Glucose Test Strips and Electroanalytical Chemistry in the Undergraduate Laboratory

Jonathon O. Howell, Bioanalytical Systems Inc., West Lafayette, IN 47906
Peter T. Kissinger, Department of Chemistry, Purdue University, West Lafayette, IN 47907
Arlen D. Kaufman and Hsiang-Jui Yeh, Department of Chemistry, University of Evansville, 1800 Lincoln Avenue, Evansville, IN 47722

Abstract

The $4 billion+ market of home glucose test devices is dominated by electrochemical based instruments. The study of these common, “simple”, and inexpensive meters can provide an interesting opportunity to teach undergraduate students the basics of analytical chemistry, biosensor development, and electrochemical techniques and show how fundamental studies relate to practical devices.

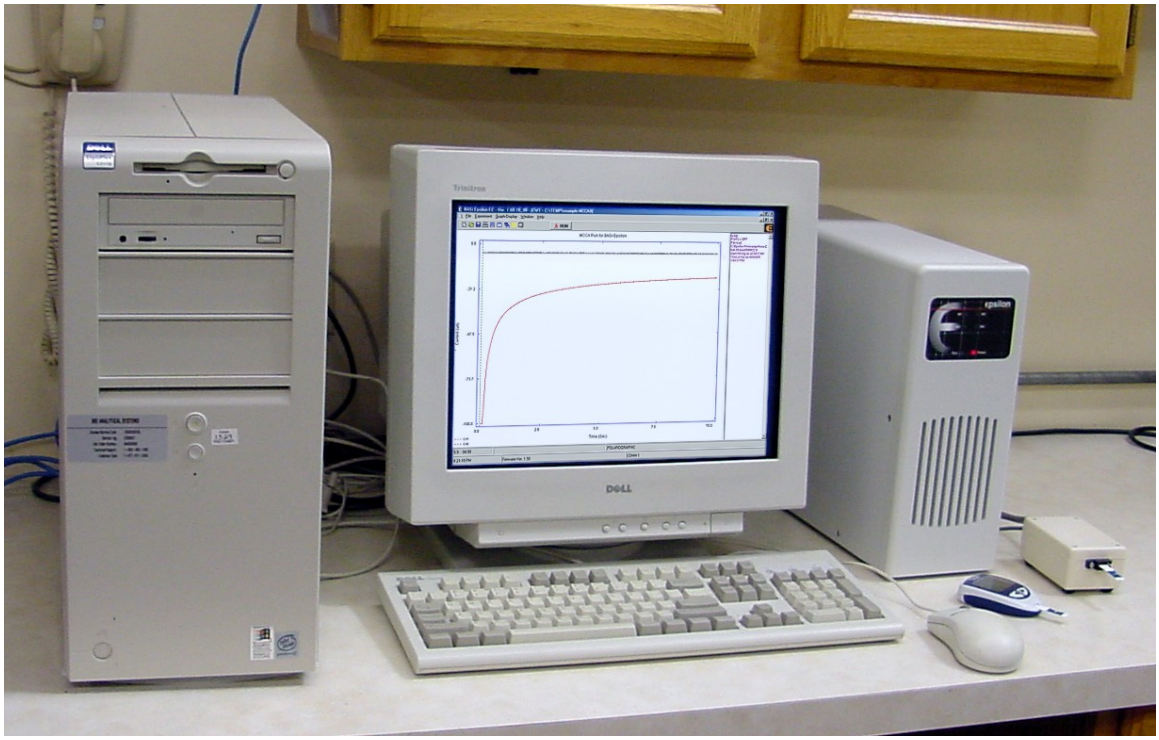
These instruments are sufficiently inexpensive to provide an opportunity for high school science at the interface between biology and chemistry, providing a “show and tell” example proven to interest students in the age of MP3 players and cell phones. The glucose meter is the first of many Point of Care Testing (POCT) devices now used in clinical chemistry and enables an introduction to this entire class of products for many analytes.

Topics covered include: sample collection, microliter volume cells, mass transport, enzyme kinetics, materials science, accuracy, precision, temperature effects, and redox properties.

Experimental

An EC epsilon electrochemical workstation (Bioanalytical Systems, Inc., West Lafayette, IN) and a custom built trigger box were employed along with the commercial Accu-Chek® blood glucose measurement system (Accu-Chek® Advantage meter with Comfort Curve test strips, Roche Diagnostics). Conventional electrochemical experiments employed a platinum wire auxiliary electrode, a Ag/AgCl reference electrode (3 M NaCl, RE-5B, Bioanalytical Systems, Inc.), and a platinum working electrode.

To perform the experiments, a modified chronoamperometry technique was created in order to study the effect of the length of homogeneous reaction time on the electrochemical response. The experiment is similar to chronoamperometry except that instead of a Quiet time (equilibration time) at the initial potential, there is an Incubation Time with the cell at open circuit.

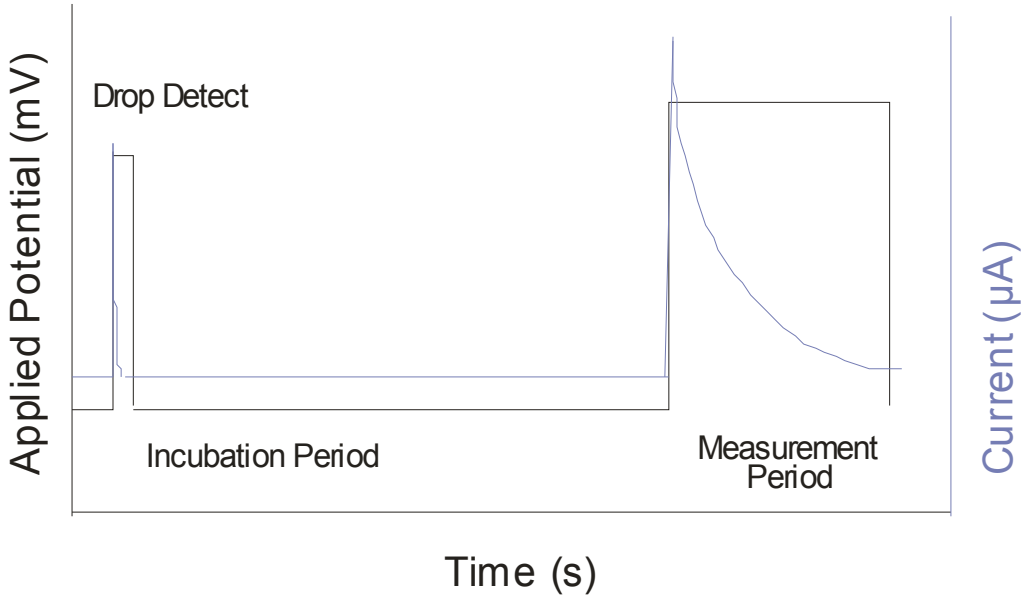


Reproducible and accurate timing between the steps is critical. When Run is clicked on the computer, the cell is at open circuit (no current can flow) and the working is grounded. At this point the epsilon is waiting for an external trigger before starting the run. When solution is added to the cell, an external circuit in the trigger box senses the cell wetting (change in cell impedance) and sends a signal to the Start In terminal on the back panel of the epsilon to initiate the experiment. After the trigger, the cell

remains at open circuit until the epsilon counts down for the set Incubation Time (controlled with millisecond resolution). At the end of the Incubation Time, the potential is applied to the electrodes, and the current is monitored based on the parameters set in the Epsilon software. A digital oscilloscope was used to investigate the timing of some of the steps during this process. The following timing can be expected.

1. For the glucose strips used, it took approximately 0.5 ms for the strip to be wetted. The trigger was set to fire midway in the wetting process, or approximately 0.25 ms after solution contacted the electrodes.
2. The Incubation (Quiet) Time has an extra 52 ms lag due to the trigger mechanism and relay closure.

Test Strip Timing Sequence

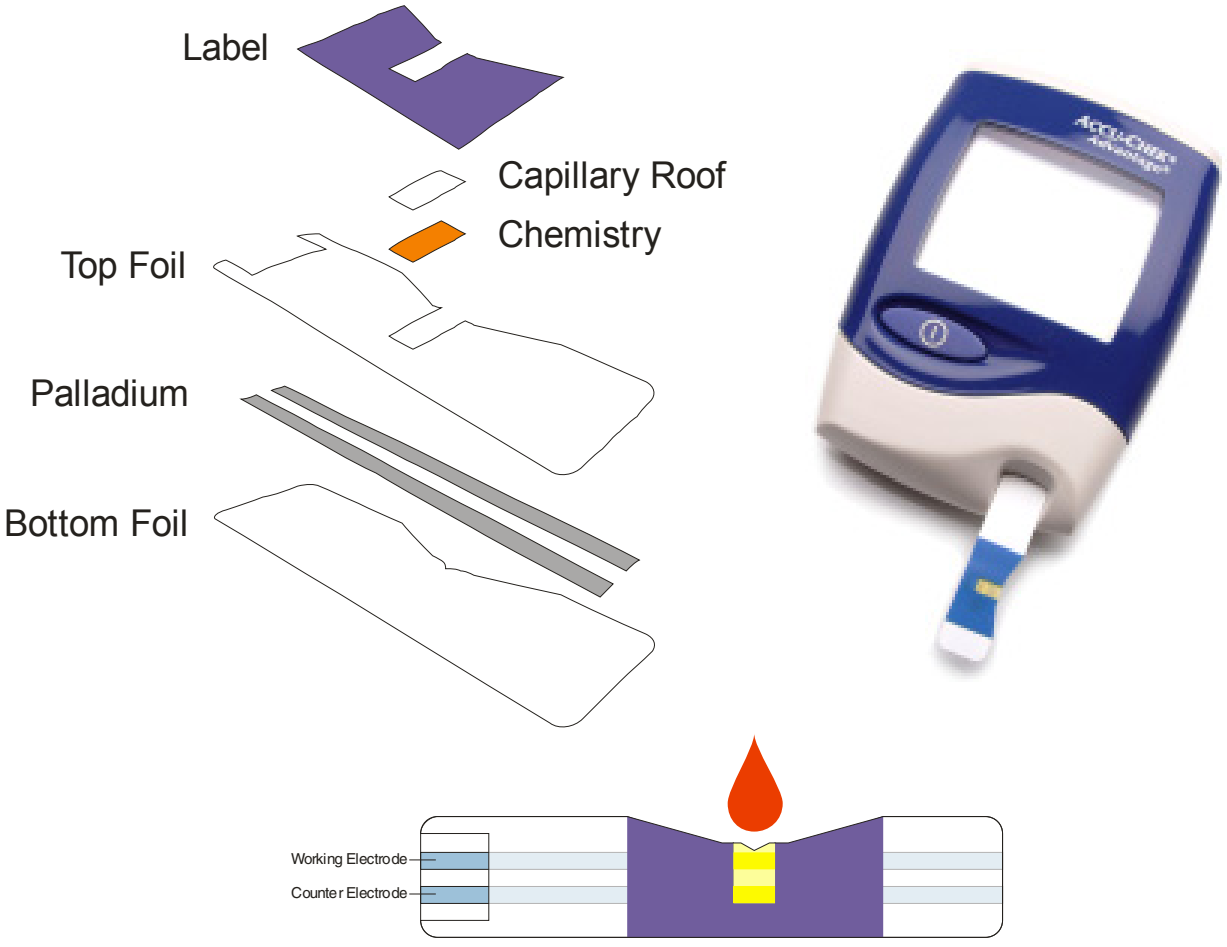


Stock solutions of β-D-glucose were prepared in PBS, pH 7.4 and allowed to mutarotate overnight before use (except during the mutarotation rate experiments). This was to establish equilibrium concentrations between the α- and β-anomers. Glucose concentrations were reported as total glucose concentrations.

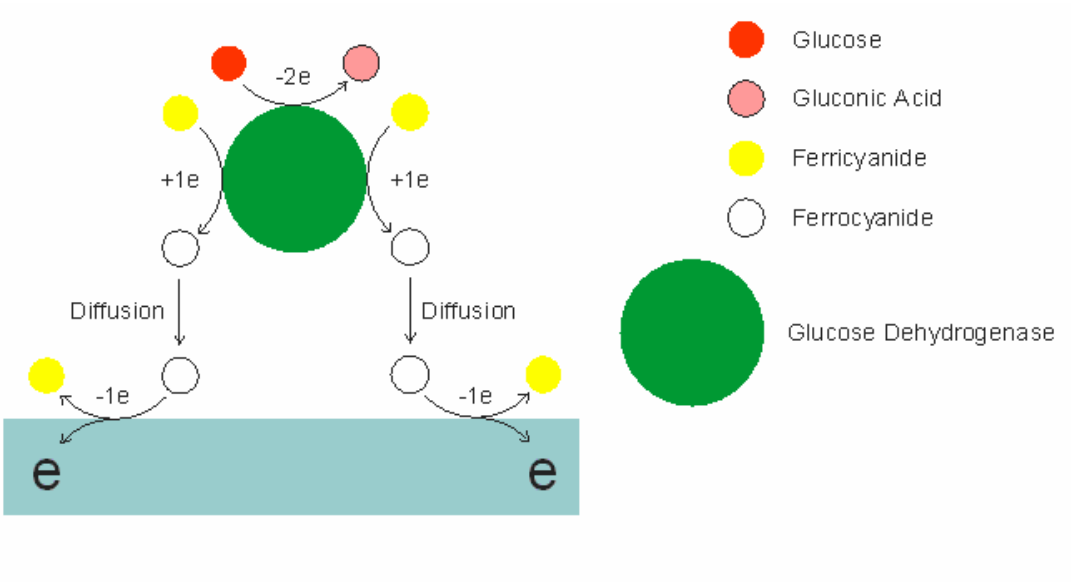
General Operation of the Glucose Meter and Sensing Strips

1. Set up meter with calibration chip for particular lot of strips used
2. Insert Strip (Disposable Biosensor). This turns meter on and it performs internal tests and applies voltage to cell.
3. Collect sample by touching drop of blood to strip opening
   a. Blood fills chamber by capillary action.
   b. Meter senses wetting (by drop in impedance), turns off cell, and starts the incubation time.
   c. The incubation time allows dissolution of chemicals, enzymatic reaction to occur and solution to become homogeneous.
   d. When incubation time is completed, potential is applied so that reacted mediator is converted back to original oxidation state. The current is monitored, compared to a calibration curve, and then concentration is reported.

Accu-Chek® Comfort Curve Glucose Strip (Figure from reference 1)



Incubation Chemistry And Detection Mechanism



The chemistry is relatively complex and the sample chamber contains many constituents (e.g., stabilizers, processing aids, etc.). However, for this discussion it will be treated more simply as an enzyme and mediator. An enzyme overcomes many of the problems associated with a variable biological sample matrix. Because glucose and enzymes do not readily exchange electrons directly with an electrode, an electrochemical measurement requires a mediator to facilitate (or mediate) the electron transfer. The chemistry is summarized as:

1. Glucose first reacts with the enzyme glucose dehyrogenase. Glucose is oxidized to gluconic acid and the enzyme is temporarily reduced by two electrons transferred from glucose to the enzyme.
2. The reduced enzyme next reacts with the mediator (Mox), transferring a single electron to each of two mediator ions. The enzyme is returned to its original state, and the two Mox are reduced to Mred.
3. At the electrode surface, Mred is oxidized back to Mox and the measured current is used to determine the concentration of glucose in the sample.

The enzyme (a protein catalyst) glucose dehydrogenase was chosen because it is highly specific for, and accelerates the oxidation of, glucose to gluconic acid. It is also less susceptible than glucose oxidase to common interferences. Its specificity enables it to selectively react with glucose in the presence of the thousands of compounds that could potentially interfere within the complex sample fluid, blood. This specificity is critical because glucose levels vary widely over time in a single healthy patient along with many other factors such as hematocrit, oxygen levels, metabolic byproducts, etc.

Furthermore, patients with diabetes often have a variety of other medical problems causing even greater variation in their blood.

The well-known mediator, potassium ferricyanide is used in this process. The redox couple ferricyanide/ferrocyanide is capable of rapidly transferring electrons with an electrode (electrochemically reversible on the timescale of the experiment). It also has a relatively low oxidation potential. This allows for a lower applied potential at the working electrode, thereby minimizing the amount of oxidation of extraneous compounds in the sample. The end result is that electrons may thus be transferred between glucose and the electrode via enzyme and mediator.

Accu-Check Advantage Sensor Characteristics

The characteristics of this medical device and sensor can be discussed from a scientific standpoint and in terms of its development to make it viable for its intended market.

Discussion Points:

- Specificity: very specific (enzyme)
- Accuracy: less than 5% error
- Linear Dynamic Range:
  10-600 mg/dL
- Environmental Conditions:
  Temperature: 10 – 40 ºC
  Humidity: up to 85% RH
  Altitude: up to 10,150 ft
- Sample variety:
  Hematocrit Variability:
  20-65% for glucose < 200 mg/dL,
  20-55% for glucose > 200 mg/dL
- Sample Site:
  venous, arterial, finger stick, capillary, neonate
- Interferences:
  Biological entities
  Drug interactions
- Precision (Lot): 2 mg/dL or 2%
- Sample Size: < 6 µL
- Ease of Use:
  Potential for impaired users
- Frequency of Use:
  Minimize sampling pain
  Small sample required
- Analysis Time: < 30 s
- Stability: long shelf life desirable
- Distribution channels:
  Consumer location
- Cost/Price: Competitive
  (~$1/measurement)
- Complexity of Manufacture
- FDA Approval

Cyclic Voltammetry of the Mediator

Performing Cyclic Voltammetry on the ferricyanide/ferrocyanide couple, the mediator used on the glucose test strips, is a very common undergraduate laboratory exercise, and it has been previously described in detail (reference 6).

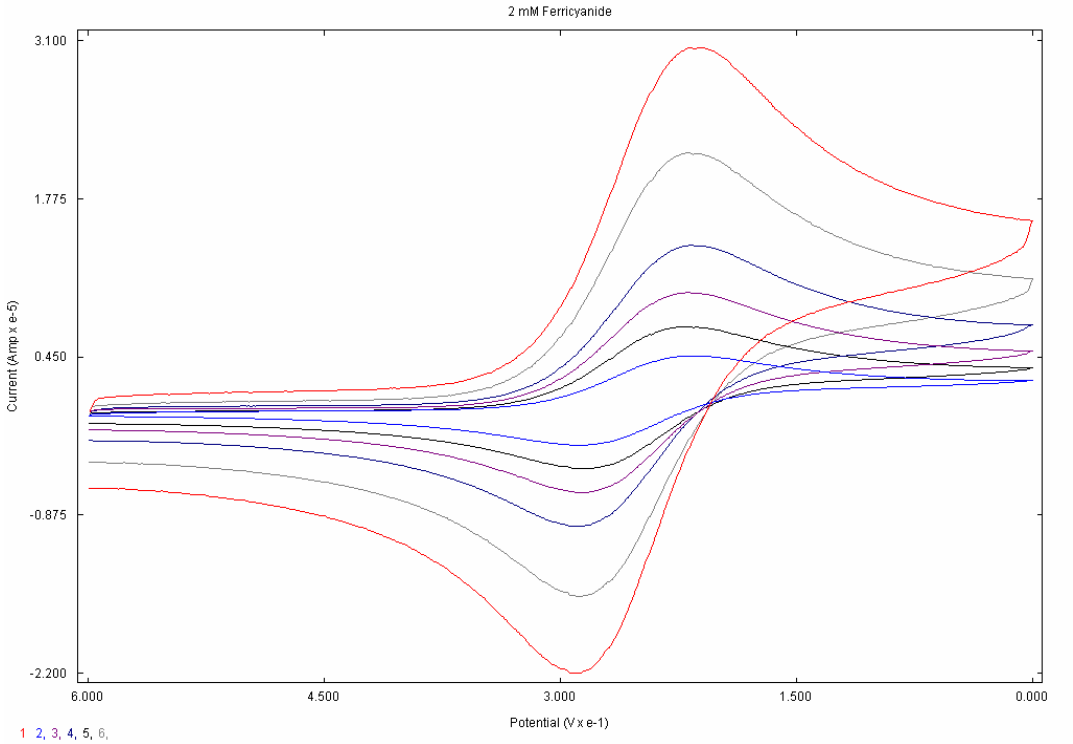
Information Obtainable from Cyclic Voltammetry:

- Thermodynamic and kinetic data on the reactants, products, and coupled chemical reactions
- Mediator characteristics (ferricyanide/ferrocyanide couple) - chemically and electrochemically reversible and has a low reduction potential
- Mass Transport Controlled Peak Current: $I_p = (2.69X10^5)n^{3/2}AD^{1/2}Cv^{1/2}$
  - n = number of electrons transferred (for ferrocyanide, n = 1)
  - A = electrode area (cm^2)
  - D = diffusion coefficient (measure reactant transport, for ferrocyanide D ~ 7 x 10^{-6} cm^2/sec at room temperature)
  - C = concentration of reactant (mol/cm^3)
  - v = scan rate (V/s) determines the time scale of the experiment and can be varied to obtain reaction rate information. Note: for an electrochemically reversible reaction, the peak current is proportional to $v^{1/2}$.
- Formal Reduction Potential: $E^{O'} = (E_{pa} + E_{pc})/2$
  - E_{pa} = Anodic Peak potential
  - E_{pc} = Cathodic Peak potential
- Difference in Peak Potential: $\Delta E_p = E_{pa} - E_{pc}$
  - The ΔE_p provides a means to determine the electron transfer rate, with a value of 0.059 V/n for a reversible electron transfer.

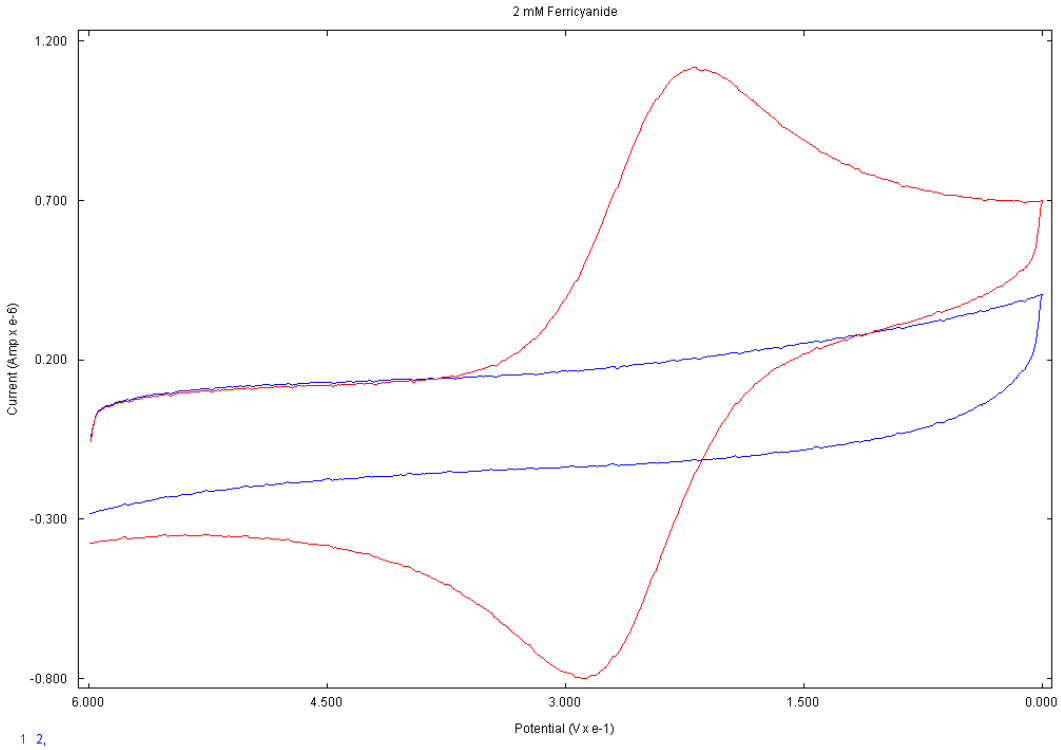
In general, the measured current response for electrochemical methods, cyclic voltammetry included, is proportional to concentration. However, cyclic voltammetry is usually not used for quantitation because of the relatively large charging current (I_c) that occurs as the result of the double layer capacitance (C_{dl}) at the working electrode. Mathematically, $I_c = vC_{dl}$. In regard to the glucose test strips, CV cannot be used for quantitation because, for a reversible couple, the signal is proportional to the sum of both species. One must be able to determine the concentration of the species in one of the oxidation states while in the presence of the other. Thus, the next section describes a better electrochemical technique for use in this device.

Laboratory experiments could include cyclic voltammetry of other compounds to determine if they would be suitable mediators or to determine if other compounds present in the blood (e.g., ascorbic acid, acetaminophen, etc.) would interfere at their required electrochemical potentials.

Cyclic voltammograms of 2 mM ferricyanide in 1 M KNO_3 at scan rates of 20, 50, 100, 200, 500, and 1000 mV/s.



Cyclic voltammograms of 0.2 mM ferricyanide in 1 M KNO_3 superimposed on a blank solution.



Chronoamperometry

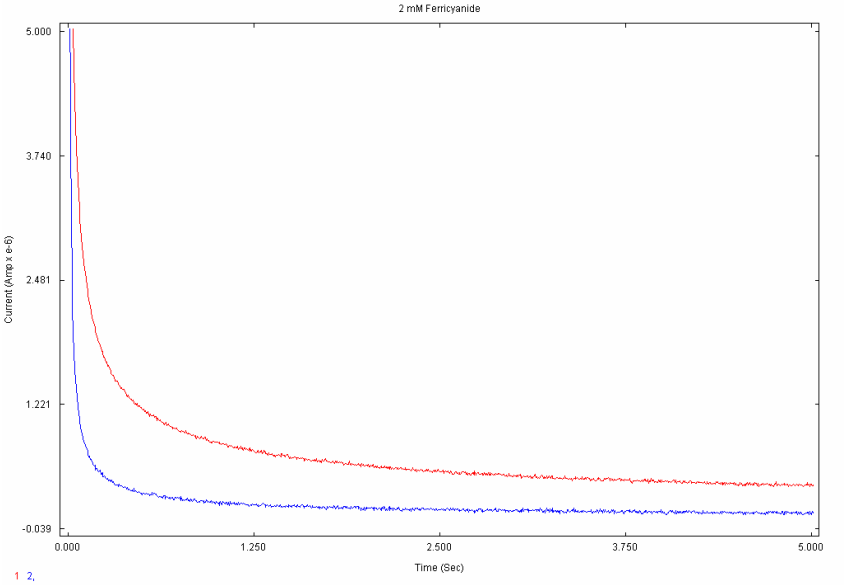
Chronoamperometry is an extremely simple and useful electrochemical method that is often overlooked in undergraduate education. This technique applies an electrode potential such that every analyte molecule or ion of interest reaching the electrode surface immediately undergoes an electron transfer reaction. The current (rate of electron transfer) is thus limited by how rapidly the reactants arrive – a diffusion-controlled current, since diffusion is the primary transport mechanism. Because the reactant is being converted (consumed) at the electrode surface, its average concentration will be decreasing in the vicinity of the electrode and the current will decay with a $t^{-1/2}$ relationship and is described the Cottrell equation:

$$i = \frac{nFAD^{1/2}C}{\pi^{1/2}t^{1/2}}$$

For quantitation of a simple and well defined system, pulse techniques, such as chronoamperometry, offer several advantages over cyclic voltammetry.

1. Better discrimination against charging current: $I_c = (\Delta E/R_s)e^{-t/Rs\ Cdl}$
   - ΔE = Potential Step amplitude
   - R_s = Solution Resistance
2. Signal is dependent upon only redox state of interest
3. It is a simpler waveform and can employ simpler data analysis, thus it is easier to implement in a device.

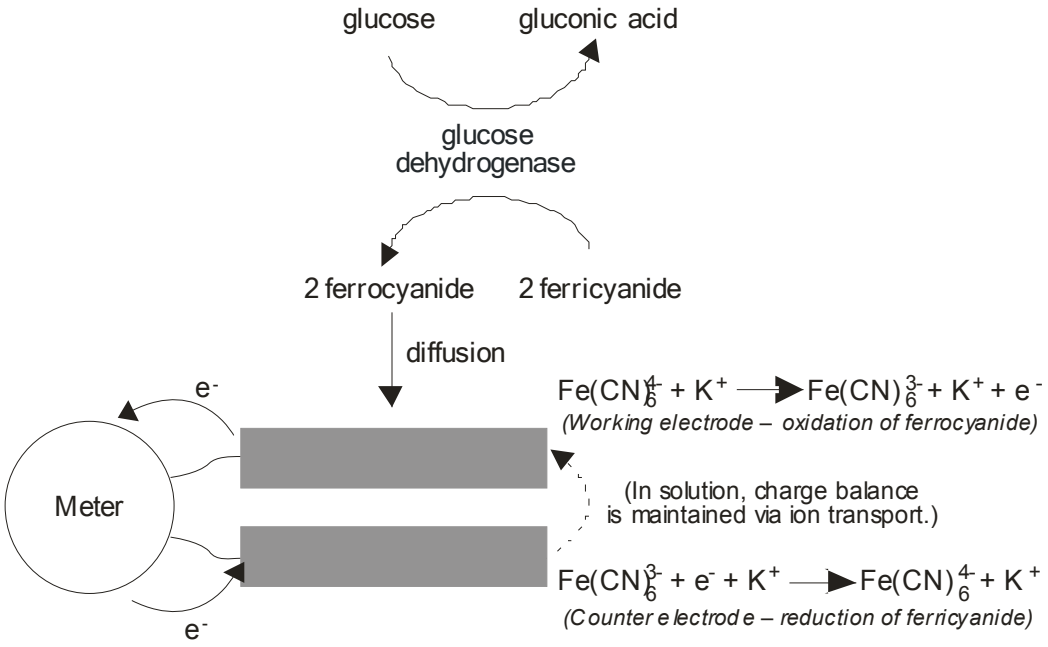
Chronoampermetry of 0.2 mM ferricyanide in 1 M KNO_3 and blank solution superimposed.



Three-electrode vs Two-electrode Amperometry

Normally, one prefers to use a three-electrode system for voltammetry/amperometric measurements because it offers good control of the interfacial potential (the driving force of the reaction) at the working electrode. However, the Accu-Chek device is engineered to get reliable results with a two electrode system. In the case of the Comfort Curve strips, this simplifies their manufacture and thus lowers the cost.

Chemical/Electrochemical Summary (Figure modified from Ref. 2)



In a two-electrode system, the working electrode is where the reaction of interest occurs and the counter electrode provides a counter reaction to maintain charge balance in the system. The potential at the working electrode interface is the applied potential relative to the interfacial potential of the counter electrode. In this device, the interfacial potential of the counter electrode is dependent upon the relative concentration of ferricyanide and ferrocyanide at its electrode surface and is given by the Nernst equation:

$$E = E^{o'} + (0.059/n)(\log([Ox]/[Red]))$$

Where Ox is ferricyanide and Red is ferrocyanide. The ferricyanide solution is such that initially only about 0.05% is ferrocyanide. In the Comfort Curve strips, the ferricyanide concentration is high enough that no more than 10% of the ferricyanide will be converted to ferrocyanide by the enzyme reaction with glucose. This will minimize

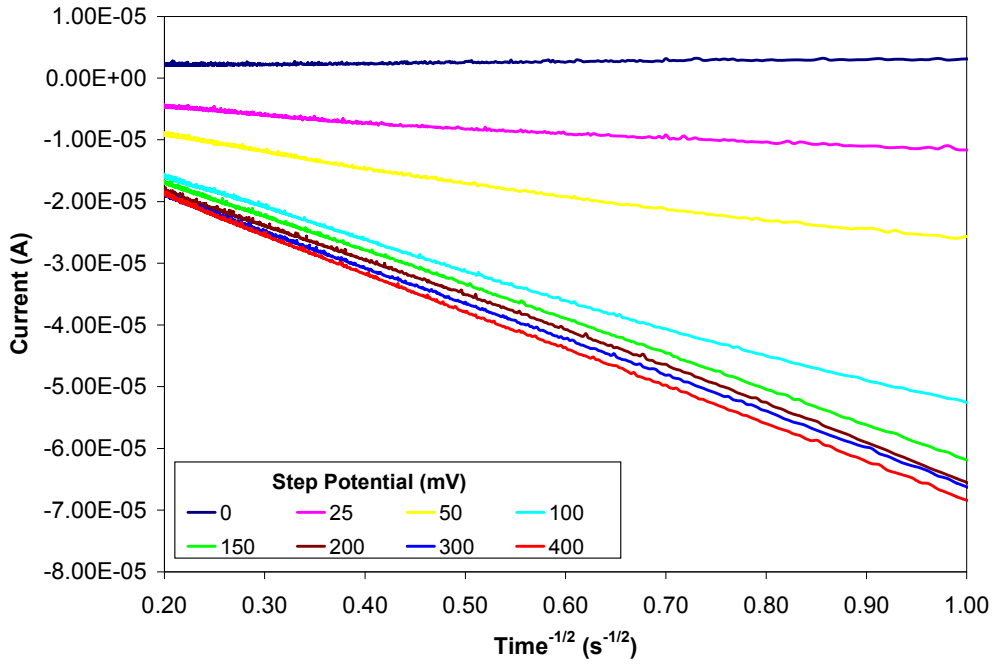
the change in the interfacial potential of the counter electrode during the electrolysis. Thus, in the worst case, the counter electrode potential will be 59 mV positive of the $E^{o'}$, and with a step potential of 150 mV, the interfacial potential of the working electrode will be 209 mV vs $E^{o'}$. The Nernst equation predicts greater than 99.9% conversion at the electrode with this voltage. Theoretically, even if the ferricyanide concentration were such that it was only 2 times the amount of generated ferrocyanide, there would still be 99.7% conversion. Since ferricyanide has very high solubility and is inexpensive, increasing its concentration to "over engineer" the device has no detrimental affects.

Chronoamperometric Experiments Using Glucose Test Strips

In order to explore the function of the glucose test strips, chronoamperometric data was collected using the custom trigger box controlled by the Epsilon potentiostat.

The Effect of Potential Step Amplitude:
Cottrell plots were created for potential steps that ranged in size from 0 to 400 mV using a glucose solution with a concentration of 200 mg/dL. The glucose standard was allowed to react on the test strip for eight seconds before the potential step was initiated.

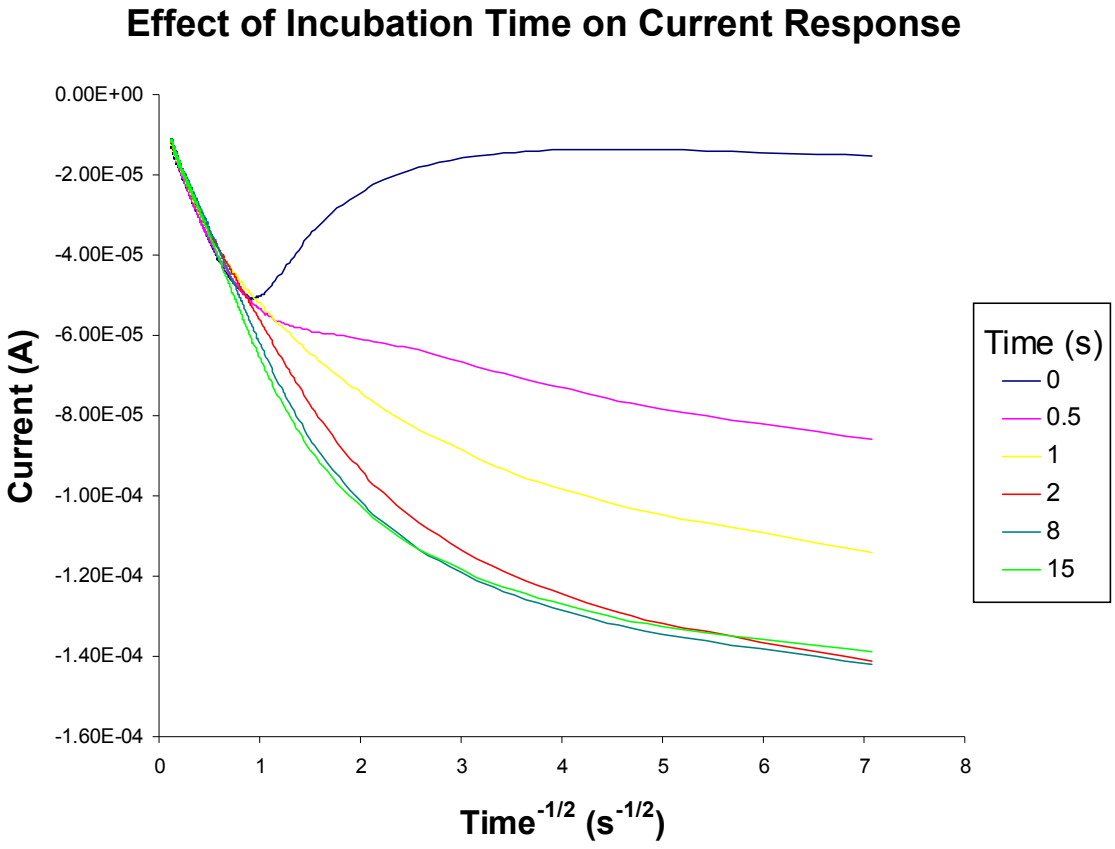
Effect of Potential Step Amplitude on Current Response



The figure above demonstrates that a potential step of at least 150 mV is required for the current to be limited by mass transport.From the standpoint of selectivity in this measurement, the potential step should be kept to the minimum so that other oxidizable species in the sample do not interfere. All subsequent chronoamperometry experiments were performed with a 150mV step potential. Note: only the data in the mass transport controlled portion of the analysis is shown in this figure.

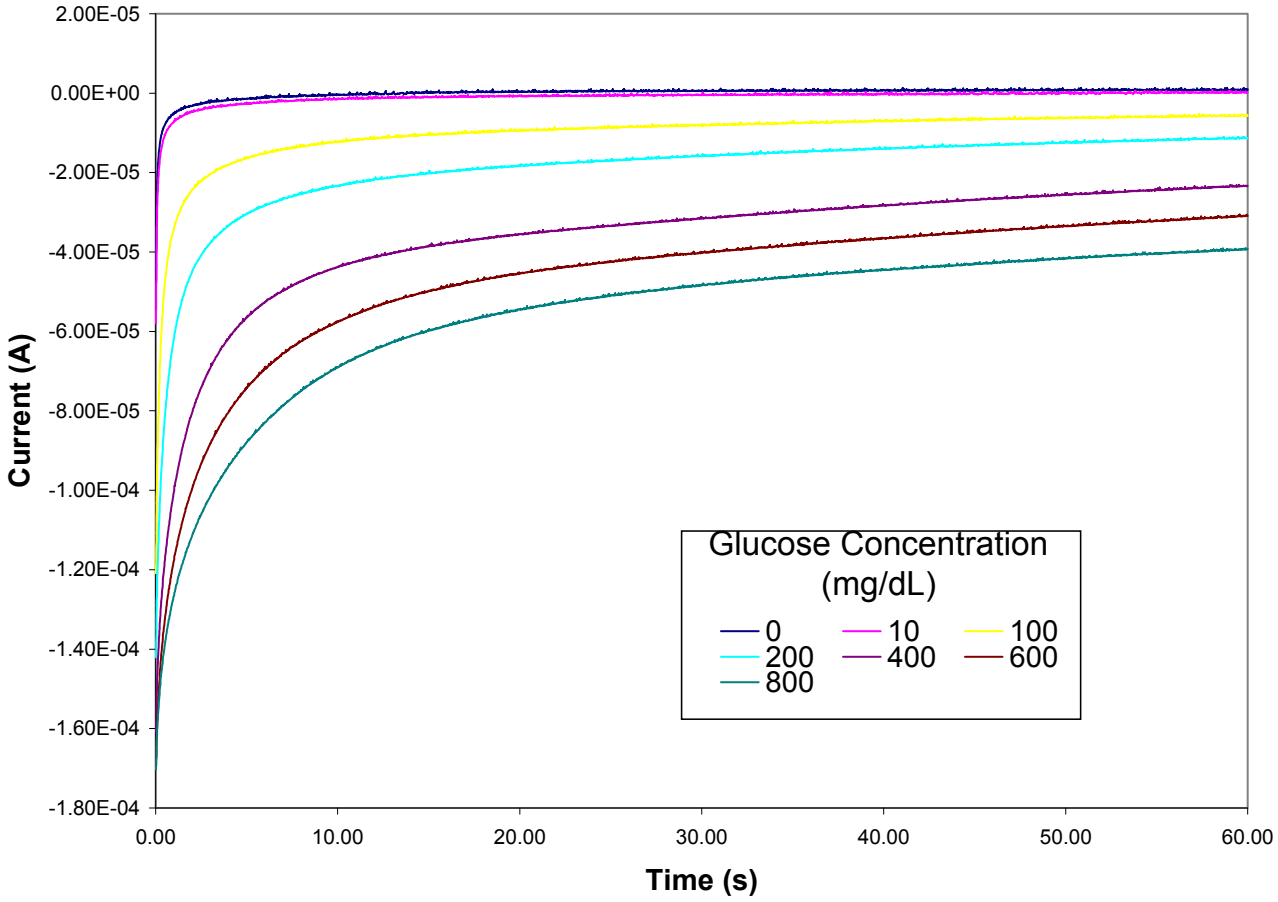
Incubation Time Study:

In order to assess the proper incubation time for glucose on the enzymatic test strips, the incubation time for a solution of 200 mg/dL glucose was studied. Cottrell plots were generated for incubation times between 0 to 15 seconds. The graph below shows the results of this study. From this graph, an incubation time of eight seconds was chosen for all subsequent experiments. The strange shape of the curve for an incubation time of zero is a result of the fact that incubation begins as soon as the glucose wets the test strip. The resulting current starts out small, because initially the reaction has not had time to proceed. However, as time increases during the chronoamperometric data collection, the ferrocyanide generated by the enzymatic reaction is detected.



Concentration Range Study:
In order to determine the useful range of concentrations that can be measured with the test strip chemistry, chronoamperometric experiments were performed using glucose concentrations that ranged from 10 to 800 mg/dL.

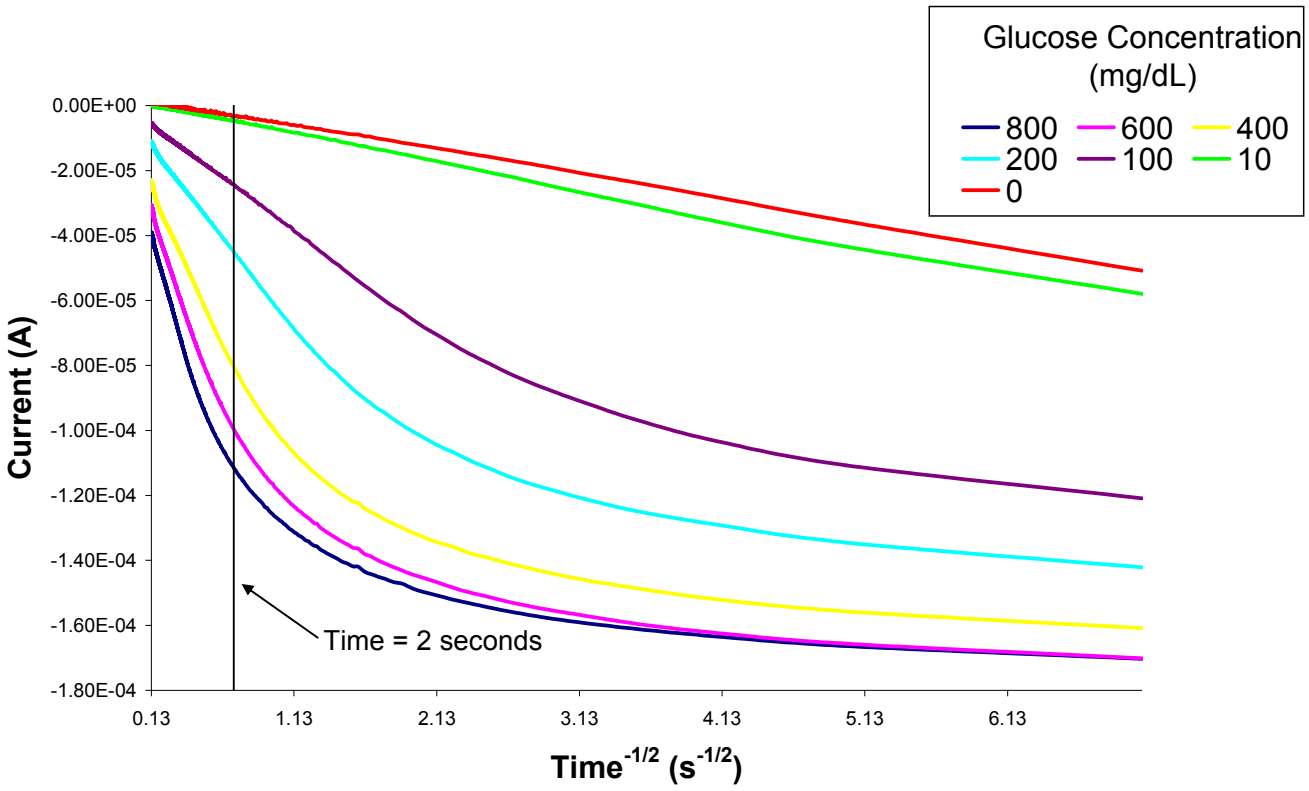
Current Profile as a Function of Time and Glucose Concentration



Cottrell plots were generated from this data. The figure shown below demonstrates that two seconds ($t^{-1/2} = 0.707$) after the potential step the current appears to become mass transport limited. Therefore, when sampling current for calibration and measurement, the integration window should be in this region of mass transport control.

The data also demonstrate that the concentration range for this analysis is limited to the 0 to 400 mg/dL range due to a lack of linearity in the Cotrell Plots above 400 mg/dL. The 600 and 800 mg/dL concentrations are less linear in this region of the plot, suggesting that the enzymatic reaction rate is limiting the current at these larger concentrations.

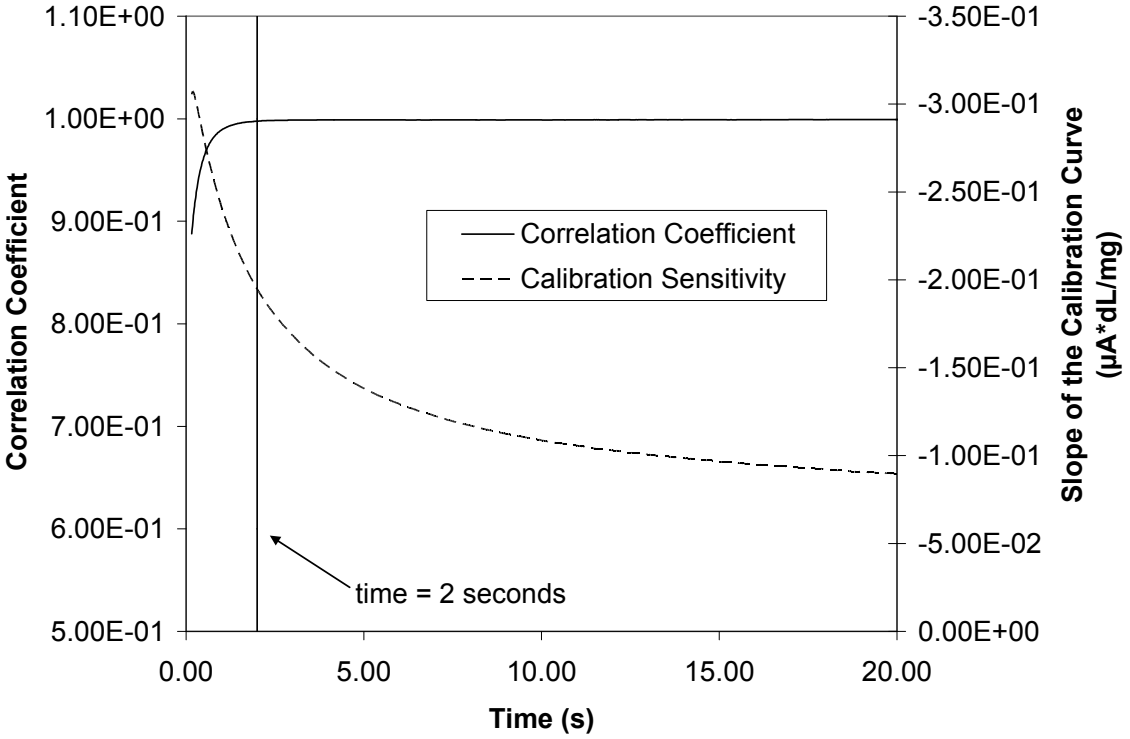
Cottrell Plots as a Function of Glucose Concentration



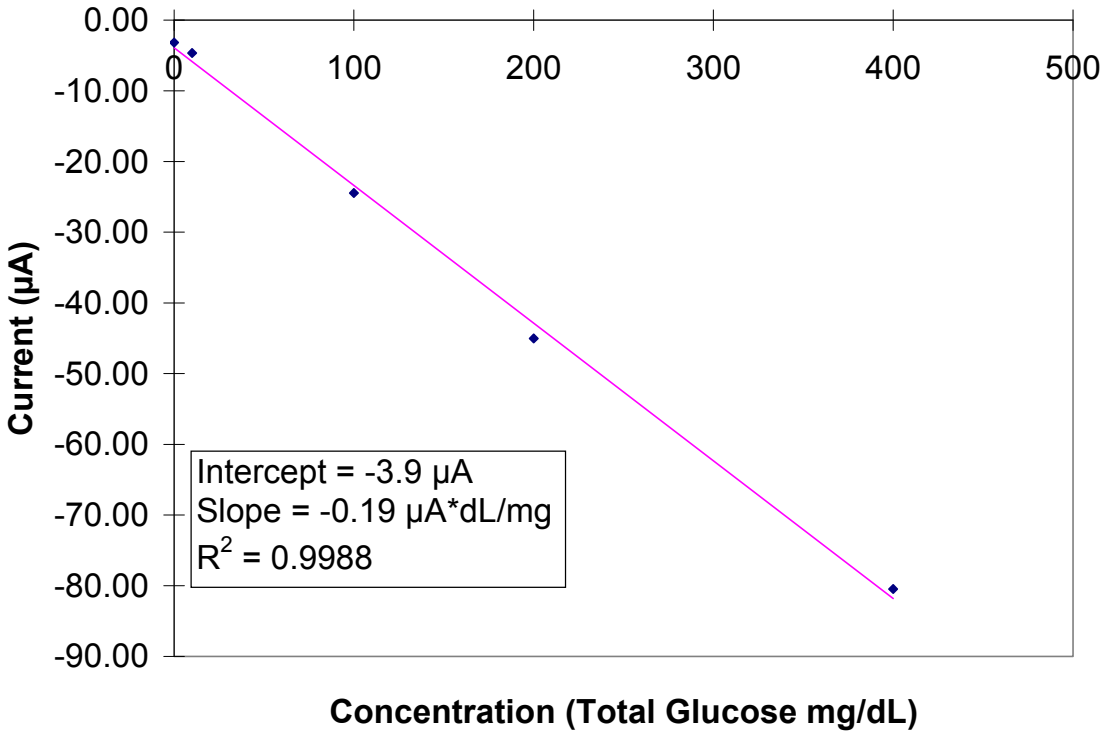
Optimization of Sampling Window:
In order to establish the best current sampling window for the analysis, the data was signal averaged (15 point boxcar smooth = 0.3 seconds of data), and the slope, intercept, and correlation coefficient of each average set of concentration - response data (~3000 sets) was calculated.

The figure below shows the slope of this calibration data (the calibration sensitivity) and the correlation coefficient plotted as a function of the average current sampling time. In order to maximize calibration sensitivity and the linearity of the data, a sampling time of approximately two seconds should be used. This correlates well with the Cottrell plot which demonstrating mass transport limited current after this time.

Calibration Sensitivity and Linearity as a Function of CA Sampling Time

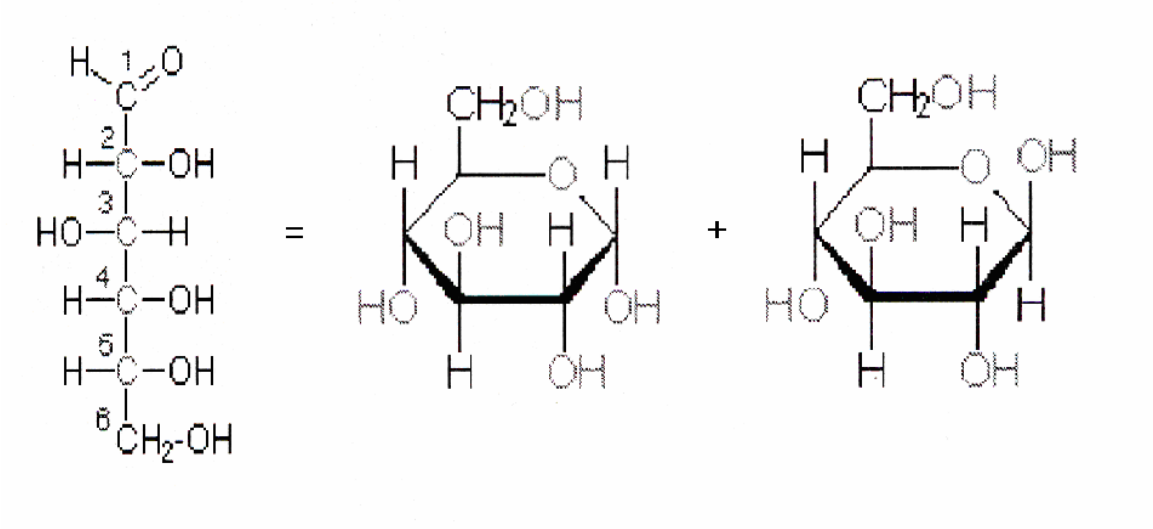


Optimized Calibration Data



Glucose Structure, Isomerization, and Mutarotation

The sugar D-glucose and L-glucose can exist as two enantiomers (mirror-image isomers), but in living organisms only the D-isomer is found. In solution at pH 7, only 0.0026% of the glucose exists in the acyclic form and the vast majority is D-glucopyranose. D-glucopyranose consists of two anomers (α and β) that will come to equilibrium with 36% α and 64% β at room temperature (25°C).



D-(+)Glucose Solid = 36% α-D-Glucose + 64% β-D-Glucose Equilibrated Solution

The conversion between the two anomers is relatively slow and follows a simple first order reversible process. This process has been well documented in the literature and proceeds via the following integrated rate equation:

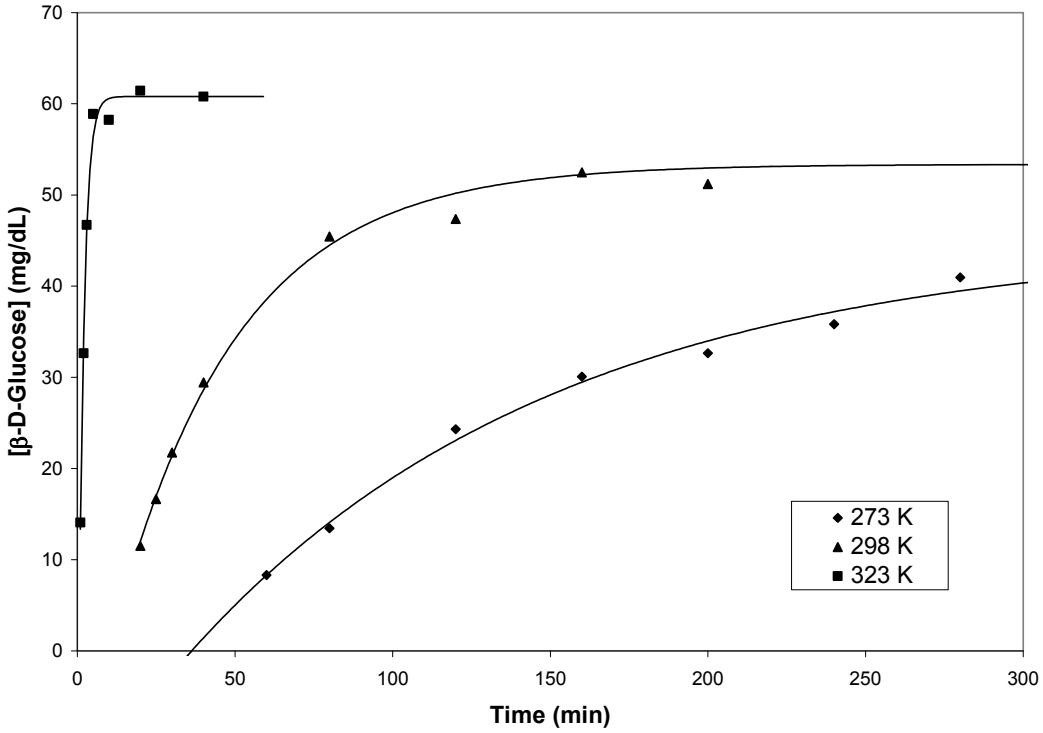
$$-\ln\left(\frac{\beta_t - \beta_{eq}}{\beta_0 - \beta_{eq}}\right) = kt$$

where β_0, β_t, and β_{eq} represents the concentration of the β anomer at time zero, at time t, and at equilibrium, respectively, and the mutarotation coefficient (k) is the sum of the forward and reverse rate constants in this conversion.

Glucose Mutarotation Study Using an Accu-Chek Glucose Monitor and Test Strips

Glucose Mutarotation Kinetics:
Since the enzyme (glucose dehydrogenase) on the test strips used by these glucose meters only reacts with the β anomer of glucose, and the conversion from a primarily α-D-glucose solution to the equilibrium mixture is relatively slow, these inexpensive meters can be used to follow the kinetics of the mutarotation process.

Effect of Temperature on the Rate of α-D-Glucose Mutarotation



The data was fit to the exponential form of the integrated rate law shown below using the Solver function in Microsoft Excel.

$$\beta_t = \beta_{eq} + (\beta_0 - \beta_{eq})e^{-kt+z}$$

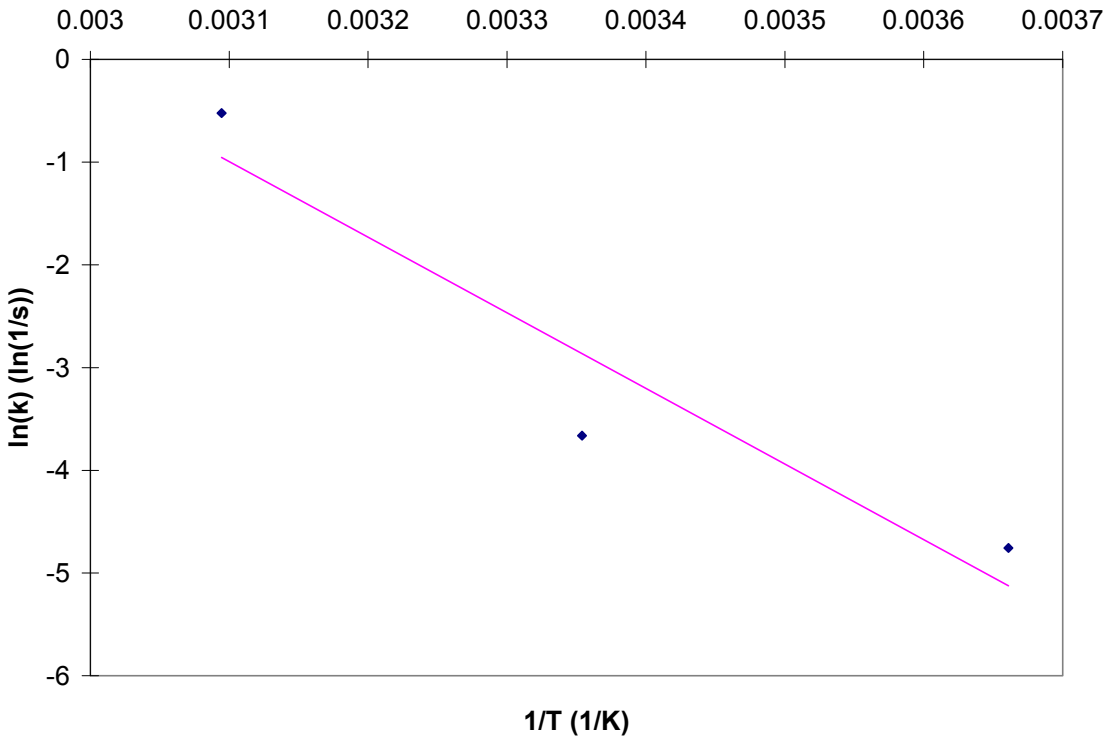
It was necessary to add a time correction factor (z) to the equation in order to compensate for the fact that the α-D-glucose begins to mutarotate as soon as the solid is added to the PBS diluent, but measurements cannot be made until all of the solid is dissolved, or the concentration will not be accurately known. Since the β form

represents ~ 5% of the total solid form of D-glucose used to make the solutions, the value for β_0 was taken as 5 mg/dL for the fit optimizations.

The mutarotation constants and the other fit parameters are shown in the table below and are consistent with literature values.

Temperature (K)	k (s^{-1})	β_{eq} (mg/dL)	z (s)
273	$1.4x10^{-4}$	45	26
298	$4.3x10^{-4}$	53	22
323	$9.9x10^{-3}$	61	26

Arrhenius Plot for the Mutarotation of α-D-Glucose



While the mutarotation for glucose is strictly non-Arrhenius in nature, the Arrhenius treatment over this small range of temperatures provides a reasonable estimate of the activation energy for this process: 61 KJ/mol.

Hopefully, this simple experiment demonstrates the utility of glucose meters for educational laboratory use based on glucose dehydrogenase chemistry. This experiment could be further expanded by studying the effect that acid or base catalysis would have on the rate and activation energy of the mutarotation.

Conclusion

This work demonstrates that the use and study of these inexpensive and “simple” glucose meters can be a powerful tool in the understanding of many chemical, electrochemical, and analytical principles.

References

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