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Accu-Chek® Advantage: Electrochemistry for Diabetes Management

This article discusses the electrochemical principles behind the Accu-Chek glucose meter.

Diabetes

When the body no longer produces insulin, or has a reduced sensitivity to it, diabetes is the result. In Indiana, over 338,000 people (7.4% of the population) have been diagnosed, and another 182,000 are thought to have unidentified diabetes. That is, roughly one in twelve Hoosiers has diabetes. The chronic elevated blood sugar which results from this condition causes long-term damage to numerous tissues and organs.

The medical and financial costs to our state, summarized in a recent State Department of Health report (1), are staggering:

- Diabetes has caused nearly 40% of end-stage renal disease in Indiana.
- Diabetes has blinded 22,000 Hoosiers over age 40 and causes over 1,700 non-traumatic amputations each year in Indiana.
- Diabetes roughly doubles a patient's risk of death and contributes to over 5,000 deaths in Indiana each year.
- Diabetes cost Indiana almost $4.5 billion in 2002.

Much of this burden may be reduced or eliminated by early detection and improved self-care. The Diabetes Control and Complications Trial (DCCT) (2), a 10-year nationwide study of 1,441 diabetics, conclusively demonstrated that improved control of blood sugar delayed or prevented many of these complications at least 50% better than with poorly-controlled subjects. Subsequent studies have corroborated this conclusion. This good control is enabled by frequent, consistent and accurate self-testing of blood glucose to optimize therapy.

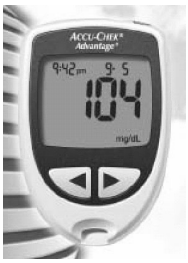
Accu-Chek Advantage

There are several essential elements in a medical device designed for patient self-monitoring. Because these systems are medical devices, used to make medical decisions or avoid potentially life-threatening incidents every day, they must be of very high quality, and the information displayed must be accurate. The sensors must be easily manipulated by sight-impaired users, and the system must be very user-friendly to encourage more frequent testing for better control. In the hospital or physician's office there are additional quality requirements, and the possibility of multiple sample types (e.g., capillary, arterial, venous and neonatal blood).

Accu-Chek Advantage meets these needs by providing accurate and dependable glucose measurement using a tiny drop of blood, for all blood sample types (capillary, arterial, venous and neonatal) over a wide range of hematocrit. It delivers this performance in a matter of seconds at temperatures from 57 to 104 °F, and at altitudes to above 10,000 feet. More patients and hospitals worldwide rely on Accu-Chek Advantage than any other glucose measurement system.

How Does the Hand-held Electrochemical System Work?

Accu-Chek Advantage technology uses two components:

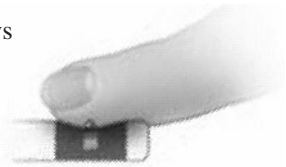


Electronic Meter
(measurement, storage and communication device)

- Applies potential differences in a programmed sequence to the sensor
- Collects biamperometric current data
- Records and displays results

Disposable Biosensor
(sample measurement reaction site)

- Collects the blood sample
- Sample undergoes an enzymatic chemical reaction followed by:
- An electrochemical reaction



The patient simply inserts a biosensor into the meter and applies a small drop of blood.

Electrochemistry Basic Principles

Let's begin with basic electrical definitions and concepts and assemble them into a *simplified* description of the measurement method.

- Current: The *rate* of electron movement or charge transfer. This is analogous to water flow in a pipe (e.g., gallons per hour for water, *amperes* for current).
- Potential: If current is *flow rate*, potential is *pressure* (e.g., pounds per square inch for water, *volts* for potential). A potential difference represents a difference in the *average electron energy* at two points.

Just as water will flow if a pressure difference exists between two points in a pipe, *current* will flow if a *potential difference* exists between two points in a conductor. Similarly, as flow increases with pressure in a pipe, current increases in a conductor as the potential difference is increased.

- **Electrochemistry** We'll limit this discussion to particular chemical and electrical interactions between two phases: a chemically inert, electrical conductor (*electrode*) and a liquid ionic conductor (*electrolyte* or *sample*) where chemical reactions occur.
- **Electrode** Although electron energy in ions or molecules is determined by chemical structure, the energy of electrons in an electrode may be easily changed by adjusting the electrode potential. There are two common methods for controlling *electrode potential*:
  1. Electronically using a meter or *potentiostat*.
  2. Chemically. If an electrode can match its electron energy with ions in solution, those ions may define the electrode potential. The *Nernst equation* defines this relationship.

Electron Transfer Reactions

Consider the *reduction* (electron addition) of potassium ferricyanide (M_{ox}) to potassium ferrocyanide (M_{red}). This is a simple *electron transfer reaction*:

$$Fe(CN)_6^{3-} + e^- \rightarrow Fe(CN)_6^{4-}$$

(shorthand) $$M_{ox} + e^- \rightarrow M_{red}$$

Since M_{ox} and M_{red} each have different electron energies, we use the Nernst equation to define the solution potential (the average energy of electrons in the solution):

$$E = E^{0'} + \frac{0.059}{n} \log \frac{[M_{ox}]}{[M_{red}]}$$

E = solution potential
$E^{0'}$ = formal potential (potential under standardized conditions, $[M_{ox}] = [M_{red}]$)
$[M_{ox}]$ = concentration of M_{ox};
$[M_{red}]$ = concentration of M_{red}
n = number of electrons exchanged in redox reaction (in this case, $n = 1$)
$\log \frac{[M_{ox}]}{[M_{red}]}$ = base 10 logarithm of the concentration ratio

Enzyme Reactions

Enzymes are protein catalysts which accelerate specific chemical reactions. In the plumbing metaphor, enzymes increase pipe diameter by removing a blockage; instead of water trickling past the blockage, it may now flow freely.

Enzymes are often highly specific, and this specificity enables organisms to manage a single type of molecule among tens of thousands within a cell or organ. Enzymes offer powerful advantages for biological analysis.

- Blood is a complex fluid and glucose levels vary widely over time in a single patient.
- Many factors besides glucose vary in blood from healthy patients (*e.g.*, hematocrit, oxygen levels, metabolic byproducts, *etc.*).
- Patients with diabetes may have a wide range of other medical problems creating even greater variation in their blood.
- Enzymes permit chemical reactions to occur at much faster rates without requiring major modifications to the biological sample (*e.g.*, increased temperature or pressure, dramatic pH changes, addition of highly reactive chemicals, *etc.*).

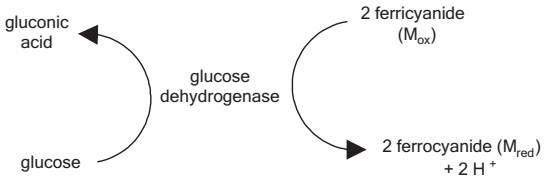
Unfortunately, enzymes rarely exchange electrons directly with electrodes. The same is true for many other biological molecules, such as glucose. An electrochemical measurement often requires a substance to facilitate (or mediate) this transfer, and such reagents are termed *mediators*.

Mediators

A well-known mediator is potassium ferricyanide. The reaction process is:

a) Glucose first reacts with the enzyme g*lucose dehyrogenase*. Glucose is oxidized to gluconic acid and the enzyme is temporarily reduced by two electrons transferred from glucose to the enzyme.

b) The reduced enzyme next reacts with M_{ox}, transferring a single electron to each of two mediator ions. The enzyme is returned to its original state, and the two M_{ox} are reduced to M_{red}:



c) Ferricyanide and ferrocyanide are capable of rapidly transferring electrons with an electrode. The electrons may thus be transferred between glucose and the electrode *via* enzyme and mediator.

Now we have a chemical mechanism for transferring electrons from glucose to the electrode. The biosensor reagent is actually more complex, containing a number of other active ingredients (*e.g.*, stabilizers, processing aids, *etc.*), but we'll view it as composed of simply the enzyme and mediator. How is the electrochemical measurement performed?

Amperometry

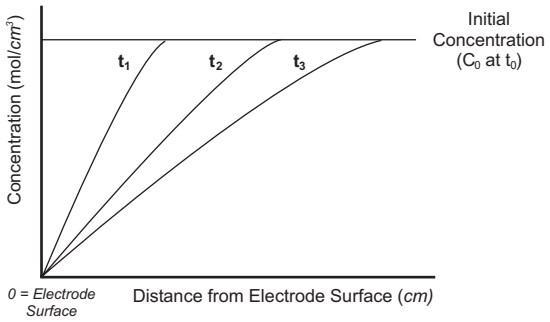
An extremely useful method is *amperometry*. This technique sets the electrode potential at a level where every molecule or ion reaching the electrode surface *immediately* undergoes an electron transfer reaction. The current (rate of electron transfer) is thus limited by how rapidly the reactants arrive – a *diffusion-controlled* current, since diffusion is the primary transport mechanism.

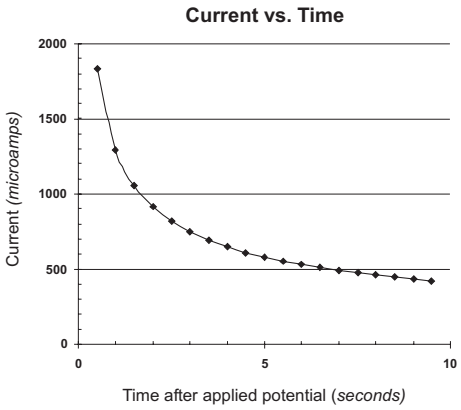
Because reactant is being converted (consumed) at the electrode surface, its average concentration will be decreasing in the vicinity of the electrode, so current should *decrease* with time. We won't go into detail, but the *Cottrell equation* describes this behavior.

Cottrell equation: $I = (n F A D^{1/2} C) / (\pi t)^{1/2}$

I = current, in *amps*
n = number of electrons transferred in the reaction (for ferrocyanide, n = 1)
F = Faraday constant (the quantity of charge carried by 1 mole of electrons = 96,485 *Coulombs/mol*)
A = electrode area (cm^2)
D = diffusion coefficient (a measure of how rapidly reactant is transported; for ferrocyanide the diffusion coefficient is ~ 7 x 10^{-6} cm^2/sec at room temperature)
C = concentration of reactant (mol/cm^3), the model assumes uniform concentration before potential difference applied
t = time (*seconds*)

It's easier to visualize this effect if we examine diagrams of the concentration and current changes with time.





- Initially (t_0), the reactant has a uniform concentration C_0.
- When the potential difference is applied, reactant concentration at the electrode surface goes to zero. Over time (t_1 t_2, t_3), consumption of the reactant decreases its concentration, creating a *diffusion layer*.
- The result is a current decreasing with the square root of time.

Putting the Pieces Together

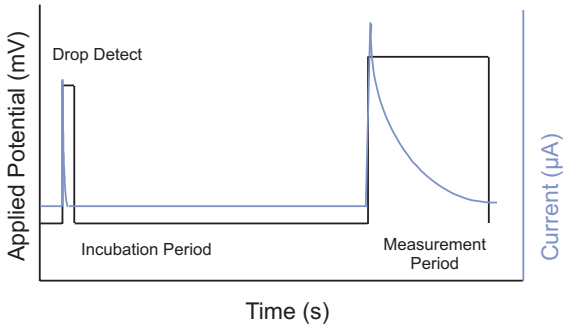
Reviewing The Measurement System Components

- An enzyme (g*lucose dehydrogenase*) rapidly transfers electrons from glucose to a mediator, which may transfer them to an electrode.
- An electrode potential is imposed on the system.
- In any circuit, current must be identical at all points. If we put two electrodes in a circuit, electron flow rate *out* of one electrode (*working electrode*) has to equal the electron flow rate *into* the other (*counter electrode*).
- If we *oxidize* ferrocyanide to measure glucose at the working electrode, we must *reduce* an equal quantity of something else at the counter electrode. Since we already have ferricyanide in the reagent, this is our best choice.
- In *biamperometry*, the two electrodes are identical. (Accu-Chek Advantage uses *palladium* electrodes, a noble metal similar to platinum.)

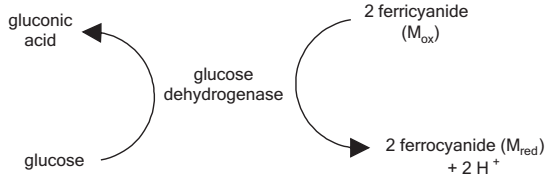
The Measurement Sequence Itself Consists of Five Time Segments

1. When biosensor is inserted, the meter automatically turns on and performs a series of tests.
2. After all tests are successfully completed, the meter applies a potential difference to detect sample (*Drop Detect*).
3. Following sample application (*Drop Detect*), electrode potential difference is removed and enzymatic reaction is permitted to proceed (*Incubation Period*).
4. After *Incubation Period,* the meter applies a potential difference and measures current (*Measurement Period*).
5. Current data are analyzed, the result is recorded and displayed.

Measurement Sequence Diagram



- *Incubation period:* After Drop Detect, glucose dehydrogenase catalyzes a selective electron-transfer reaction between glucose in the sample and M_{ox} (potassium ferricyanide) in the reagent layer:



Each molecule of glucose reduces two molecules of ferricyanide, creating two molecules of ferrocyanide. ***The final ferrocyanide concentration is directly correlated to the sample glucose concentration.***

- *Measurement period:* During the measurement period, the meter applies a potential difference between the working and counter electrodes. The counter electrode potential is defined by the ratio of ferricyanide and ferrocyanide at the electrode surface:

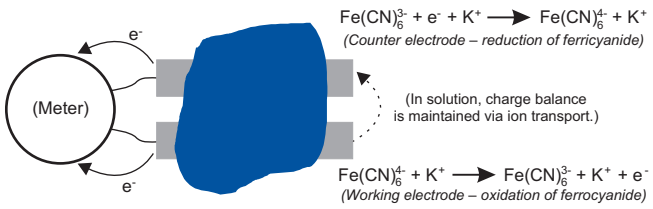
$$E = E^{0'} + \frac{0.059}{n} \log \frac{[M_{ox}]}{[M_{red}]}$$

Since the amount of ferrocyanide is small relative to the amount of ferricyanide, the concentration ratio (and hence the

counter electrode potential) is effectively constant.

This applied potential difference is sufficient to provide a diffusion-limited current at the working electrode, so the ferrocyanide concentration may be determined by biamperometry.

The meter measures working electrode current, which is linked to ferrocyanide concentration by the Cottrell equation. Because the ferrocyanide concentration is coupled to glucose concentration, the current measurement permits calculation of blood glucose.



Conclusion

- Accu-Chek Advantage illustrates many fundamental chemical and electrochemical reaction concepts. Although we've only skimmed the science and engineering considerations, the system illustrates elements of enzymatic and charge transfer reactions, potentiometry, amperometry and diffusion.
- By carefully optimizing a design based on fundamental principles, this handheld device provides a robust electrochemical system for blood glucose measurement.
- Because of this, the instrument is a medical management tool improving the lives of millions of diabetes patients across the world.
- Accu-Chek Advantage delivers remarkable performance with a wide range of sample types and conditions, performing tens of millions of measurements each year:
  - Glucose measurement range: 10-600 mg/dL
  - Temperature: 10 - 40°C
  - Hematocrit: 20-65% for glucose < 200 mg/dL; 20 - 55% for glucose > 200 mg/dL
  - Humidity: up to 85% RH
  - Altitude: up to 10,150 feet
  - Samples: venous, arterial, finger stick capillary, neonate
  - Typical within-lot precision: SD/CV ~ 2 mg/dL or 2%
- As electronic technology evolves, so do electrochemical measurement systems. The latest Accu-Chek Aviva (currently pending U.S. Food and Drug Administration 510(k) clearance) will be available soon.

References

1. *The Burden of Diabetes in Indiana, Ind. State Dept. of Health Diabetes Prevention and Control Prog., April 5, 2004.*
2. *The Diabetes Control and Complications Trial Research Group, New England J. Med. 329, 977 (1993).*