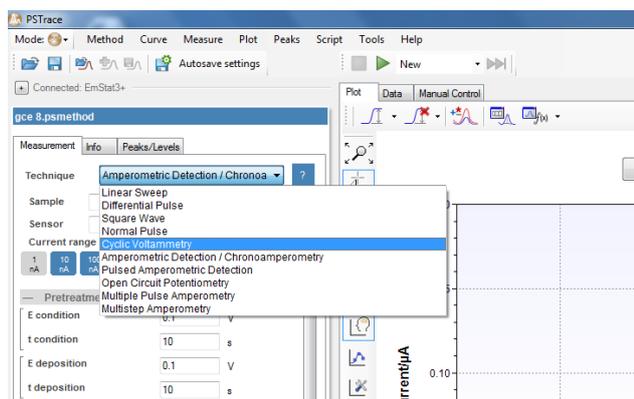
**Figure 3.** Data entries for electrodeposition of nickel

This should ensure the successful deposition of nickel (Figure 4).



**Figure 4.** Representative data collected during electrodeposition of nickel

**Step 11.** Following the electrodeposition of nickel, the Ni-GC electrode will be removed from the electrodeposition solution, rinsed (but not wiped!) with deionised/ultrapure water, and then placed in 10 mL of a non-degassed 1 M KOH aqueous solution for conditioning. Change the technique in the software, as shown in Figure 5, to Cyclic Voltammetry.

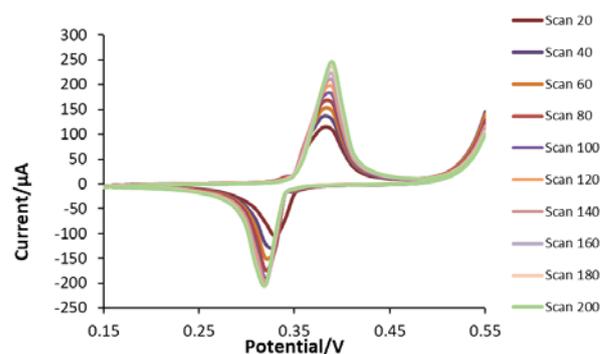


**Figure 5.** Software setup for cyclic voltammetry

**Step 12.** The electrode must be conditioned by cycling between 0.15 and 0.55 V in the alkaline medium ca. 200 times to settle the crystalline phases of Ni(OH)<sub>2</sub> into the stable aged beta phase. These settings are as follows:

- Set the start potential and lower limit for the cycle window (E begin and E vertex 2) to 0.15 V (i.e. the negative limit of the cycle window).
- Set the upper, positive limit of the cycle window (E vertex 1) to 0.55 V. This will ensure that both the oxidation and reduction peaks of the Ni(OH)<sub>2</sub> will be recorded in completion.
- The step potential (E step) is the frequency of digital measurements and should be *at most* 0.005 V to obtain smooth data.
- Finally, set the scan rate to 0.2 V/s and number of scans to 200.

Save the data by exporting to a spreadsheet (using the MS Excel button to the left of the graph), then you should plot every 20<sup>th</sup> scan (rather than all the voltammograms obtained), see Figure 6 for representative data.

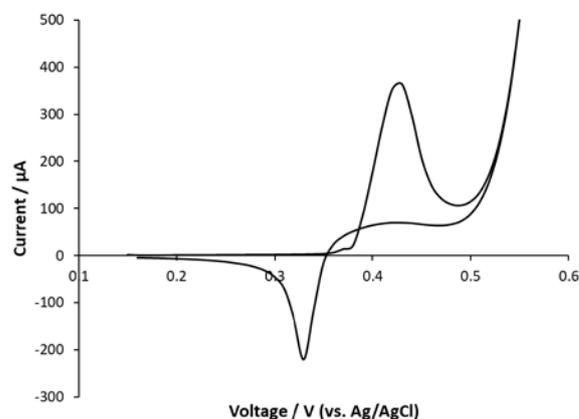


**Figure 6.** Representative CV during conditioning step

## 2.2.2. Electrochemical Quantitation of Organic Molecules in Solution: Optimisation and calibration

**Step 13.** Once the conditioning process is complete, ensure all the data is saved, then alter the settings to take 2 scans. Add a stirrer bar to the KOH solution (a standard 1.0 M solution of potassium hydroxide (ca. pH 14) will be used, prepared using potassium hydroxide pellets in deionised water). You are to record 6 cyclic voltammograms over a range of 0-5 mM glucose, adding the 1 mM of glucose each time. The calibration stock solution of glucose has a concentration of 200 mM and your beakers contain 10 mL of KOH. Work out the volume of stock glucose needed to make sequential 1 mM additions of glucose to the 10 mL of KOH. Assume the volume added for each addition makes a negligible change to the 10 mL volume. Once you have set the pipette to this volume, run the first scan with 0 mM glucose and save the data by exporting to a spreadsheet. Then add the first 1 mM addition, stir the solution briefly, then with the stirrer OFF obtain another CV, repeating this procedure up to 5 mM, each time saving the data by exporting to a spreadsheet.

**Step 14.** From an overlay of the scans from **Step 13** determine the potential best used to obtain a calibration plot by ascertaining the most linear increase in current. This will be after the peak, at approximately 0.45 V, but is also dependent on the reference electrode and quality of the Ni-GC electrode (see Figure 7).



**Figure 7.** Plot to ascertain potential to run subsequent experiments at.

**Step 15.** Return to chronoamperometry mode (Figure 2).

**Step 16.** To obtain a calibration plot over the range of 0-100 μM follow the instructions that follow. The

amperometric analysis of glucose concentrations will be conducted at the potential determined in step 14 (approximately 0.45 V), recording a current vs. time plot. Make sure you change the electrode potential value ( $E_{dc}$ ) in the chronoamperometry set up to the potential determined in step 14. You will also need to adjust the measurement time to 300 s.

Empty and rinse the beaker with deionised water and add a fresh 10 mL of KOH solution into the beaker remembering the magnetic stirrer. Replace the parafilm and electrodes as previously. You are now going to make smaller additions of glucose from the stock solution.

Adjust the volume of the pipette accordingly to make the 10 or 20  $\mu\text{M}$  additions over the range of 0-100  $\mu\text{M}$  (dependent on the accuracy of the pipette you are using). Get the magnetic stirrer stirring steadily, start the scan, and after 60 seconds begin making the 10 or 20  $\mu\text{M}$  glucose additions (over the range of 0-100  $\mu\text{M}$ ). Make a new addition every 20 seconds up to 100  $\mu\text{M}$ . The systematic noise caused by the magnetic stirrer can be easily removed by averaging the current value over the 20 s interval, which should provide a highly linear calibration plot of  $R^2 > 0.95$ . There may be spikes at the start of each addition caused by the addition, disregard the spikes when calculating the current average. Save the scan. Plot a current vs. time plot (representative data is depicted in Figure 8).

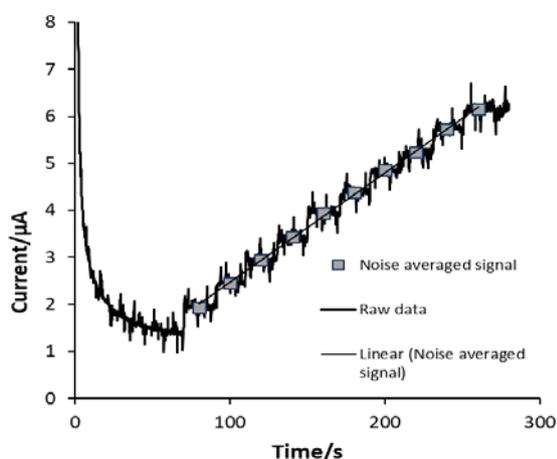


Figure 8. Calibration plot over the concentration range 0-100  $\mu\text{M}$

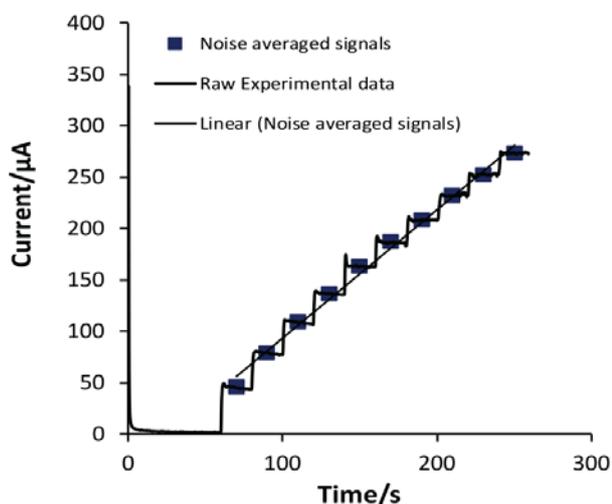


Figure 9. Calibration plot over the concentration range 0-10 mM

**Step 17.** Calibration plot over the range of 0-10 mM. Step 16 will be repeated this time recording a current vs. time plot of the 1 mM glucose additions made to a stirred 1 M KOH solution over the range of 0-10 mM. Adjust the volume of the pipette to make 1 mM additions of glucose. Save the scan. Plot a current vs. time plot (representative data is depicted in Figure 9). This is a good point to take a short break.

**Step 18.** This is a good point to take a break (optional).

### 2.2.3. Electrochemical Quantitation of Organic Molecules in Solution: Measurements of Unknown Samples

**Step 19.** Experimental determination of the concentration of glucose in an “unknown” sample: Step 17 will be repeated however, this time recording a current vs. time plot upon the 1 mM glucose additions made to a stirred 1 M KOH solution over the range of 0-3 mM. Thereafter a sample of liquid with an “unknown” concentration of glucose will be added and the current vs. time recorded, after which a further 5 additions of 1 mM glucose can be made and current vs. time recorded (representative data is depicted in Figure 10).

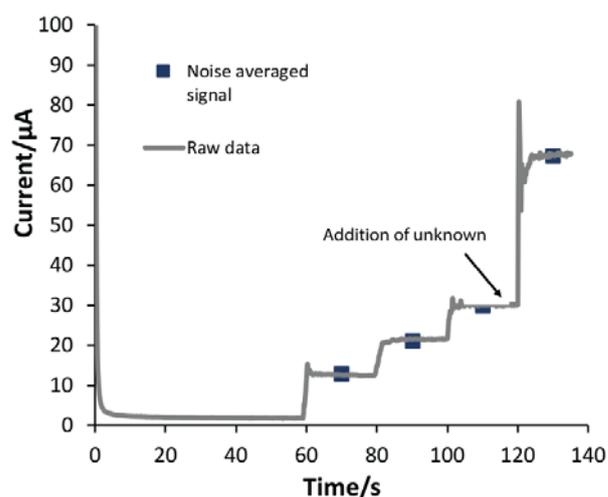


Figure 10. Current vs. time plot recorded on addition of glucose solution of unknown concentration

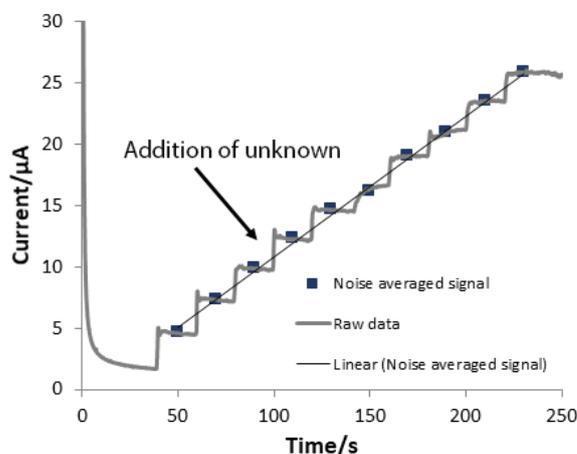


Figure 11. Current vs. time plot recorded on addition of 10-fold diluted glucose solution of unknown concentration

**Step 20. Optimisation:** If the current generated upon the addition of the “unknown” is outside the linear range

for the calibrations (0-10 mM, or 0-100 mM), then step 19 can be repeated with a lower/higher amount of the “unknown” sample as appropriate (representative data is depicted in Figure 11). Ensure any higher volumes remain a negligible influence (less than 1%) of the whole sample volume.

**Step 21.** Glassy carbon electrodes will be polished, dried and stored for other experiments.

**Step 22.** Solutions will be disposed of safely and glassware rinsed for other experiments.

#### 2.2.4. Report

**Step 23.** Read the paper from Toghil and co-workers [33].

**Step 24.** Plot the cyclic voltammetry data for the conditioning step. Pay specific attention to Figure 3 in the paper from Toghil and co-workers [33].

**Step 25.** Plot the data from steps 13 and 14.

**Step 26.** Plot the current vs. time calibration curves. Hint: the steps recorded for the known solutions during the calibration should be reproducible (as noted above yielding a highly linear calibration plot of  $R^2 > 0.95$ ).

**Step 27.** Plot the current vs. time curves obtained when adding the solution of “unknown” concentration from which the concentration of glucose in the “unknown” sample will be determined. Pay specific attention to Figure 8 and 9 in the paper from Toghil and co-workers as it will help with plotting to determine the unknown [33]. *Hint:* if you are adding the unknown solution and the signal you record in response to its addition is greater than that of the known solution you use for the calibration curve, then the concentration of the unknown solution is by extrapolation higher than that of the known solution by an amount that is proportional to the increase in step size.

**Step 28.** When responding to the questions in the report include a section titled: Answers to questions 1-3.

#### 2.2.5. Example Questions

1. What is the rationale for using non-enzyme functionalized electrodes for determining the concentration of glucose?
2. What might interfere with determining the concentration of glucose in the “unknown” samples?
3. What factors might affect the stability and lifetime of the Ni(OH)<sub>2</sub> coated glassy carbon electrode?

#### 2.2.6. Example Marking Scheme

The mark for the lab report will be given out of 100% (with partial marks available where a particular criterion has been partially but not fully met) according to the criteria in Table 1.

Table 1. Example Mark Scheme

Weighting	Criteria
10%	Abstract, introduction and references sections are accurately written and appropriate
10%	Well-written experimental section, including layout of complete analytical data and correct calculations
10%	Clear discussion of electrochemistry data
10%	Clear/correct calibration plots
20%	Clear/correct assessment of the concentration of glucose in the “unknown” sample as judged from own data
10%	High quality of writing and the overall presentation of the report
30%	Clear/correct answers to questions 1-3

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