Welcome to Lecture 9

Announcements

Exam date: Friday, 19 December (midday)

This is NOT 100% certain yet; the registrar should confirm some time next week and it will be posted on our website

Quiz results: Now posted but there was one mistake

to Relish Shah

My apology

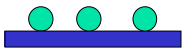
On the spreadsheet I posted Relish score appeared as XXX due to cheating on quiz

It was not Relish but another student with whom I spoke prior to posting the results – I just marked the wrong raw

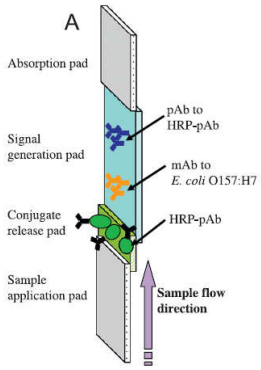
Relish score has been fully restored and I regret any distress it might have caused

Last time

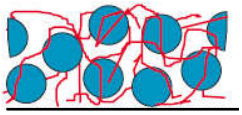
Physical methods:



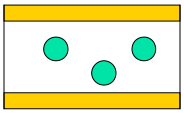
adsorption



Immobilization



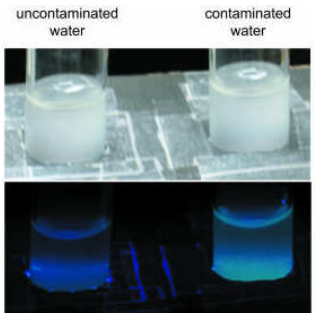
gel entrapment



membrane entrapment

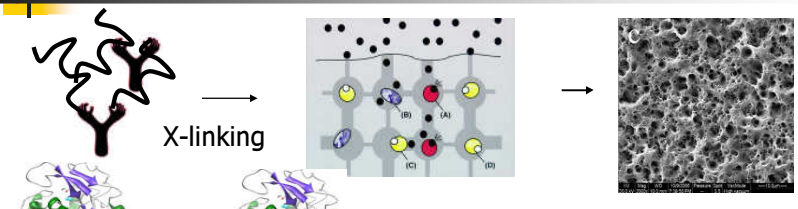
Not too different ☺

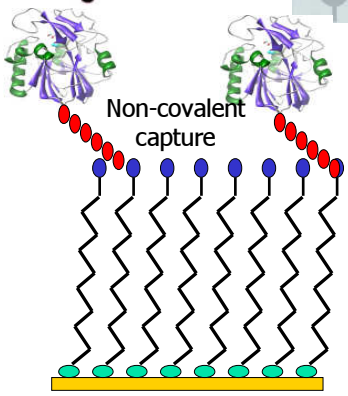
Gelatin dessert



Environmental biosensor ☺

Also last time





SAMs: self-assembled monolayers

Various surfaces

COOH → Covalent attachment

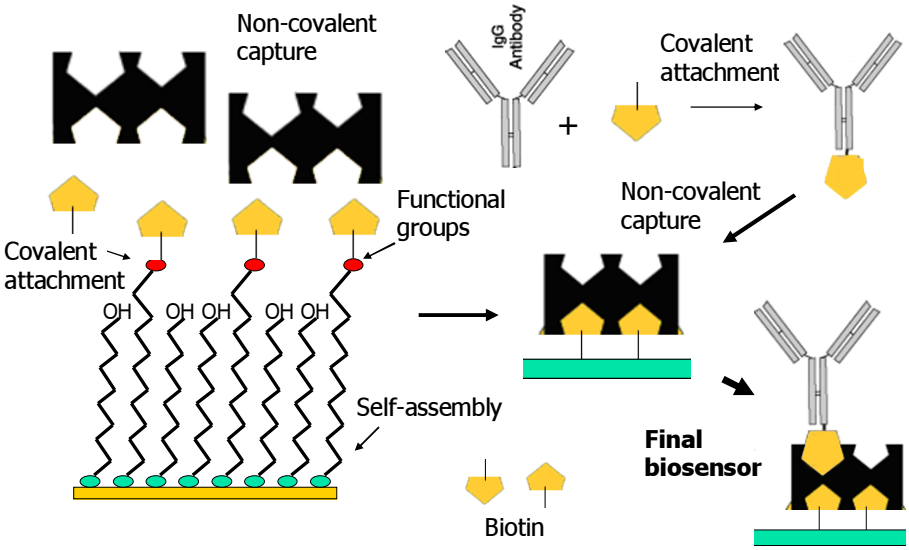
H_2N-R

HS-R

HO-R

Orientation on the surface

Combination of methods



Plan for today:

Glucose biosensors

but also cholesterol, alcohol and $$$

Diabetes

Diabetes is a disease in which the body can no longer regulate the level of glucose in blood due to the failure to produce enough insulin

It is one of the leading causes of death by disease. If untreated or improperly managed, it results in major complications:

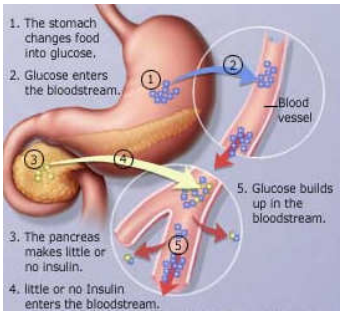
- 2-4 fold increase in incident of heart disease
- Over 40% all new cases of serious kidney disease
- Over 60% of all non-traumatic limb amputations
- Major cause of adult blindness, erectile dysfunction in man, etc, etc, etc

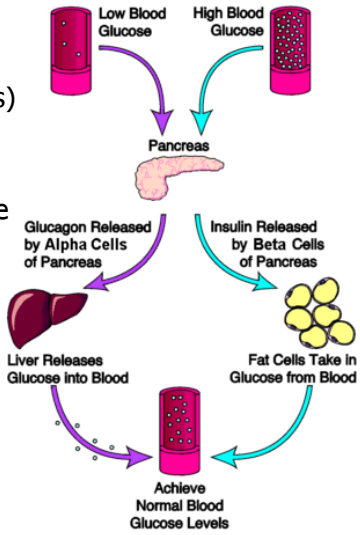
There are estimated **20 million children and adults** in the US alone, (7% of the population) who have diabetes; 2/3 diagnosed and the other ~**6 million being unaware of the problem**

Currently there are 170 mln diagnosed diabetics worldwide; this number is expected to reach 300 mln (!) by 2025

Glucose regulation

- Normally the human body maintains blood glucose in a very narrow range
- Insulin and glucagon (produced by pancreas) are the hormones responsible
- People with diabetes mellitus are either unable to produce insulin (type 1), or unable to produce it in sufficient amounts (type 2)





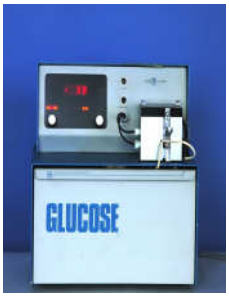
The early "sensors"

- Sugars are not normally present in the urine of healthy people
- Uroscopy – a gross examination of urine by color, consistency, smell and taste, was one of the earliest methods of diagnostics

XIX century: Moore's potash test - boiling urine with potash (K_2CO_3) led to the development of dark coloration in the presence of glucose

Unreliable, especially for the detection of small amounts of glucose, since most urine darkens on boiling with potash

Clinical analysis



This model of glucose analyzer dates back to 1975 and its role as the first whole blood analyzer has earned it a listing as one of the fifty most influential scientific instruments of the XXth century

Many early instruments utilized Hexokinase (HK)-based systems:

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{HK, Mg}^{2+}} \text{G-6-PO}_4 + \text{ADP}$$

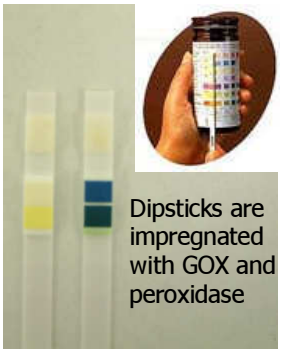
$$\text{G-6-PO}_4 + \text{NADP}^+ \xrightarrow{\text{G-6-PD}} \text{G-6-PD} + \text{6-P-gluconate} + \text{NADPH} + \text{H}^+$$

Signal: increase in absorbance of NADPH at 340nm

Modern clinical analysis

Modern analyzers use the same or similar detection principles but more sensitive, offer much higher throughput and INTEGRATION

Qualitatively: Modern equivalent of boiling urine with potash



Dipsticks are impregnated with GOX and peroxidase

E 170 module for MODULAR ANALYTICS, a Roche/Hitachi modular system offers a seamless integration of Pre-Analytics, Clinical Chemistry, Special Chemistry, Immunochemistry and Post-Analytics in a single, easy-to-use workstation

But diagnosis is not the only reason

Rational for Glu monitoring

Diabetes Control and Complications Trial

- The study spanned a period of 10 years (1983-1993) and involved 1441 type I diabetics
- Two groups: (1) standard regime insulin shots and glucose measurements and (2) frequent glucose monitoring and [often] more frequent insulin injections
- **Bottom line**: Over time group (2) had significantly fewer diabetes-related complications
- Computer simulations: People in group (2) will have an additional 5 years of life, 8 years of sight, 6 years free of kidney disease and 6 years free of amputations

Hence, regular monitoring is a critical part of effective health care system for diabetics

Self-monitoring

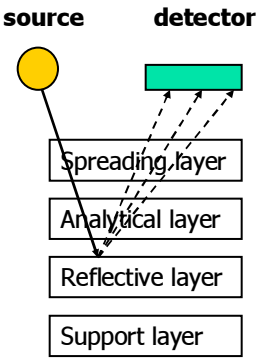
- Regular monitoring is critical for properly regulating the blood glucose level
- The consequences of poor glucose regulation are at best, long term damage to organs from hyperglycemia and, at worst coma or death caused by insufficient glucose reaching the brain (hypoglycemia)
- Portable/home glucose biosensors are most convenient (and inexpensive!) way to monitor blood glucose
- This is ~$6-7 bln pa business with dozens of different glucometers available in the market place
- Big pharmaceutical/diagnostics companies (Roche, Abbott, Bayer) hold the lion share of the market but the real innovators were the small guys…

First commercial glucometer

- The first commercial glucometer relied on glucose oxidation to generate a colored product
- In the literature it is referred to as "reflectance photometry," or "light reflectance" method
- Capillary blood glucose is oxidized on the surface of the glucose meter's strip leading to a color change
- The amount of color reflected from the strip is then measured photometrically
- The color change is proportional to the amount of glucose in the blood - the darker the test strip, the higher the blood glucose content

Ames reflectance meter

Principle of operation:

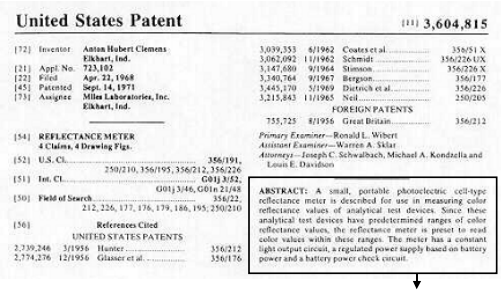


DIRECTIONS: Must be followed exactly

1. Freely apply a large drop of capillary or venous blood sufficient to cover entire reagent area on printed side of strip
2. Wait exactly 60 sec (the use stopwatch recommended)
3. Quickly wash off blood (in 1 or 2 sec) with a sharp stream of water, using a wash bottle and blot once gently on a lint-free paper towel
4. Read result within 1 or 2 seconds after washing. Hold the strip close to the Color Chart and interpolate, if necessary

Ames reflectance meter

Patented in 1971 but hit the market a little earlier



United States Patent [11] 3,604,815

[72] Inventor: Anton Hubert Clemens, Elkhart, Ind.
[21] Appl. No. 723,102
[22] Filed Apr. 22, 1968
[45] Patented Sept. 14, 1971
[73] Assignee Miles Laboratories, Inc., Elkhart, Ind.

[54] REFLECTANCE METER
4 Claims, 4 Drawing Figs.

[52] U.S. Cl. 356/191, 250/210, 356/195, 356/212, 356/226
[51] Int. Cl. G01j 3/52, G01j 3/46, G01n 21/48
[50] Field of Search 356/22, 212, 226, 177, 176, 179, 186, 195; 250/210

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Primary Examiner—Ronald L. Wibert
Assistant Examiner—Warren A. Sklar
Attorneys—Joseph C. Schwalbach, Michael A. Kondzella and Louis E. Davidson

ABSTRACT: A small, portable photoelectric cell-type reflectance meter is described for use in measuring color reflectance values of analytical test devices. Since these analytical test devices have predetermined ranges of color reflectance values, the reflectance meter is preset to read color values within these ranges. The meter has a constant light output circuit, a regulated power supply based on battery power and a battery power check circuit.

Abstract: A small, portable photoelectric cell-type reflectance meter is described for use in measuring color reflectance values of analytical test devices. Since these analytical test devices have predetermined ranges of color reflectance values, the reflectance meter is preset to read color values within these ranges. The meter has a constant light output circuit, a regulated power supply based on battery power and a battery power check circuit.

Not as big as it looks…

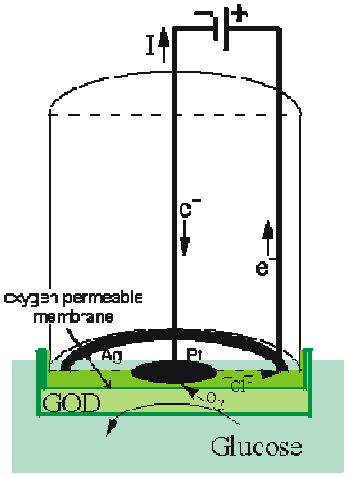
The Ames glucometer was primarily meant for use in physicians' offices, not at home

•Weight about 1 kg •Not very convenient to use •Required prescription

Nevertheless, it was a **GREAT COMMERCIAL SUCCESS** with many products to follow

Electrochemical sensors followed

Clark O_2 electrode

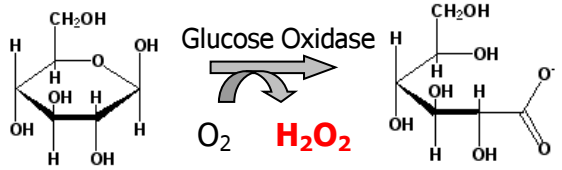


Operating principle – as discussed ☺

- A potential is applied between the central platinum cathode and the annular silver anode
- This generates electric current which is carried between the electrodes by means saturated KCl
- The electrode compartment is separated from the enzyme by a thin membrane, permeable **only to oxygen**
- The test solution is separated from the enzyme layer by another membrane, which is permeable to the analyte and the reaction products

Glucose oxidase

From a practical standpoint, it is better to follow the reaction electrochemically by oxidation of hydrogen peroxide



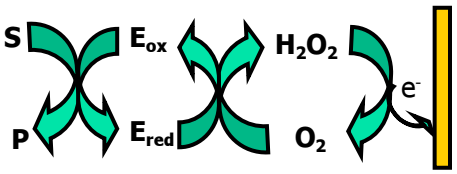
For hydrogen peroxide:

Polarization range +0.6/0.7V
Anode (Pt or Au) reduction
$H_2O_2 = 2H^+ + O_2 = 2e^-$

Ag/AgCl reference electrode completes the circuit:

$2AgCl + 2e^- = 2Ag + 2Cl^-$

Peroxide electrode



YSI 23A Blood Glucose Analyzer

Introduced in 1975 by Yellow Spring Instrument Company (Ohio, USA), this instrument is still on the market today!

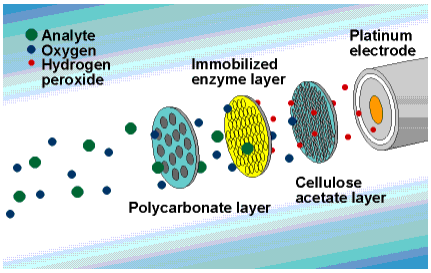
YSI 2300 STAT Plus Glucose & Lactate Analyzer

- Robust but relatively expensive
- Great, time-proven technology but not easy to miniaturize

YSI 23A and its successors have become a standard for clinical diagnostic work in many hospitals

YSI Immobilization technology

- An enzyme is immobilized between two membrane layers, polycarbonate and cellulose acetate
- Porous polycarbonate limits the diffusion of the substrate into the 2nd layer (immobilized enzyme), preventing the reaction from becoming enzyme-limited
- The substrate is oxidized as it enters the enzyme layer, producing H_2O_2, which passes through cellulose acetate to a platinum electrode where it is oxidized



- The third layer, cellulose acetate, permits only small molecules, such as H_2O_2 to reach the electrode, eliminating many electrochemically-active compounds that could interfere with the measurement
- The resulting current is proportional to the concentration of the glucose

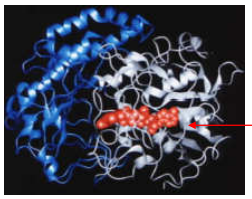
GOX is a redox enzyme

The majority of redox reactions in vivo are catalyzed by enzymes

Glucose oxidase (GOX)

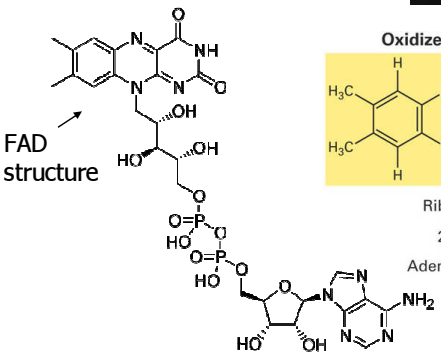
Glucose + O_2 =
Gluconic acid + H_2O_2

But how? Which amino acids are redox active?

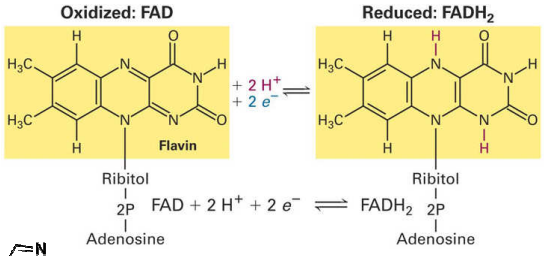


Redox chemistry is carried out by **coenzymes**

FAD* is an e- carrier in GOX



FAD structure

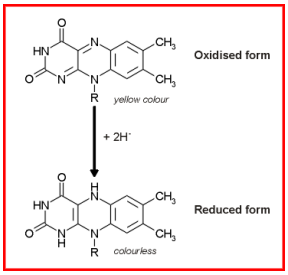


*FAD: flavin adeninedinucleotide

GOX half reactions

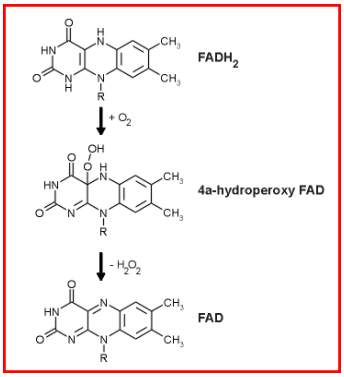
Coenzymes **must be re-generated** after each reaction cycle

Glucose + FAD + H_2O =
Gluconic acid + $FADH_2$



- GOX is a dimeric protein of ~160 kDa
- Each monomer has one tightly bound FAD (Ka = $1x10^{10}$) i.e. two FAD-sites per enzyme

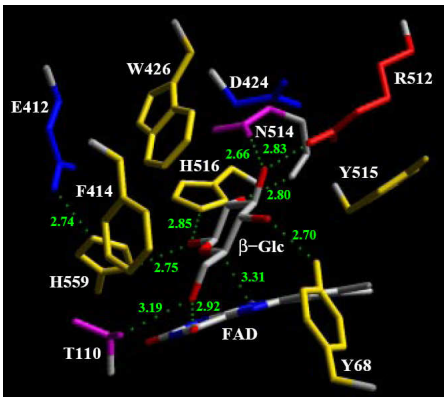
$FADH_2$ + O_2 = FAD + H_2O_2

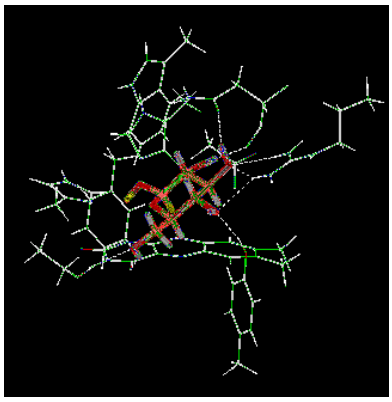


Glucose + O_2 =
Gluconic acid + H_2O_2

Active site of GOx

Selectivity: The precise amino acid arrangement and hydrogen bond network holding ß-D-glucose in the active site of GOx





However, when it comes to the 2nd substrate – O_2, there is flexibility…

Evolution of glucometers

Introduction of RedOx mediators

- Although very specific to glucose, GOx (and other enzymes) is much less fussy about the second substrate – the electron acceptor - O_2 for GOx
- Hence, it would be sensible to replace O_2 with another redox couple and/or organic dye (mediator) as e^- sinks for the enzyme
- Such a **mediator** would re-oxidize the coenzyme and will then be regenerated (re-oxidized) at the electrode; thus the mediator **is not consumed** in the course of the reaction
- This would enable (a) operating at much lower potentials and independently of [O_2] and (b) eliminate problems associated with interference from other optically or electrochemically active species present in blood

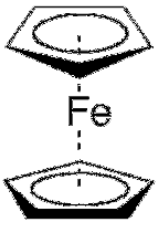
Mediators: ferrocene

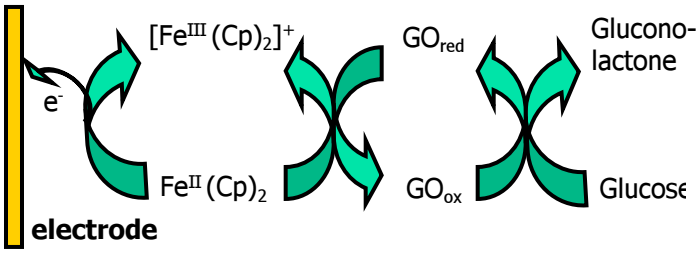
Ferrocene (iron cyclopentadienyl) is one of the most widely used mediators in electrochemical biosensors*

$Fe^{3+} + e^- \longrightarrow Fe^{2+}$ $E^0=+0.53V$

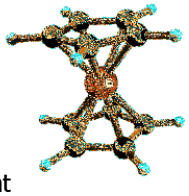
$[Fe^{3+}(CN)_6]^{-3} + e^- \longrightarrow [Fe^{2+}(CN)_6]^{-4}$ $E^0=+0.45V$

$[Fe^{3+}(Cp)_2]^{+} + e^- \longrightarrow Fe^{2+}(Cp)_2$ $E^0=+0.165V$



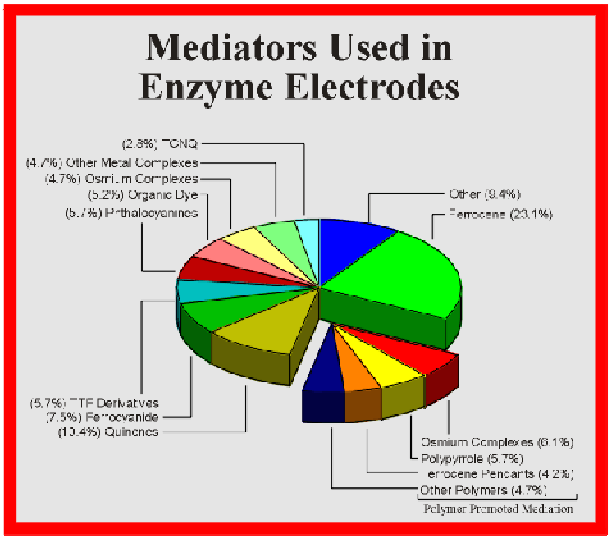


$Fe(C_5H_5)_2$



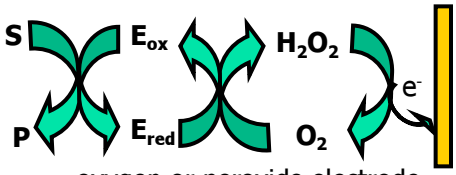
*Ferrocene is easy to make and E^0 is pH independent

Commercial practice



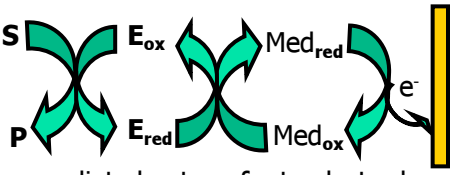
1st *vs* 2nd generation sensors

1st generation sensor:



oxygen or peroxide electrode

2nd generation sensor:



mediated e^- transfer to electrode

Enzyme:

$FADH_2$-oxidase + O_2 =
FAD-oxidase + H_2O_2

Electrode:

$H_2O_2 = O_2 + 2H^+ + 2e^-$

Enzyme:

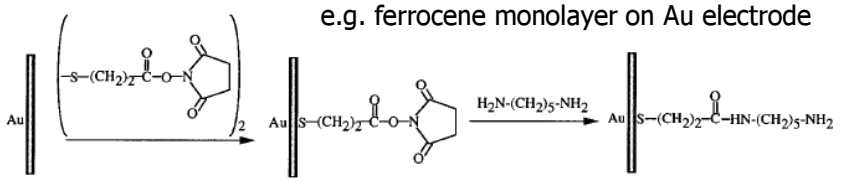
$FADH_2$-oxidase + $2[Fe^{III}(Cp)_2]^+$ =
FAD-oxidase + $2Fe^{II}(Cp)_2 + 2H^+$

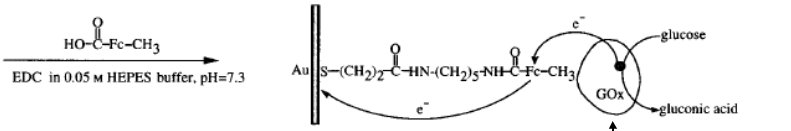
Electrode:

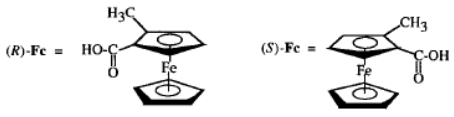
$2Fe^{II}(Cp)_2 = 2[Fe^{III}(Cp)_2]^+ + 2e^-$

Ferrocene monolayers

The mediator chemistry is easily adapted to other formats…
e.g. ferrocene monolayer on Au electrode







A monolayer away from the electrode

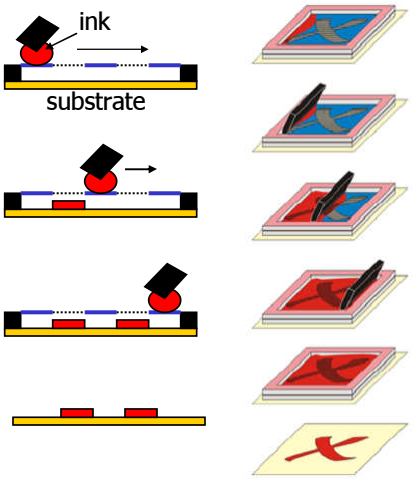
However, what really made glucose sensors **BIG** products is…

Screen printing

Another major development in electrochemical sensors

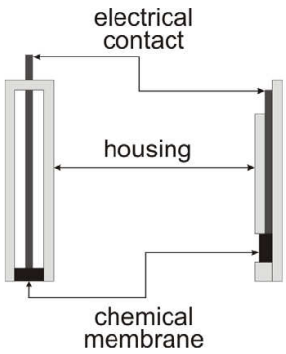
- Screen printing is technology based on squeezing a thick suspension ("ink") through a pattern screen onto a solid support ("substrate"), held on the back of the screen
- Ink is based on carbon and metals; substrates are either ceramic or polymer-based
- Typically, a multiple repeat patterns are designed on a single screen enabling the production of a large number of electrodes in a single pass

making pattern on a substrate



Printing electrodes

Comparison of a conventional electrode (left) and planar structure of the screen-printed sensor (right)



A three steps operation:

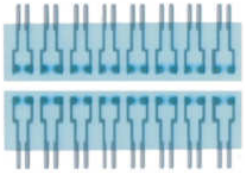
1. printing the paste that will form an electric contact of the sensor
2. printing the paste that will make a biosensing layer
3. printing the paste that will form an insulating layer – analogous to conventional electrode body

Very simple!

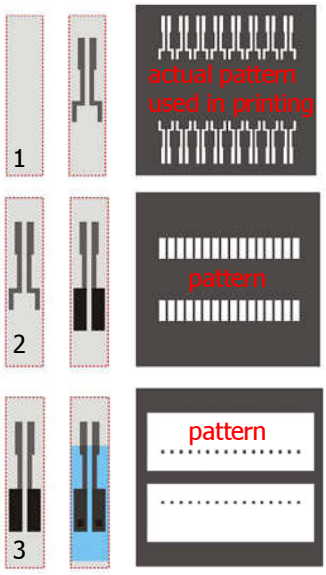
Screen printed electrodes

Schematic view of the patterns used for electrode fabrication by screen-printing

1. pattern for fabrication of electrical contact of sensors
2. pattern for fabrication of sensing area on screen-printed electrodes
3. pattern for the insulation layer fabrication

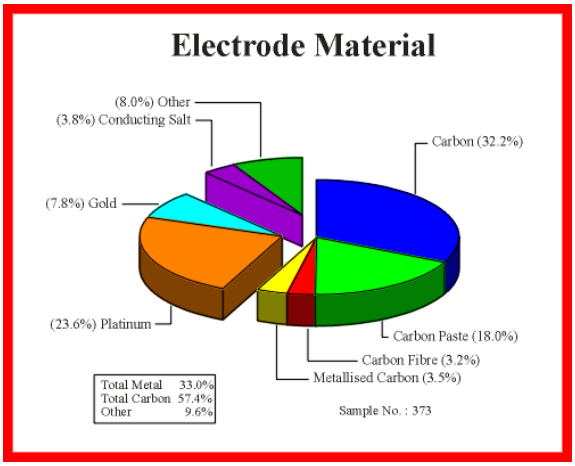


Very small:



Commercial practice

Very inexpensive and widely used technology today



Mainly carbon-based electrodes but other materials are used too

Screen printing: hardware

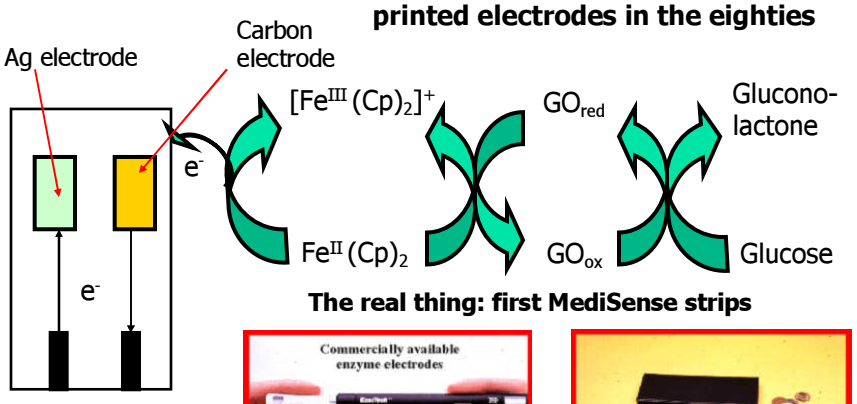
Simplicity, low-cost, high reproducibility and efficiency in large-scale production – essential for success in the home use market!

pilot scale screen-printers produce many 1000s/ day

A modern production scale printer

First commercial product

MediSense introduced first ferocene-based screen-printed electrodes in the eighties



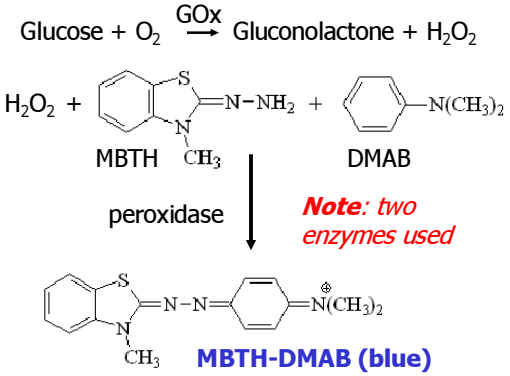
glucometer

The real thing: first MediSense strips

MediSence (acquired by Abbot) produced over 1 bln sensors pa!

2nd generation optical sensors

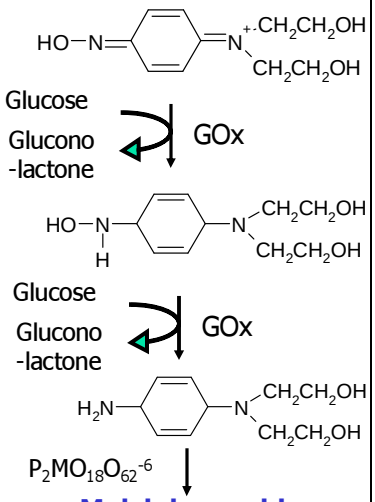
J&J Lifescan



MBTH: 3-Methyl-2-benzothiazolinone hydrazone;

DMAB: 3-dimethylamino benzoic acid

BMC AccuChek



Optical strip production

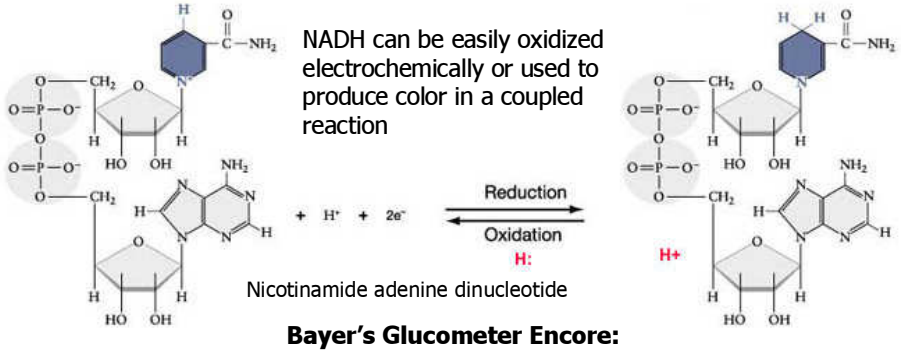
Not too difficult to make either ☺

Lab procedure for making Lifescan type glucose sensing strips

Step	Procedure
Dye solution	40 mg MBTH, 80 nm DMAB, 5 ml water
Initial dip	Dip a piece of membrane into the dye solution, blot off excess of liquid and dry at 56°C for 15 min.
Enzyme solution	6 ml water, various buffer salts and two enzymes, glucose oxidase and horse radish peroxidase
Final dip	Dip the membrane into enzyme solution, blot off excess of liquid and dry at 56°C for 15 min

Alternative enzyme systems

$$\text{Glucose} + NAD^+ \xrightarrow{\text{dehydrogenase}} \text{Gluconolactone} + NADH + H^+$$



NADH can be easily oxidized electrochemically or used to produce color in a coupled reaction

Nicotinamide adenine dinucleotide

Bayer's Glucometer Encore:

$$\text{Glucose} + ATP \xrightarrow{\text{hexokinase}} \text{Glc-6-P} + ADP$$

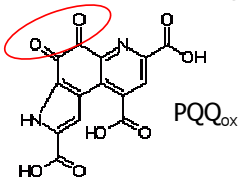
$$\text{Glc-6-P} + NAD^+ \xrightarrow{\text{Gl-6-P DH}} \text{6-PG} + NADH + H^+$$

$$NADH + \text{tetrazolium} \xrightarrow{\text{diaphorase}} \text{formazan (brown)} + NAD^+$$

Which enzyme?

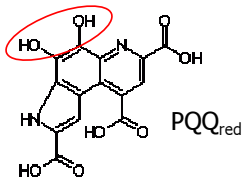
- GOX is inexpensive and works well with mediators - there is no requirement for oxygen; optical and electrochemical detection
- NAD^+-dependent GDH does not need O_2 and it is widely used for monitoring biochemical reactions; the co-factor is expensive but not much needed…
- **New DH**: PQQ-GDH is an efficient enzyme with rapid electron transfer rate, but it is more expensive than the rest

$$\text{Glucose} + PQQ_{ox} \xrightarrow{\text{Glucose DH}} \text{Gluconolactone} + PQQ_{red}$$



PQQ_{ox}

The discovery of this enzyme has stimulated the development of the 3rd generation sensors



PQQ_{red}

Make your pick ☺

Testing Made **Small and Simple**

LifeScan by Johnson & Johnson

Therasense/Abbott Laboratories

New

TrackEase and Prestige by Home Diagnostics Inc

Ascencia by Bayer

Accu-check by Roche Diagnostics

What's the difference?

Commercial biosensors comparison

	LifeScan One J&J**	Roche Accu-check	Bayer's Elite	TheraSense Abbot labs*	MediSense Abbot labs
Alternate site	Yes	No	No	Yes	No
Sample size	1 μL	3-4 μL	2 μL	0.3 μL	3.5 μL
Test time	5 sec	40 sec	30 sec	15 sec	20 sec
Temperature	5-44°C	8-39°C	10-39°C	10-35°C	18-30°C
Test memory	150	100	120	250	125
Data d-load	Yes	Yes	Yes	Yes	Yes
Capillary act	Yes	Yes	Yes	Yes	No

*PQQ-GDH: most sensitive **Optical detection: very fast

Why is there so many technologies?

Everybody wants to get a slice of the cake, but they need to get around other people's patents

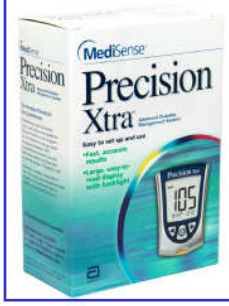
Does the technology matter?

Which technology?

New Flat Screen Automatic B Pressure Monitor from Walmart!

←**Just like choosing a cell phone ☺**

LifeScan's Sof-Tact to be completely painless



Large easy to read display with backlight, does 2 things at once

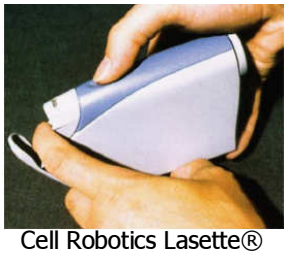
How do they differentiate?

Alternative sites, low volume, no pain…

LifeScan's Sof-Tact

Analysis of interstitial fluid:
Laser ablation, ultrasound reverse iontophoresis – painless methods!

Bayer's Vaculence



Cell Robotics Lasette®

Amira AtLast®

Pelikan Sun®

Glucowatch

The market realities

- Biosensors' technology per se is not a primary consideration any more – all of these biosensors do a pretty good job
- Other issues dominate the competition – comfort for the patient (e.g. pain, alternative site), ease to use, integration with PCs (data download), etc
- Commoditization – Wal-Mart is there!

and regular price wars…

Alameda, Calif., Sept. 29, 2006 – Abbott today announced it will provide a **free Abbott blood glucose meter** and a starter supply of strips to all Becton, Dickinson and Company blood glucose monitoring patients. Abbott made this decision after Becton, Dickinson's announcement that it intends to exit the blood glucose monitoring market by the end of 2007

Indianapolis, Ind. October 2, 2006 - Roche Diagnostics Offers ACCU-CHEK® Blood Glucose Systems. Users can receive **a free ACCU-CHEK** blood glucose system (**meter and strips**) by calling 1-888-355-4242

And **free** Bayer's CONTOUR® and **free** LifeScan's OneTouch®

After the break

- How companies make money on biosensors
- Third generation of glucometers
- Competition, need and other uses for this platform

Quiz time

How to make $$$?

There are two things one can sell: hardware and sensors (e.g. disposable)

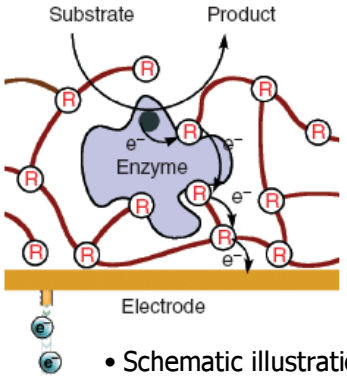
Which one is better?

It depends…

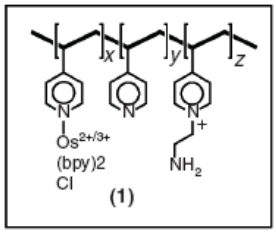
HP printer model

Third generation sensors

Enzyme wired electrodes



Electrons travel to the electrode via polymer wires – no mediators

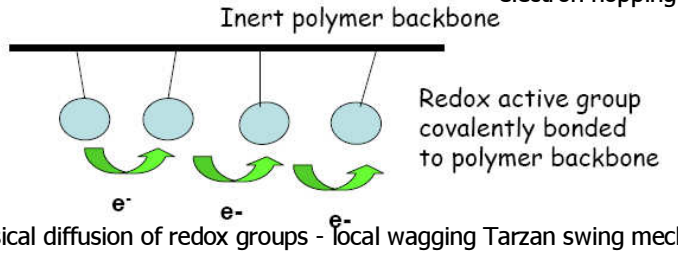


- Schematic illustration of a redox-enzyme wired to an electrode with an electro-active polymer
- This system is an amperometric biosensor

ET in polymer materials

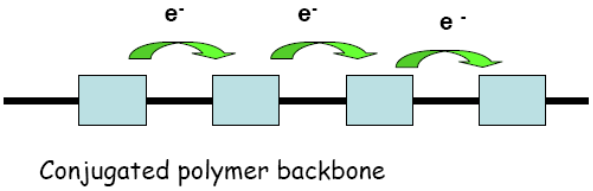
Redox polymer material.

Nearest neighboring electron hopping



No physical diffusion of redox groups - local wagging Tarzan swing mechanism

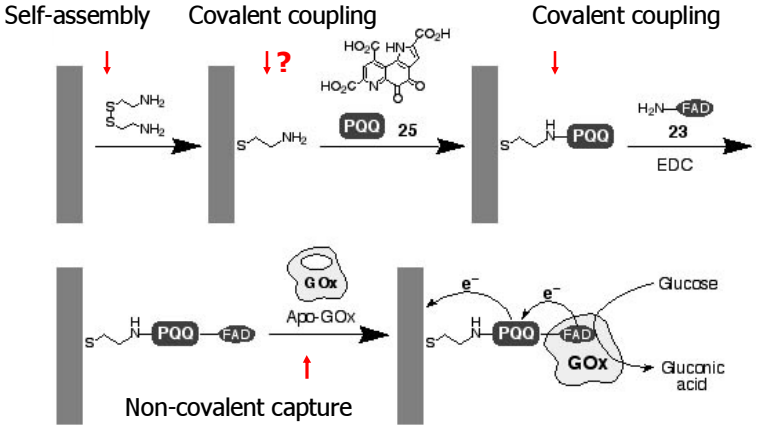
Electronically conducting polymer material.



Polymer backbone intrinsically conductive

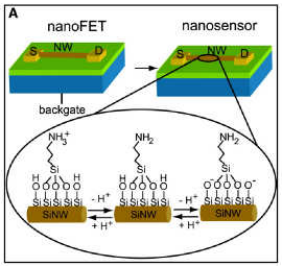
SAMs are good too

The surface-reconstitution of apo-GOx on a PQQ-FAD monolayer assembled on a Au-electrode



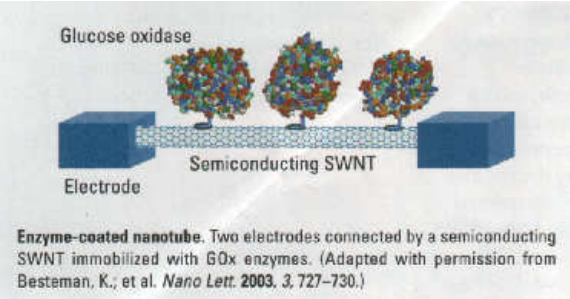
What chemistry is used?

Semiconductor designs



Semiconductor nanoelectrodes with familiar technologies:

Self-assembled monolayers and field-effect transistors (use voltage to control conductivity – remember?)



Enzyme-coated nanotube. Two electrodes connected by a semiconducting SWNT immobilized with GOx enzymes. (Adapted with permission from Besteman, K.; et al. *Nano Lett.* **2003**, *3*, 727–730.)

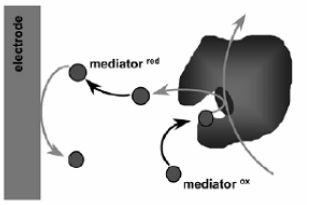
How about this one?

Single wall carbon nanotubes

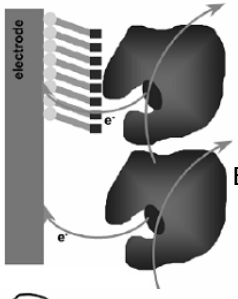
Yi Cui, et al. Science, 2001, 293, 1289-1292

Possible architectures

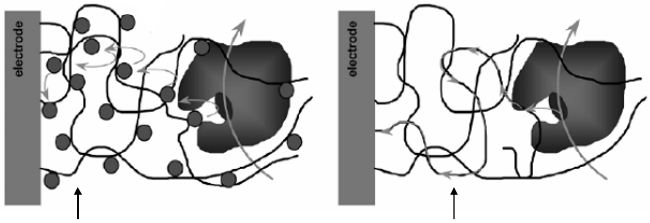
Electron transfer schemes:



2nd generation: diffusion of mediators

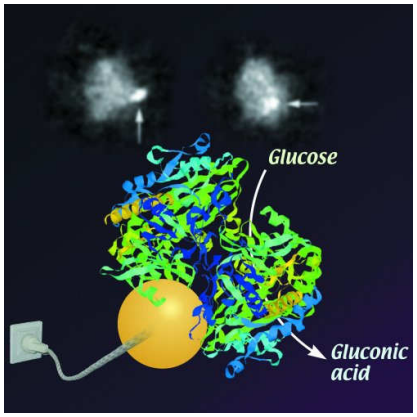


ET at monolayer



ET "hopping" in a redox relay and transfer via conducting polymer

Nanotech and bioelectronics



- Three-dimensional structure of the GOX with "plugged-in" gold nano-particles
- The bottom part is a scheme showing the operating principle
- The upper part is a TEM micrograph image of the protein with an implanted nanoparticle (marked with an arrow)

Which ones of the three sensors you like best ☺?

What about their commercial prospects?

Generation comparison

1st generation:
Enzyme:
$FADH_2$-GOx + O_2 = FAD-GOx + H_2O_2

Electrode:
H_2O_2 = O_2 + $2H^+$ + $2e^-$

- The 1st generation electrode utilizes H_2O_2 produced in the reaction. (E^0 = +0.68 V)

2nd generation:
Enzyme:
$FADH_2$-GOx + 2 Ferricinium = FAD-GOx + 2 Ferrocene + $2H^+$

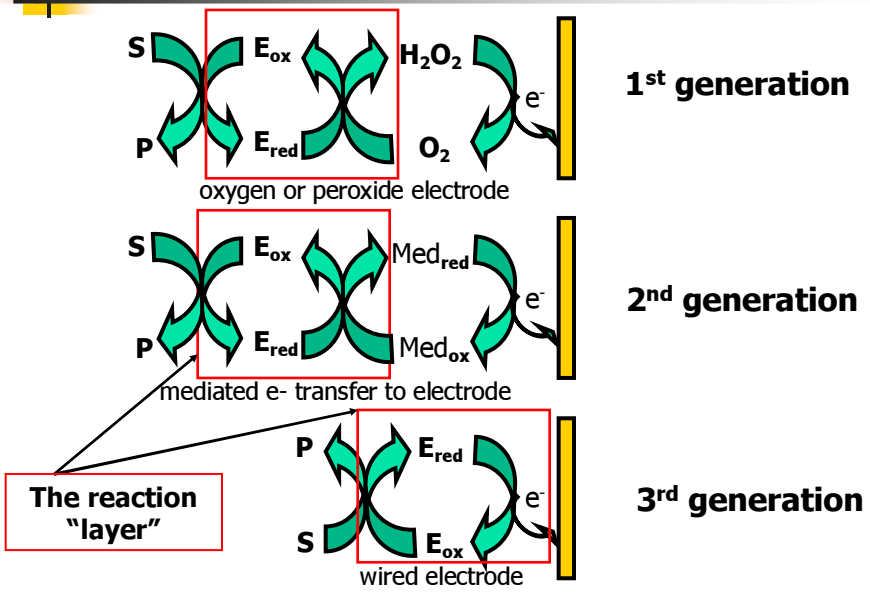
Electrode:
2 Ferrocene = 2 Ferricinium$^+$ + $2e^-$

- The 2nd generation electrode relies on a mediator (ferrocene) to transfer the electrons produced in the reaction to the electrode. (E^0 = +0.19 V)

3rd generation:
Enzyme/electrode
$FADH_2$-GOx = FAD-GOx + $2H^+$ + $2e^-$

- The 3rd generation electrode directly utilizes the electrons (E^0 = +0.10 V)

Generation comparison



The ultimate goal

Frequent non-invasive measurements

Objectives:

To create a closed loop glycaemic control by providing insulin injections on demand e.g. via a portable delivery coupled with continuous monitoring device

A reliable biosensor component (implantable?) is clearly a key component of such system

Issues:

- Sensor stability*
- Calibration*
- Biocompatibility

*especially for implants

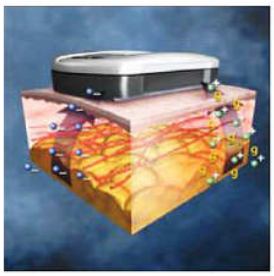
Technologies:

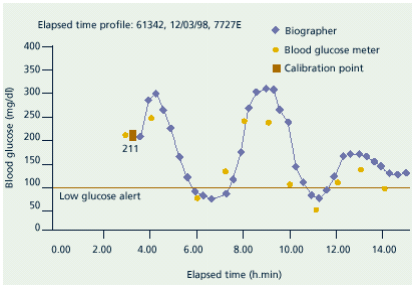
Needle type subcutaneous electrodes

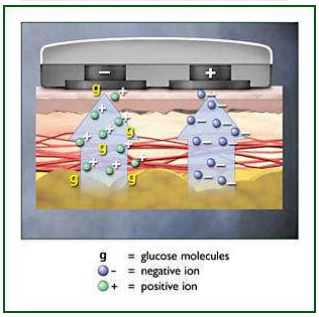
Optical e.g. near/mid IR, RAMAN and photoacoustic spectroscopy

Non-invasive through the skin e.g. reverse iontophoresis

Glucowatch Biographer™

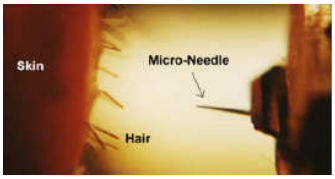






Implantable/non-invasive sensors

- Minimed (Sylmar, CA) – subcutaneous implant (needles)
  Trying to integrate with an insulin pump – 1 in 3 mo refill with projected 10 year (!) battery life
- Therasense (Alameda, CA) – same but much smaller needles
- Kumetrix (Union City, CA) – silicone micro-needles
- SpectRx (Norcross, GA) – laser-based sampling/sensor in a patch
- Animas (Frazer, PA) – near IR, tissue transparent implant that gives wireless alarms in hyper- and hypo-glycaemic situations
- Pendragon (Zurich, ZW) – non-invasive continuous monitor based on the impedance pattern of the skin with an open resonance circ



⟵ Kumetrix

Pendragon ⟶

Sensing in tears

Tear glucose tracks blood glucose with ~30 min lag

The concept: glucose-sensing material to determine glucose concentration in tear fluid

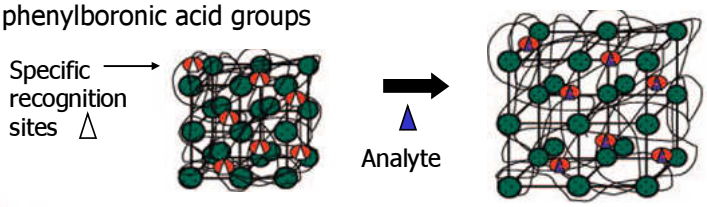
- The photonic crystal sensing materials would be contained in a contact lens or ocular insert
- The color diffracted changes with the tear glucose concentration
- A simple mirrored compact-like device would illuminate the sensor material with white light

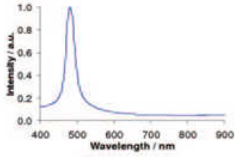
- The color of the sensor would be determined by viewing the reflected (diffracted) light and comparing it with an exterior color wheel calibrated in terms of the blood glucose concentration

Clinical Chemistry 50:12 2353–2360 (2004)

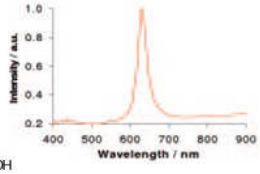
Working principle

Crystalline colloidal array embedded within a polymer network of a polyacrylamide-poly(ethylene glycol) hydrogel with pendent phenylboronic acid groups

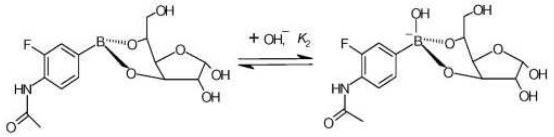




A red shift in the wavelength of diffracted light from hydrogel swelling induced by interaction of the analyte with sensor's molecular recognition elements

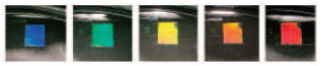


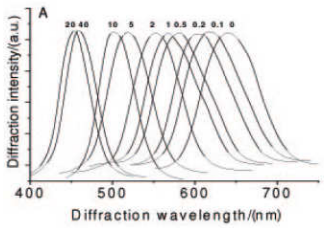
Chemistry: boronic acids

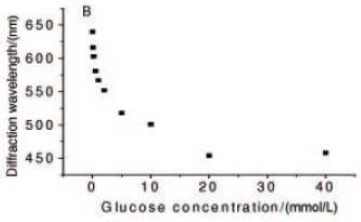


Remember covalent imprinting?

Actual measurements







Effect of glucose concentration on diffraction of the AFBA-AAPEG PCCA sensor

Top: diffraction color changes from red to blue with increasing glucose concentration

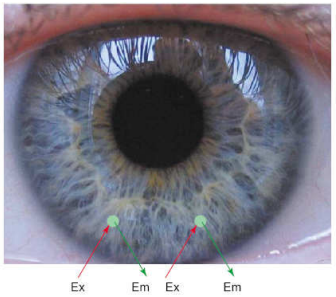
A: dependence of diffraction wavelength on glucose concentration. The diffraction peaks are labeled with the corresponding glucose concentration (mmol/L)

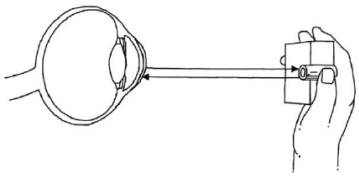
B: dependence of diffraction peak maxima on glucose concentration

Note that in this case the gel shrinks; hence the blue shift

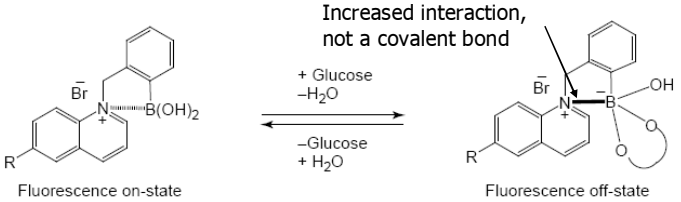
Clinical Chemistry 50:12 2353–2360 (2004)

Fluorescence works too





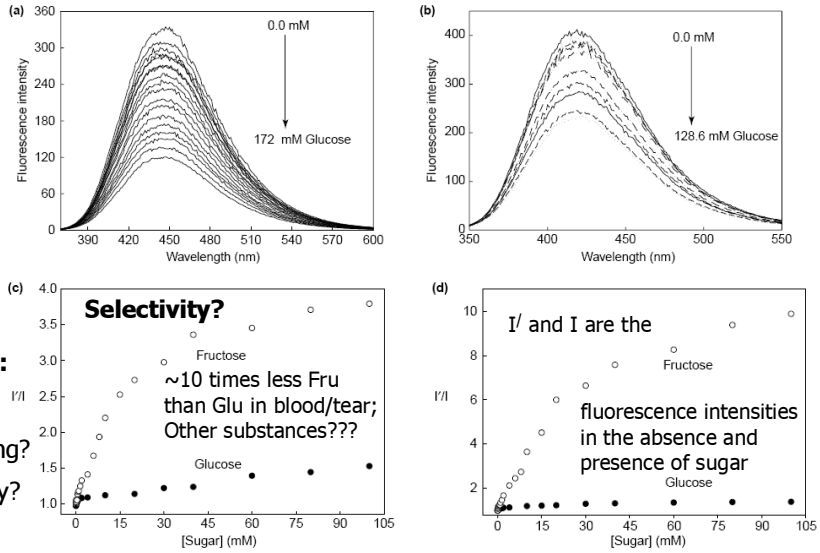
Or perhaps even "dope" commercially available lenses with boronic acid probes to produce a response



Current Opinion in Biotechnology 2005, 16:100–107

Not too bad a sensor

Sensor response to glucose in tear (different boronic acids)



Other issues:

Leaching?

Stability?

Glucose sensing

- Technologically and financially glucose biosensors have been a great success, although their medical benefits are not that easy to quantify
- Glucose monitoring is still an exciting development area for biosensors – **REAL NEED and BIG MARKET**
- The hotspot is the development of implantable/non-invasive biosensors and their integration with insulin pumps – this should have a **VERY** significant impact on the management of diabetes

OK, let's get back to hand held glucometers

Platform technology

- The cell phone-like device is "generic" - it is designed to record a signal and convert it into a number according to calibration i.e. it does not care what is being measured
- Screen printing technology is available and can applied to manufacturing of practically any redox enzyme sensor
- Why not make others? **Which ones?**

Introducing The **Two-Minute Home Cholesterol Test**

CardioChek®

Is Your Cholesterol in Chek?™

http://www.cardiochek.com/

Why is the glucose biosensor so much bigger product than the cholesterol one?

Alcohol sensors

Alcohol oxidase catalyses the conversion of ethanol to acetaldehyde $CH_3CHOH + O_2 = CH_3CHO + H_2O_2$

Dream enzyme/reaction for a biosensor developer ☺

Drink driving breath test for alcohol?

Are biosensors used in breathalysers? **No**

Why? **Other technologies are available…**

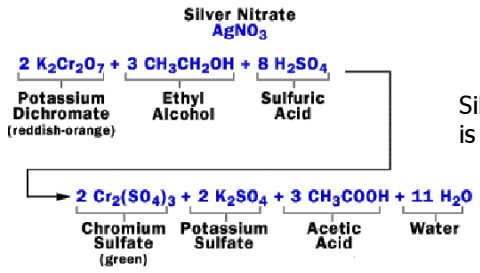
Blood Alcohol Concentration

- Alcohol is rapidly absorbed from the mouth, throat, stomach and intestines; it shows up in the bloodstream in about 30 min
- Alcohol is not digested or metabolized in the bloodstream and, as the blood circulates through lungs, some of it diffuses through alveoli into the air
- The concentration of alcohol in the alveolar air is directly proportional to the concentration of the alcohol in the blood - ratio of breath to blood alcohol is 2100:1
- As alcohol is exhaled, it can be detected by a breath testing device rather than having to draw driver's blood for the test; cops can do the breath test instantly on the spot
- The legal BAC limit in NY is 0.08% i.e. 0.08 grams of alcohol per 100 ml of blood

Determination of BAC

There are three major types of breath alcohol testing devices, and they're based on different principles:

- [Old] Breathalyzers - used colorimetric chemical reactions

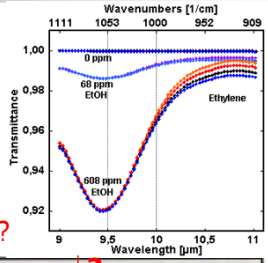


Silver nitrate is a catalyst

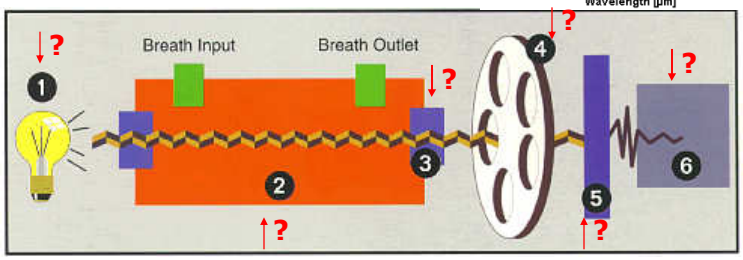
- Alcohol detection by infrared (IR) spectroscopy
- Alcohol detection in a fuel cell-based electrochemical device

IR-based breath analysis

IR spectroscopy deals with the infrared region of the electromagnetic spectrum (0.8-30 μm). It is based on analyzing specific frequencies at which molecules (functional groups) rotate or vibrate



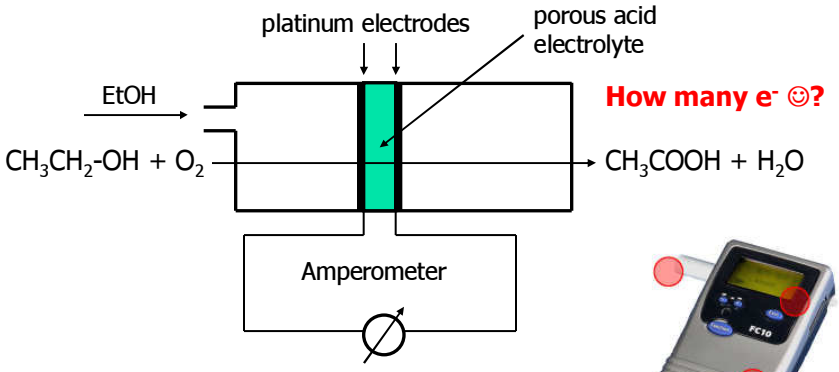
What do the numbers on the scheme refer to?



1) Light source - quartz lamp 2) Sample chamber 3) Lens
4) A wheel with narrow band IR filters 5) detector 6) microprocessor

Electrochemical devices

Fuel cell technology



Typical spec:
Negative response: 5 sec
Positive response: 30-40 sec
Operation temp: 0-40°C
Linearity up to 400 BAC
Weight: 6oz (171 g)

Alco-Sensor

Intoximeters Inc: Alco-Sensor line of handheld products for law enforcement and workplace portable breath alcohol detection

The Alco-Sensor line of products offers instruments for both screening applications and applications where gathering court accepted evidence is required.

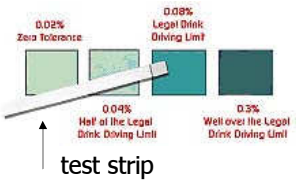
ALCO-Screen

ALCO-Screen™ produces a color change in the presence of saliva ~1:1 relationship between the saliva and blood alcohol content

$$CH_3CH_2\text{- }OH + O_2 \xrightarrow{\text{Alcohol Oxidase}} CH_3CH{=}O + H_2O_2$$

$$H_2O_2 + \text{Colorless}^* \xrightarrow{\text{Peroxidase}} H_2O + \text{Blue color}$$

~1:1 relationship between the saliva and blood alcohol content

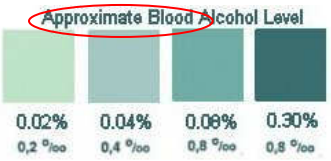


test strip

*Tetramethyl benzidine

ALCO-Screen: test procedure

- Abstain from placing anything in the mouth for 15 min prior to beginning the test
- Open the foil package and remove the test strip
- Saturate the reactive pad with saliva from the test individual's mouth or sputum cup
- At exactly two minutes observe the color change, if any, in the reactive pad (results obtained after more than 2.5 min may be erroneous and should not be used)
- Estimate the approximate blood alcohol concentration



Do you think cops would be happy with this?

Same technology?

Good enough for home use

Alcohol in breast milk

Homework from L1

Come up with a "cool", new biosensor

Remember?

The rules of the game:

- It has to be something NEW and COOL so that people may really want to use it (or better buy!)
- You need to come up with a concept/idea – no technical details are necessary
- However, you have to (1) describe what your biosensor does, (2) why it is useful and (3) specify why the "bio" as a recognition element is required
- **B&E students only**: estimate the market for your device (assume it can be built easily) in $$$

But now the rules have changed ☺

New rules

I am looking for a concept/idea but with sufficient **technical details** for a skillful person to be able to build it

How to go about it:

1. In a few lines state clearly what your sensor is designed to measure and why (the need)
2. Provide an overall design scheme, stating what your bioreceptor and transducer are and why – the choice of both MUST be clearly justified (use your imagination!)
3. Provide a scheme explaining how your receptor is immobilized on the surface and justify your choice of the immobilization strategy
4. Use pbd viewer for the justification and to highlight your target residues (e.g. SH – are there any? NH_2 – does your protein have NH2 groups in the active site?, etc). All these amino acids can be individually colored in the pdb viewer

New rules

5. Using pdv viewer illustrate the orienattion of your protein on the surface - you will need to find out which amino acid residues constitute active/binding site

6. Those who decide to use DNA in their sensor will have to pick up a DNA binding protein (or something) to do this - you must have at least one illustration done in the pdb viewer

7. State what kind of interfering molecules you would expect in your samples and how they would affect the performance (briefly and to the point)

8. Illustrate what signal(s) your sensor will generate in the absence/presence of interfering molecules

How to submit?

PPT: no more than three slides and no background; just white

Deadline: Wednesday, December 3 (midnight ☺). If you want me to comment on your design – by Wednesday, November 26

That’s all for now

Have fun

and see you next week