Med TechNet
Online Continuing Education for the Clinical Laboratory Professional
Med TechNet Online Services✦ East Amherst, NY June 1998

Clinical Instrumentation Refresher Series: Ion Selective Electrodes

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At a glance ...

Introduction

The set of analytical tools known as **ion selective electrodes** (ISE's) is based on the principles of electrochemistry, and the majority fall under the category of *potentiometry*, where an electrical potential (voltage) is measured and related to some unknown analyte concentration.

The simplest example of the principles involved can be seen in **Figure 1**. Here a bar of metallic zinc is placed in a solution of copper sulfate. A short time later, we would find that the zinc had dissolved (gone into solution as Zn^{++}), and metallic copper would have precipitated.

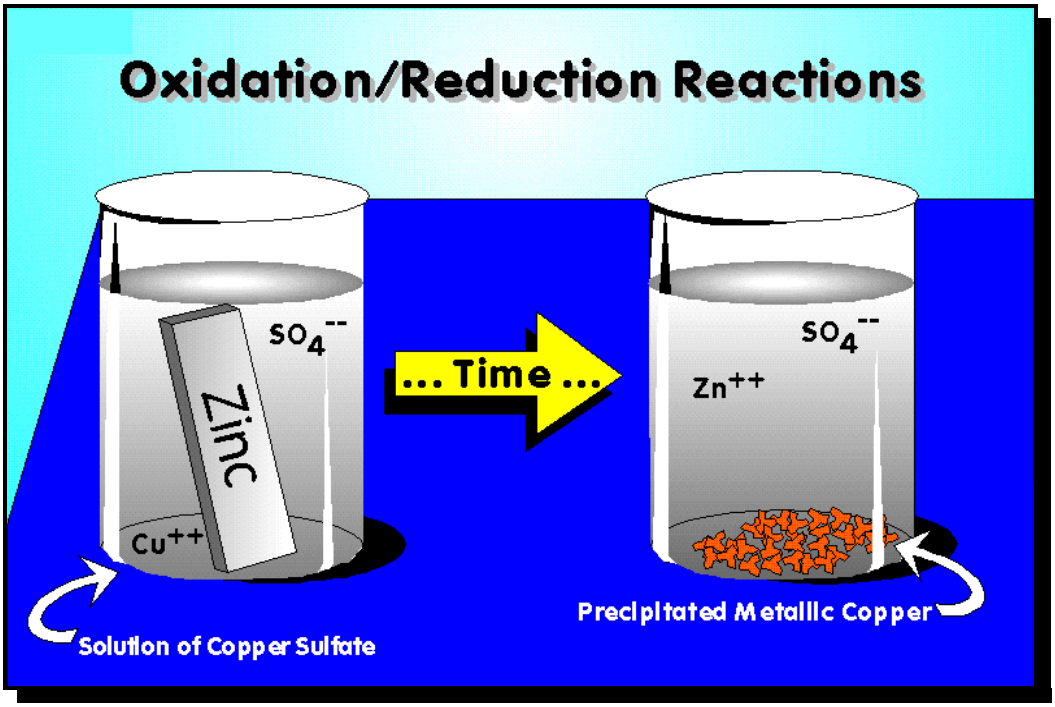

Figure 1

More accurately, we should be referring to ion "activities" instead of concentrations. For sufficiently dilute solutions (<0.001M), ion concentrations and activities are equal. When concentrations reach higher values, activities fall off and do not rise as rapidly. The reason for this is that at higher concentrations, ions do not act independently. The relation between concentration and activity is a simple one, shown as Equation 2, **Figure 2**. The activity coefficient can be determined empirically, and generally ranges between 0 and 1, although at very high concentration, some substances exhibit coefficients greater than 1.

What happened? In this case, the Cu ions in solution have a greater attraction for electrons than the zinc atoms (ie, the copper is more "electrophilic" than the zinc atoms). Each copper ion "stole" two electrons from each zinc atom. The zinc atoms lost two electrons, which is the process of oxidation. The copper ions gained two electrons, which is the process of reduction.

We probably all have fond memories of general chemistry and "redox" reactions (oxidation / reduction reactions), but this is crucial to understanding the electrochemical basis of ISE's. The overall redox reaction depicted in **Figure 1**, is represented by Equation 1, in **Figure 2**. (Figure 2 contains various formulas we will use in this paper.)

Remember that a redox reaction is composed of an oxidation reaction (where a species loses electrons) and a reduction reaction (where a species gains electrons). To write an overall balanced redox equation, the number of electrons lost must be equal to the number of electrons gained. Then, the two half-reactions can be added. The electrons on each side of the equation (which are equal) can then be dropped.

How "strong" the attraction is for electrons to leave zinc for copper is primarily controlled by the concentration of the copper ion (the "concentration" of the zinc, which is not ionized, is defined as 1.000). The total number of electrons transferred is limited by the total mass of the least available reactant.

Harnessing the Electron Transfer

If we could somehow measure the "strength" of the transfer of electrons depicted in **Figure 1**, we could relate that to an ionic concentration: the stronger the attraction, the higher the concentration. In order to accomplish this, we need to rearrange things a bit.
Figure 3 illustrates the necessary rearrangement of components. Now, in order for the electrons to leave the zinc and reduce the copper, they are forced to flow through an external circuit; in our case, this has an electrical measuring device attached (meter).

The chemical reactions in **Figure 3** are essentially the same as in **Figure 1**, however, electrons leaving the zinc bar, as zinc ions go into solution, must travel through the wire and meter, over to the copper bar. At the surface of the copper bar, copper ions in solution will pickup electrons, and "plate out" onto the copper bar. So, over time, the zinc bar loses mass and the concentration of Zn^{++} increases, while, the copper bar gains mass and the concentration of Cu^{++} decreases. The salt bridge is necessary to complete the electrical circuit. It is usually composed of a high salt concentration (such as KCl) and must be in electrical contact with the other solutions, while not allowing any degree of mixing.

The meter in **Figure 3** could be either an ammeter (measuring current flow, or "volume of electrons per unit time") or it could be a voltmeter (measuring the electrical "pressure" of the electrons). Let's see which would be

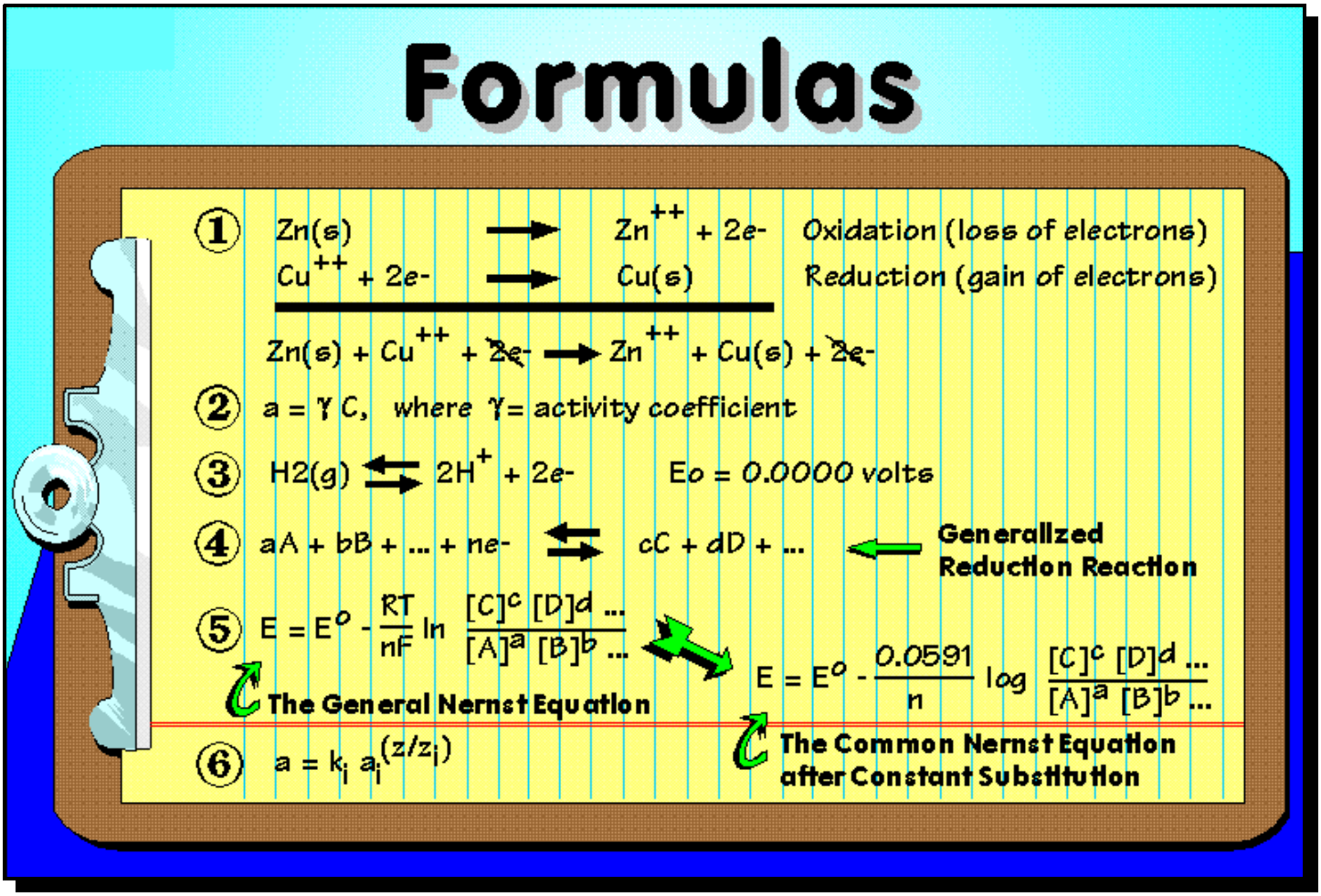

Figure 2

more beneficial to us, given that what we want to ultimately determine are the concentrations of ions in solution.

The Ammeter. The ideal ammeter has zero electrical resistance, similar to a short length of wire. Connecting an ammeter to our setup, electrons would flow as quickly as possible from the zinc side to the copper side until something was depleted (the zinc bar dissolves completely, or the Cu^{++} is depleted). The meter would indicate a large initial current that decreases quickly, and approaches zero. There are always difficulties when attempting to measure changing values.

The Voltmeter. The ideal voltmeter has an infinite electrical resistance, and theoretically allows zero current to flow. It displays only the electrical pressure between the two sides. Without current flow, the chemical composition of our setup never changes, therefore the chemical pressure remains unchanged and displayed by the meter. This is the type of parameter we want to measure.

This is also the basis of the formal definition for potentiometry: an analyte concentration is related to a measured potential (voltage) and assumes zero current flow.

Terminology

The apparatus depicted in **Figure 3** is actually an electrochemical cell, not unlike the batteries in your flashlight, or the pH electrode in your laboratory. Electrochemical cells can be classified into one of two types: Galvanic (spontaneous current flow) and Electrolytic (requires an external energy source). The one in **Figure 3** is Galvanic, however it could be turned into an electrolytic cell by replacing the meter with an external power source (such as a battery) and applying enough energy to force the electrons in the opposite direction: reducing the Zn^{++} and oxidizing the copper bar.
Our electrochemical cell is composed of two "half-cells": a zinc half-cell and a copper half-cell. The half-cell where reduction occurs is called the "cathode" (copper) and the

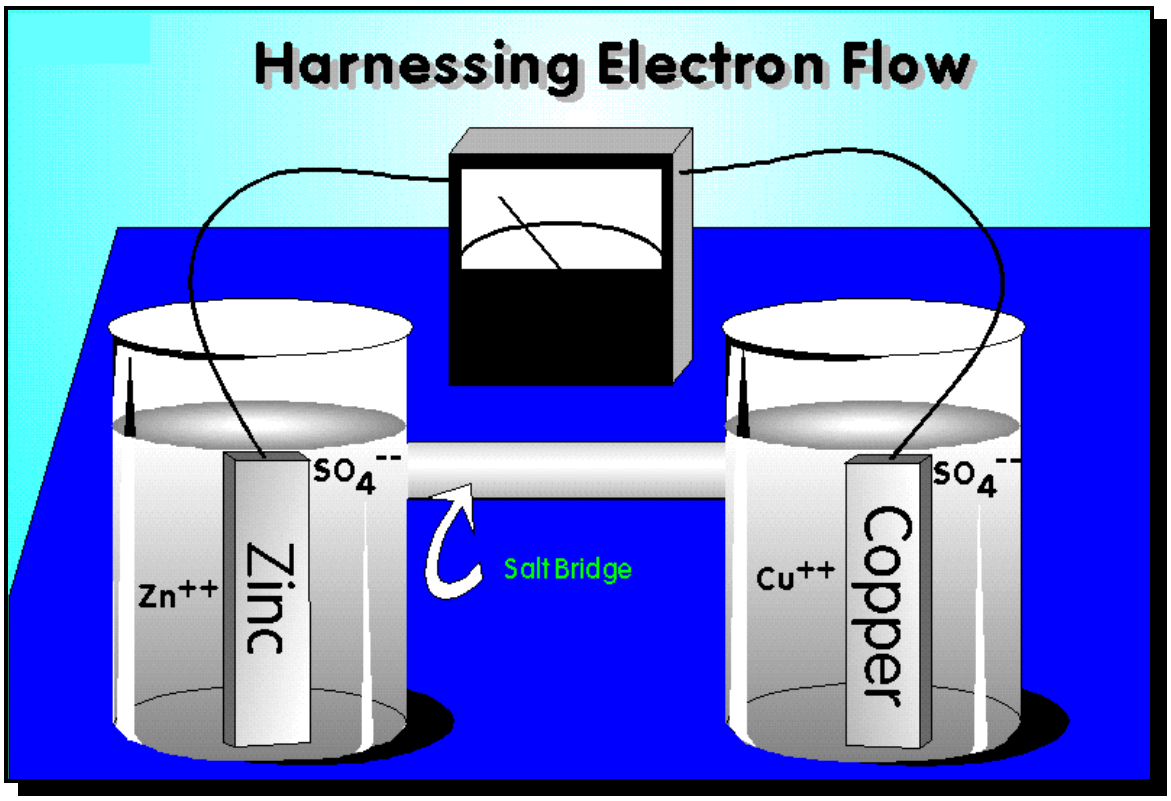

Figure 3

half-cell where oxidation occurs is called the "anode" (zinc). For galvanic cells, the electron flow in the external circuit (wire/meter) is from anode to cathode.

When used for analytical purposes, whole half-cells are also referred to as "electrodes".

Measurements

The measured voltage of the electrochemical cell in **Figure 3** will depend on both the ionic concentrations of the solutions, and the type of reactants. That is, the measured potential would be different if either half-cell were replaced with a cadmium half-cell (cadmium metal in a solution containing Cd ions), or any other type. The measured potential represents the DIFFERENCE between the potentials of each half-cell. Knowing the potential of any two half-cells, the measured potential of the resulting electrochemical cell can be predicted.

So, how can the potential of a half-cell be measured? The answer is: it can't! It is impossible to measure the potential of a single half-cell without introducing a second half-cell that will affect the measured potential. For example, if you had a beaker filled with copper sulfate, along with a bar of copper, then tried to measure the potential using a voltmeter, you might touch one lead to the copper bar and the other to the solution. However, most test meter probes are nickel plated; submersing that in the solution produces a second (nickel) half-cell. The measured potential would be that of a copper/nickel electrochemical cell -- not simply the copper half-cell, as intended.

Since half-cells can never be measured directly, it was decided to pick a "standard" half-cell and measure everything else against it. With a common reference, all other values could be compared.

The Standard Hydrogen Electrode

The standard half-cell that was chosen to compare all other half-cells was the Standard Hydrogen Electrode (SHE) or Normal Hydrogen Electrode (NHE). This is a carefully defined cell and is shown on the left in **Figure 4**. The solution must have a hydrogen ion activity of 1.0, and gaseous hydrogen is pumped in at a pressure of 1 atmosphere, which bubbles through the solution, over a piece of metallic platinum. The reaction that takes place in the SHE is shown in **Figure 2** as Equation 3. Note the platinum is not part of the reaction; it functions solely as a site for the hydrogen to react. Note also that the reaction is written with a double arrow. The reaction can go either way depending on the nature of the other half-cell. If the other half-cell is MORE electrophilic than hydrogen, then Equation 3 will run to the right and produce electrons which will flow away from the SHE. Similarly, for half-cells LESS electrophilic, Equation 3 will flow to the left, accepting electrons produced from the other half- cell.

Since the measured potential will be the difference between the two half-cell potentials, the SHE was conveniently defined to have a potential of 0.0000 volts. By doing so, the measured potential will be numerically equal to the other half-cell potential. By convention, the sign of the measured potential will depend on whether

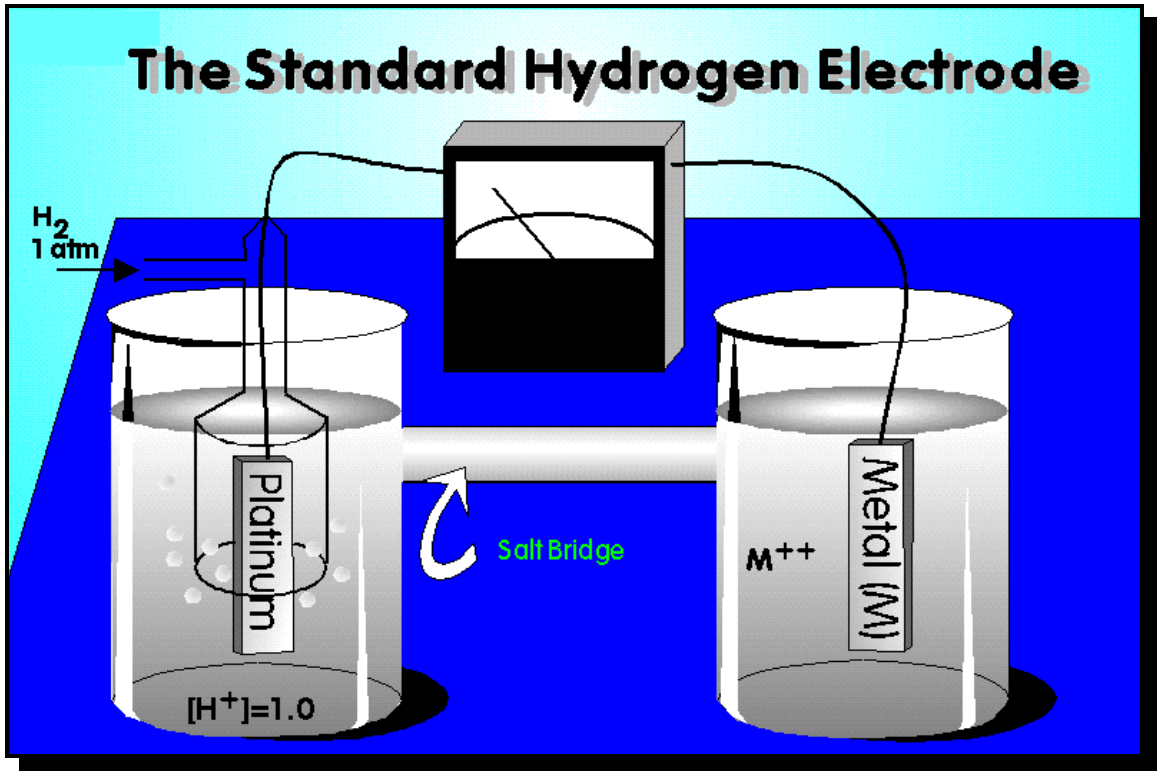

Figure 4

hydrogen is being reduced (negative potentials) or oxidized (positive potentials).

For example, (assume all activities to be 1.0 molar) if zinc were the other half-cell, metallic zinc would oxidize, releasing electrons, which would flow to the SHE and reduce the hydrogen ions. The measured potential of 0.76 volts would actually be -0.76 volts, since the SHE was accepting electrons.

Conventions

The potentials of many different half-cells have been experimentally determined and tabulated in a number of books. Depending on the nature of the book, tables will write all reactions as either oxidations or reductions and express the potentials for half-cells with unit activities, as either "standard oxidation potentials" or "standard reduction potentials". Most analytical chemists are more "at home" with reduction potentials, and that is what we will use here. The notation for "standard reduction potential" is usually, E^0 (a capital E with a post-superscripted degree symbol). This is also the convention used by the popular **CRC Chemistry and Physics Handbooks**. When comparing any two half-cells, the one with the MORE NEGATIVE E^0 is the one which will give up its electrons to the other half-cell.

Calculations

It would be helpful, at this point, to look at a typical calculation. Let's go back to our original zinc/copper electrochemical cell (**Figure 3**), where all ions are at unit activity (1.0000); what would be the measured potential? **Figure 5** lists the steps involved.

First, we find the reactions and associated standard reduction potentials in a reference, such as a CRC Handbook.

Second, we write the reaction with the MORE negative E^0 as an oxidation and reverse the sign of the listed E^0. If the number of electrons in the two equations are equal (in this case, 2), they can be added to yield the overall redox reaction. If the number of electrons is not equal, one or both of the equations must first be multiplied by an appropriate factor before summing. Note that when an equation is multiplied, the E^0 value is NOT affected.

The resulting potential is referred to as the "standard cell potential" and must be positive in order for the reaction to proceed, as written. (When using standard reduction potentials, unit activities, and following the steps above, the result will always be positive. The importance of looking for a positive value becomes more important as we explore concentration effects.)

Concentration Effects

Up to this point, we have been considering only unity concentrations. However, concentrations (activities) play an important role in determining cell potentials. The "master" equation which relates concentrations, voltage and other parameters is the **Nernst equation** and is shown in **Figure 2**, Equation 5, and is based on the generalized reduction equation (**Figure 2**, Equation 4).

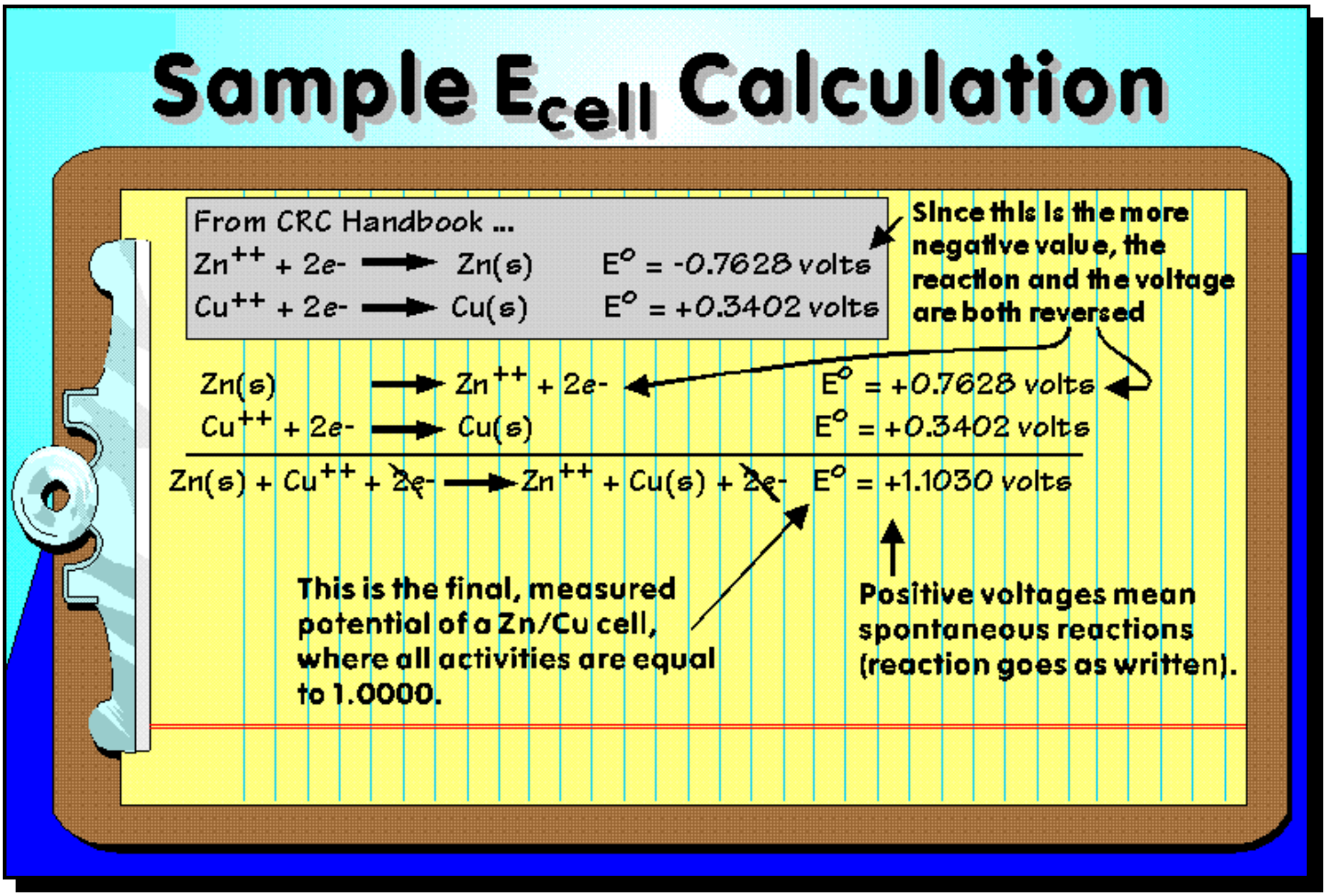

Figure 5

The variables are:

E^0 = standard (reduction or cell) potential
R = Universal gas constant
T = Temperature, degrees Kelvin
n = number of electrons in reaction
F = Faraday constant (96,493 coulombs)

If room temperature (298° Kelvin) is assumed, and all constants are substituted (R, T and F), and multiplied by 2.303 to convert the natural log term to a $base_{10}$ log, the result is the form of the Nernst equation that is more commonly used for calculations (see far right side of Equation 5, **Figure 2**). Sometimes the ratio after the log term is expressed as:

$$\frac{[\ Reduced\ Species\ ]}{[\ Oxidized\ Species\ ]}$$

In words, the Nernst equation says that the voltage will change by 59.1 millivolts (0.0591 volts) for each decade change in the ratio of [reduced species] / [oxidized species]. Note also that this defines a linear relationship; the slope of our line is 0.0591 volts and the intercept is determined by E^0.

Using the Nernst Equation

This is a good time to try YOUR hand at the Nernst equation! We will be using a cell composed of a magnesium (Mg) half-cell and a manganese (Mn) half-cell. From the CRC Handbook:

$$Mg^{+2} + 2e \rightarrow Mg(s) \quad E^\circ \quad 2.375\ volts$$

$$Mn^{+2} + 2e \rightarrow Mn(s) \quad E^\circ \quad 1.029\ volts$$

1) If $[Mg^{++}]$ = 0.010M, and $[Mn^{++}]$ = 0.002M, what will be the measured potential of this cell? (Hint: first find the

overall redox and standard cell potential, then use the ratio of [products]/[reactants] in the Nernst equation.)

Now try it from the other direction:

2) If $[Mg^{++}]$ = 0.010M, and the measured potential is 1.3076 volts, what is the concentration of Mn^{++}?

(*Find the answers at the end of this paper, page 15.*)

In our example, we could keep concentrations of either half-cell constant and use the setup to measure the ion concentration in the other half cell.

So, an unknown ion concentration in an electrochemical cell can be easily determined by measuring the generated potential. In order for this to be a viable, "bench-level" tool, the electrochemical cell must be simplified, and produce reproducible, reliable results. The first step is to find a half-cell that can be used as a "reference" electrode, against which, other half-cells can be measured. A reference electrode is simply a half-cell that can produce a reasonably constant potential, even when it accepts or donates electrons to another half-cell.

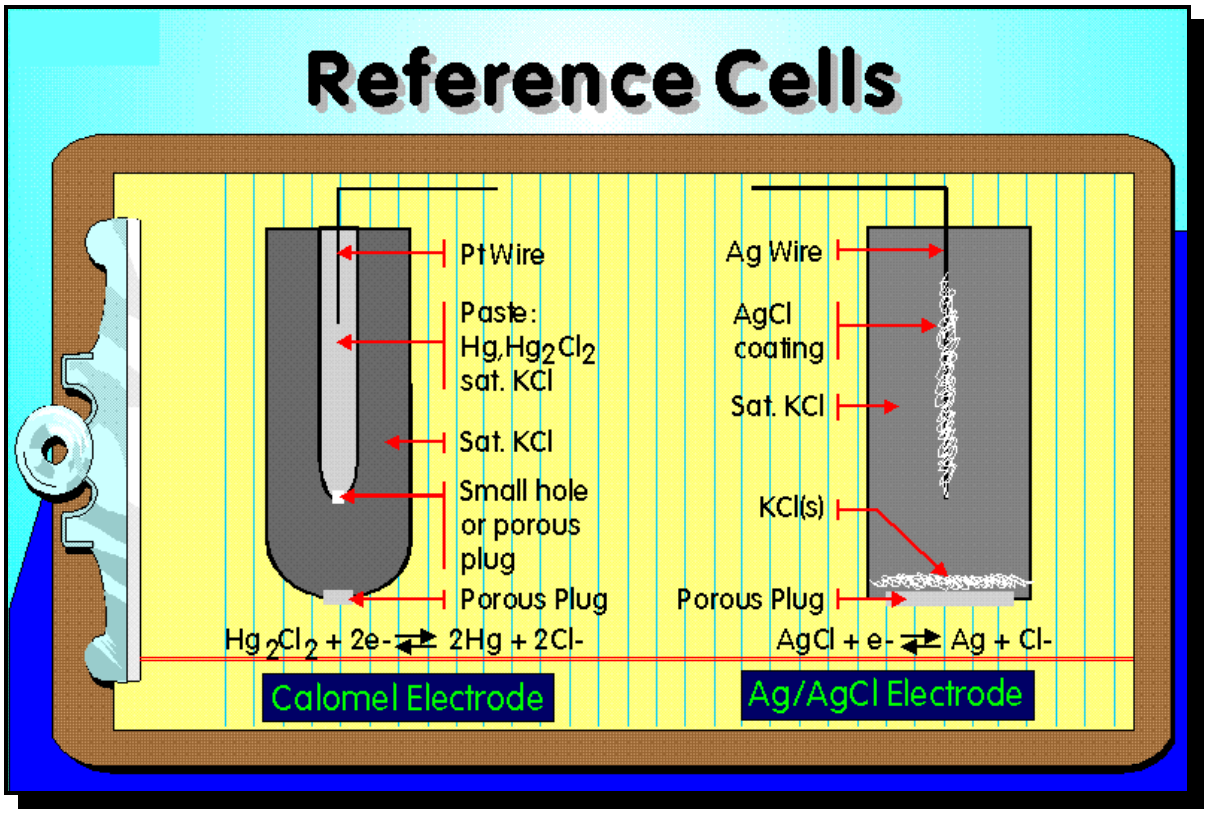

Figure 6

The SHE is not a good choice simply because it is cumbersome. As a result, other, more practical reference electrodes were developed. One of the more popular, early reference electrodes was the calomel electrode (**Figure 6**, left). The half-cell reaction for calomel is:

$$Hg_2Cl_2(s) + 2e \leftrightarrow 2Hg(l) + 2Cl$$

The Nernst equation ratio, determining potential is:

$$\frac{[Hg(l)]^2\ [Cl\ ]^2}{[Hg_2Cl_2(s)]}$$

The chloride ion is the only ionized species; the other terms have unity activities (by definition) and drop out. This means that the potential of the calomel half-cell (the calomel electrode) is directly dependent on the square of the chloride ion concentration. However, remember that in the calomel electrode, the calomel "paste" is saturated with KCl. So, as Cl^- is depleted or produced, additional Cl^- is ionized (from solid KCl) or precipitated (to solid) KCl, respectively. As a result, the $[Cl^-]$ concentration remains essentially constant, which, in turn, means that the potential of the calomel electrode remains essentially constant -- even during small current flows into or out of this electrode. This is the premier feature of an effective "reference electrode".

Today, the more common reference electrode is the silver/silver chloride (Ag/AgCl) electrode (**Figure 6**, right). It is very simple; it consists of a silver wire with a thin AgCl coating, in contact with a solution containing chloride ion. The reaction is:

$$AgCl(s) + e \leftrightarrow Ag(s) + Cl$$

The Nernst equation ratio, determining the potential of this electrode is:

$$\frac{[Ag(s)]\ [Cl\ ]}{[AgCl(s)]}$$

Again, Ag(s) and AgCl(s) have unity activities; so, the voltage of the Ag/AgCl electrode depends solely on chloride ion activity. In most Ag/AgCl electrodes the solution is either saturated with Cl^- or is at a very high concentration (over 2 molar). In either case, small currents into or out of this electrode produce insignificant $[Cl^-]$ changes, and, in turn, the potential remains constant.

The typical potentiometric method utilizes a reference electrode in conjunction with a "measuring", "sensing" or "indicator" electrode, which, itself, may be another half-cell or another type of electrode, such as a glass electrode (see below). The measured potential of the overall cell represents the sum of all potentials:

$$E_{cell} = E_{reference} + E_{indicator}$$

Since the reference electrode has a constant potential ($E_{reference}$), the cell potential (E_{cell}) will be dependent on the indicator electrode ($E_{indicator}$).

pH Measurements

Probably the most common scientific application for ISE's is measuring the concentration of hydrogen ion ($[H^+]$) in solutions, to determine pH. The ultimate reference technique for such determinations is by using a hydrogen electrode connected to another half-cell, whose activities are known and remain constant, allowing the H^+ concentration in the hydrogen electrode to vary. Clearly, this is at very least an inconvenient setup, requiring, among other things, a well regulated supply of hydrogen gas. Other drawbacks include high variability to temperature, and the oxidation or reduction of H gas by other solution constituents. Add to that the problems with a platinum electrode in proteinaceous biological solutions that coat the surface, and we are left with a very unsuitable pH measurement system.

The more reliable, convenient and reproducible method commonly employed uses what is known as a "glass membrane" electrode.

Glass Membrane Electrodes

Glass membrane electrodes are wholly different than those discussed so far, as they do not rely on oxidation or reduction reactions for measurements. A glass membrane is a very thin, ion-sensitive sheet of glass that is fused with a thicker, electrically isolated glass for support. In a modern "combination electrode", the pH sensitive glass membrane is the "bulb" at the end (see inset 1 in **Figure 7**).

The 3-dimensional molecular structure of an ion-sensitive glass membrane is represented by an irregular matrix of silicate tetrahedra. Each oxygen atom is connected to two silicon (Si) atoms. Other atoms, such as sodium, calcium, lithium, etc., may be scattered around, depending on the ingredients added to the glass in its molten state (see inset 3 in **Figure 7**). Notice that I described this as "ion-sensitive", not "pH sensitive"; there is good reason. The sensitivity of a glass membrane for various ions can be manipulated by varying the composition of the glass. It is also important to realize that these are used to produce "ion-selective" electrodes (varying sensitivities to a range of ions) and not "ion-specific" electrodes (sensitive ONLY to a specific ion). (There are actually very few "ion-specific" electrodes, one of which being the LaF_3, lanthanum fluoride, electrode used to quantitate F^- ions -- not a common clinical parameter.)

The following table shows three glass compositions and the resulting electrode response for various ions:

Constituents (Composition)	Sensitivity (Relative Response)
Na_2O-CaO-SiO_2 (21% - 6% - 72%)	**H^+ >>> Li^+, Na^+ > K^+**
Na_2O-Al_2O_3-SiO_2 (11% - 18% - 71%)	**Ag^+ > H^+ > Na^+ >> (350) (100) (1) Li^+,K^+, Cs^+ > Rb^+, NH_4^+ (0.001) (0.00003)**
Na_2O-Al_2O_3-SiO_2 (27% - 4% - 69%)	**K^+, Rb^+, NH_4^+, Na^+, H^+, (33) (17) (11) (4) (3) Li^+, Cs^+, Cu^+ (2) (1)**

TABLE I - Glass Electrode Composition / Response

All of those above are sensitive to H^+ to some extent; the first one being the better choice for pH measurements as it is the most sensitive to H^+ and gives much smaller responses to changes in Na^+ and K^+ ion concentrations. Note the last two listed which have the same constituents, but at different proportions. A dramatic change in the sensitivity is exhibited; in the case of H^+ and Na^+, the second glass shows a 100 to 1 response to H^+ compared to Na^+, while, the third glass has, effectively, equal responses to the same two ions.

Another feature evident in this table is that the response of all of the these glasses is to monovalent ions. Glass membranes show very little response to divalent (or greater) ionic species.

Glass Membrane Theory

The outermost surfaces of a glass membrane, in contact with a solution, are hydrated, much like a sponge, but to a much smaller extent (see inset 2 in **Figure 7**). This "gel" layer, which, typically is only 1 nanometer thick, interacts with the solution in a way very similar to an ion-exchange mechanism (see inset 3 in **Figure 7**). Various cations will populate available sites in the gel layers, on either side of the membrane, and generate an electrical potential, due to the uneven distribution of charge.

Inset 4 in **Figure 7**, illustrates Na^+ and H^+ ions, distributed on either side of a fixed section of a glass membrane, separating two solutions that we term internal and external solutions (IS and ES). The left side accommodates a total of 5 charges, while the right side (simulating a solution of lower $[H^+]$ / higher pH) "fits" only 4 charges in the same linear section. Because of this uneven distribution of charge, a potential difference exists across the membrane, which can be measured and ultimately determine pH. More accurately, the DIFFERENCE in pH between the solutions determines the potential difference. As long as one solution has a constant, known pH, then changes in the measured potential will be due solely to the other solution.

It should also be noted that NO ions pass into or out of the pH electrode through the glass membrane. This has been confirmed by studies using radioisotopes.

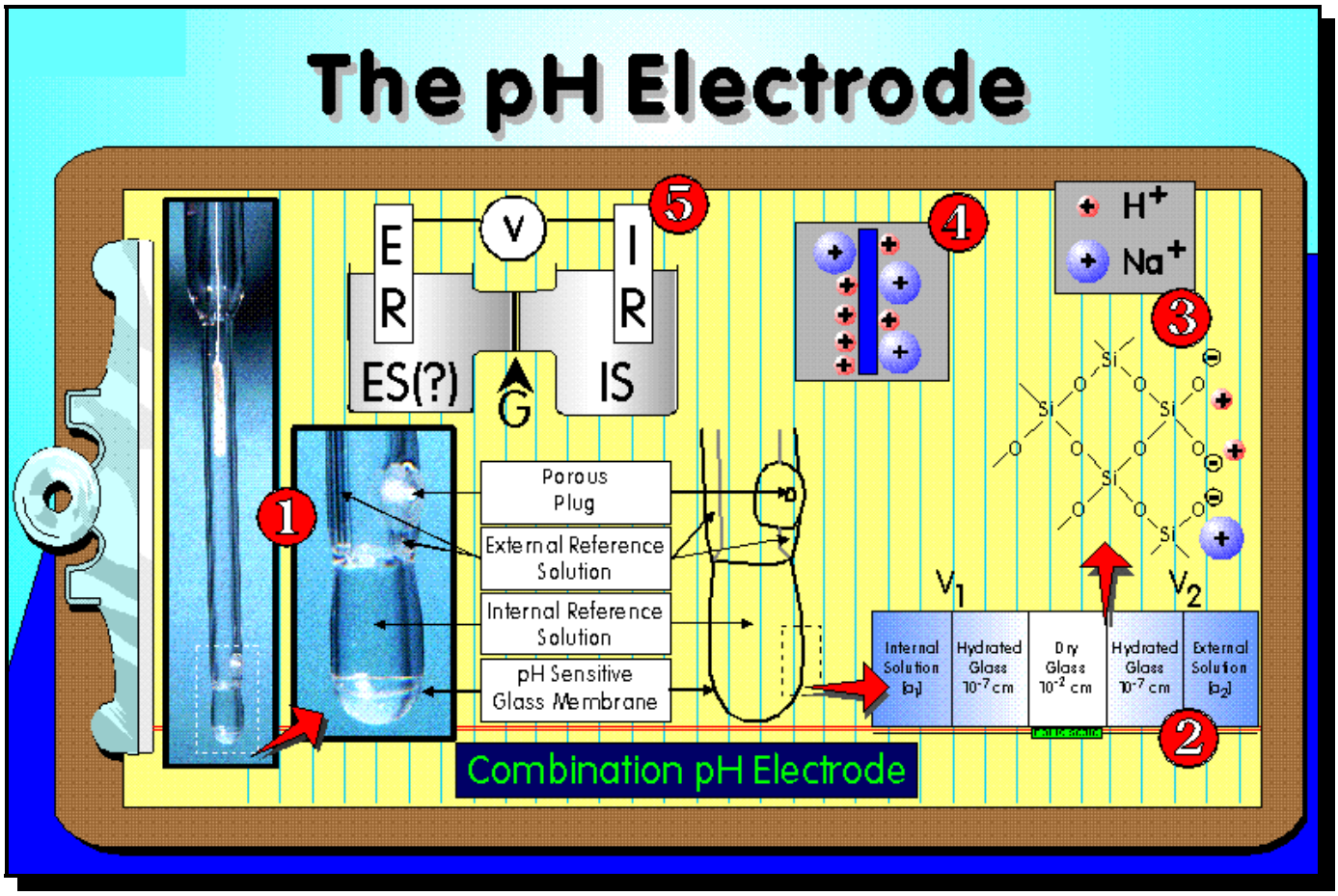

Figure 7

To measure the potential across the membrane, an electrical contact must be made with each of the two solutions. This is accomplished with two reference electrodes. Inset 5 in **Figure 7** schematically illustrates the complete setup. The solution under test, with an unknown pH, is "ES(?)". The solution with a constant, known pH is "IS" and is separated from the test solution by a pH sensitive glass membrane, "G". "ER" and "IR" are reference electrodes that are in electrical contact with the external and internal solutions, respectively. The voltmeter, "V", is connected between the two reference electrodes and measures the sum of all potentials in the system, E_{total}:

$$E_{total} = E_{ER} + E_b + E_{IR}$$

The potential across the membrane, E_b or the boundary potential, is composed of E_{V1}, E_{V2} and E_A. E_{V1} and E_{V2} are potentials between the solutions and the gel layers (see inset 2 in **Figure 7** for V1 and V2). E_A is an asymmetry potential which is unique to each glass membrane and due to the asymmetry between the two sides of the membrane. It is impossible to manufacture a glass membrane with identical surfaces on either side.

Expanding the equation above for E_b, we now have:

$$E_{total} = E_{ER} + [\ E_{V1} + E_{V2} + E_A\ ] + E_{IR}$$

Of all the above parameters, only E_{V2} will vary as the test solution varies; all other potentials will (or should) remain constant. So, changes in the measured potential (E_{total}) depends only on E_{V2}. Note that it is virtually impossible to predict E_{total}, and even with a constant set of solutions, will differ from membrane to membrane due to changes in the asymmetry potential, E_A. However, what IS predictable is that the measured potential will CHANGE by 59.1 millivolts for each unit change in pH for the solution under test.

Why 59.1 millivolts? Because changes in the measured potential are solely dependent on E_{V2}, which is a function of the hydrogen ion activity in the external solution. **Figure 8** shows the calculation.

Line 1, **Figure 8** is the hydrogen half-cell reaction. Line 2, **Figure 8** is the resulting Nernst equation. E is the overall measured potential; E' is a compound term: hydrogen's standard reduction potential (0.0000 volts), plus all other constant potentials, such as the internal and external reference electrode potentials.

The definition of pH (pH = -log [H+]), multiplied by -1 gives:

$$-pH = \log [H^+]$$

Substituting this into line 4, **Figure 8**, gives the expression in line 5. The final form in line 6 shows a linear relationship between measured potential and pH. The "y-intercept" of this line is the catchall E' parameter and will be different for any given set of electrodes and pH sensitive glass membrane. The "slope" of the line is 0.0591 volts (59.1 millivolts). So, as shown in inset 7, **Figure 8**, although we generally do not know what voltage is produced at any given pH, we do know that there will be a 59.1 mV CHANGE in potential for each unit change in pH.

pH Measurements

In the early days, pH sensitive glass membrane electrodes had an internal reference electrode, but measurements

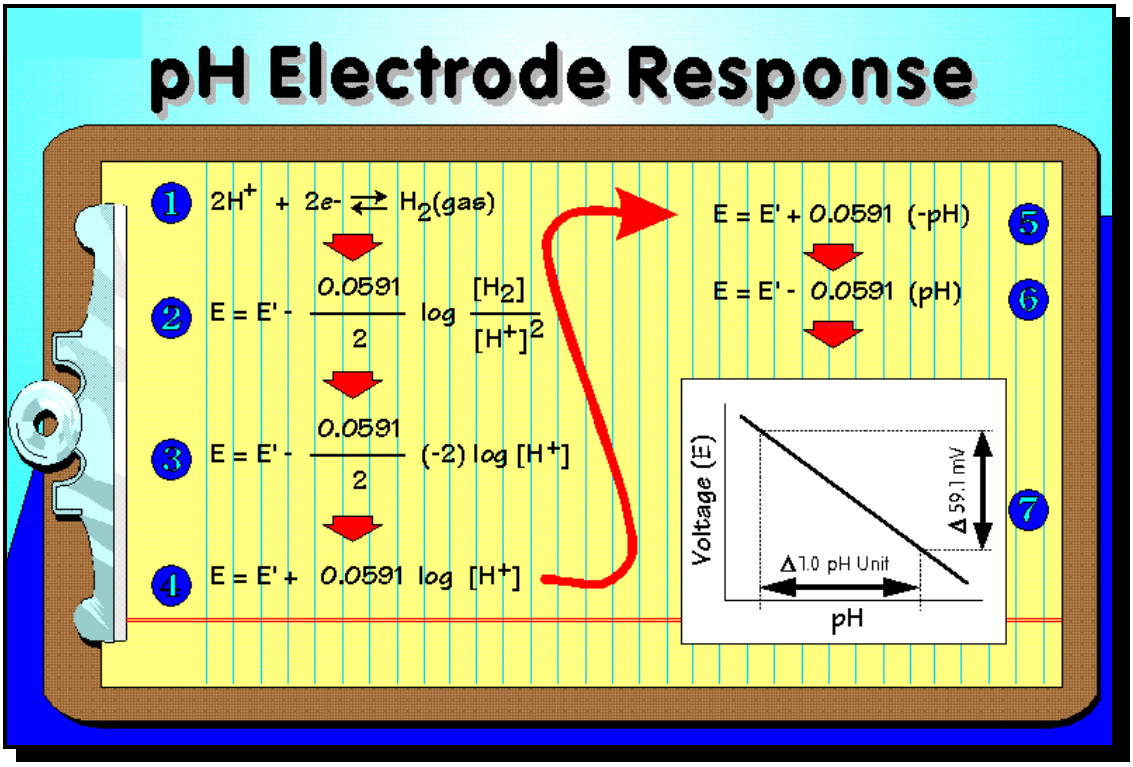

Figure 8

still required a separate external reference electrode. So, a dual-electrode arrangement was commonly seen. As with any glass membrane setup, a special voltmeter was required with an extremely high input impedance (on the order of 10^{15} ohms, or a thousand, million, million ohms), due to the high resistance of the glass membrane. A typical $30 voltmeter from Radio Shack has an input impedance of around 1 million ohms (10^6 ohms).

Remember that we are trying to measure the voltage (potential difference) across the glass membrane produced by a simple, uneven distribution of charge. No electrons are actually produced or consumed, as is characteristic with redox reactions in classic potentiometric techniques. If any significant amount of current is drawn by the attached voltmeter, we say that it "loads" the electrode and the measured potential drops to zero. The same effect may be observed if you try to start your car using a 12 volt lantern battery.

Early pH meters were expensive primarily due to the high input impedance requirement: a difficult feature to achieve using the discreet components of a few decades ago. Today, FET OP-AMP IC's (Field Effect Transistor Operational Amplifier integrated circuits) offer a "one-chip" solution to designing an amplification circuit with input impedances in the 10^{17} ohm range.

With off-the-shelf products available today, it is entirely possible to build a complete pH meter (sans electrode) for under $50!

Electrode Optimization

Most modern pH measurements use the so-called "combination electrode" (inset 1, **Figure 7**); this cleverly places the required external reference electrode right on the main indicator electrode. It is situated as a "collar" around the measuring electrode. Most combination electrodes use Ag/AgCl reference electrodes as both the external and internal types. They are simple, reliable and inexpensive.

When the combination electrode is placed into a test solution, the level of the fluid must be high enough to cover the pH sensitive glass membrane and the porous plug on the side; this insures electrical contact between the solution and the external reference electrode.

Trace the electrical paths in insets 1 and 5, of **Figure 7**:

Voltmeter -> ER -> ES(?) -> Glass membrane -> IS -> IR -> Voltmeter

pH Dynamics

Before leaving the subject of pH, let's consider the calibration procedure for a pH meter. At minimum, most pH meters will have two controls: CALIBRATION and TEMPERATURE (these may have slightly different labels on various instruments). The intent is to use a pH buffer standard and adjust the CALIBRATION control to read the proper value, while the TEMPERATURE control is set to the temperature of the solution. Right?

The answer depends on how accurate you want your pH readings. To perform a precision measurement, the TEMPERATURE control should NOT be set to the temperature of the solution. The preferred method is to use a pH 7.000 buffer and use the CALIBRATION control to set the read out to exactly 7.000. Then, using a different standard (pH 4 or pH 10 buffers are popular), use the TEMPERATURE control to actually set the read out to the value of this second standard.

Some may ask, "if that's the case, why did the manufacturer ***label*** the control the way it did; why is it ***labeled*** with TEMPERATURE?" To answer this, go back to the original Nernst equation (equation 5, in **Figure 2**). Notice that the Nernst equation can be categorized as a linear equation of the form: y = mx + b; where, m is the slope of the line and b is the y-intercept. Our independent variable (x) in the Nernst equation is the log term, while the dependent variable (y) is the total potential. The slope of the line is the term, RT/nF; and for a given reaction (fixing n) the only term that can vary is T -- the temperature! So, the slope of the line is proportional to temperature.

Why not set the TEMPERATURE control to the temperature of the solution? Generally, the scale used on this control is not terribly accurate. So, to define the line of voltage versus pH, we need to set two points. The first point is usually pH 7.000, the other point is usually some other pH in the range that the instrument will be used.

Electronically, the CALIBRATE control usually adjusts an amplifier offset voltage, moving our calibration line up and down, parallel to itself. The TEMPERATURE control is an amplifier gain control and adjusts the slope of the line (pivoting around 0 volts). It is important to always adjust CALIBRATE using the pH 7 standard first, then, use another buffer, adjusting the TEMPERATURE second. Reversing this procedure will require that you go back and re-adjust the TEMPERATURE control a second time. Do you see why?

If you have the **ph-cal.avi** file, accompanying this presentation, click the reel icon at left to view a movie of a typical pH meter calibration. In this example, we start with an uncalibrated instrument

(wrong intercept and slope). First, the pH electrode is immersed in a pH 7.00 buffer, and the CALIBRATE control is adjusted until the line crosses 0.00 volts. Next, the electrode is immersed in pH 4 or 10 buffer and the TEMPERATURE is adjusted until the line is at a 45° angle. The TEMPERATURE control pivots the line around the point where it intersects the 0.00 volt point.

After seeing how the line moves when calibrated properly, can you imagine what this would look like if we started with the pH 4 or 10 buffer, adjusted the TEMPERATURE control; then used the pH 7 buffer and adjusted CALIBRATE control?

If you look in the back of the manuals, buried in an obscure appendix, for some of the pH meters on the market, you'll see this procedure (setting a pH value with the TEMPERATURE control) described as a "precision measurement" technique.

Vitros® Potentiometric Slides

One of the most impressive fetes of optimization in ISE technology is seen in the *Vitros Chemistry System* from **Johnson & Johnson Clinical Diagnostics** (formerly, Kodak's Ektachem line). The "slides" for various ion measurements are actually disposable ISE's. A Vitros potentiometric slide is approximately 1" square, and 1/16" thick; it contains two complete ISE's, side by side. Two round holes are where a solution under test and a reference solution are applied. These two "wells" are connected by a piece of filter paper that functions like a salt bridge.

Each of the two electrodes is composed of four layers: 1) the top layer is an appropriate ion selective membrane, 2) an internal reference layer, 3) a layer of silver chloride and 4) and layer of silver. After the solutions are applied in the instrument, the slide is incubated for 3 minutes to allow for equilibration. The instrument then places two electrical contacts on two areas of the slide that look like small mirrors (actually metallic contacts to the electrodes). The voltage is measured, the analyte concentration is determined and recorded, and the whole slide is discarded!

Other Types of Membranes

An interesting array of ISE's exist to measure various other ions (**Figure 9**). The liquid membrane electrode uses a porous, hydrophobic plug, which is in contact with a reservoir containing a liquid, organic ion-exchanger. This saturated barrier separates the internal (aqueous) solution from the solution under test. The internal solution also has the requisite reference electrode.

The ion-exchanger and membrane can be changed to yield selectivities to various ions. For example, when the calcium salt of bis-(2-ethyl hexyl) phosphoric acid (d2EHP) is dissolved in a variety of long chain (straight chain) alcohols, the resulting electrode is selective for changes in Ca^{++} ion. The undissociated $Ca(d2EHP)_2$ molecules in the matrix of the membrane are free to diffuse throughout the membrane, but are not soluble in the aqueous solutions on either side of the membrane. At each membrane interface, these "transport" molecules are free to exchange their Ca^{++} ions with those in the aqueous solutions -- moving Ca^{++} between the internal solution and the solution under test. This continues until an equilibrium is established.

A variation on this idea is very similar to the way in which biological membranes exhibit their extraordinary selectivity for certain ions. Biological membranes are similar to "neutral extraction membranes" which are liquids with a low dielectric constant. An example would be decane with a micromolar to millimolar concentration of "neutral carriers", such as, valinomycin, nonactin or monactin. These molecules do not have charged groups and are extremely non-polar, however, they are abundant in ring structures that are rich in oxygen. The size of these groups plus the energetics involved allow an ion-dipole interaction whereby the hydration shell of a cation can be replaced by the ring structure -- ie, certain ions at a membrane-aqueous interface can "drop into" various slots in the normally non-miscible membrane. Once again, an equilibrium is established as ions are transported across the membrane.

Valinomycin membranes show extremely high selectivity for K^+, compared to Na^+ (close to 4000 times more selective), or H^+ (18,000 times more selective). Compare that to an optimized K^+ selective glass membrane which has, at best, a 30 fold selectivity over Na^+.

The selectivity of these electrodes is predominantly dependent on the selectivity of the ion-exchange process, and is not always impressive, however, by selecting an appropriate ion-exchanger, electrodes can be produced with selectivities for both cations and anions, and for multivalent ions -- not just the monovalent species of glass membrane electrodes.

Precipitate-Impregnated and Solid State Electrodes

These two types are grouped together because they are very similar. A precipitate-impregnated electrode is characterized by some sort of highly insoluble salt, imbedded into the matrix of some sort of non-reactive material (see **Figure 9**). For example, pellets of a silver

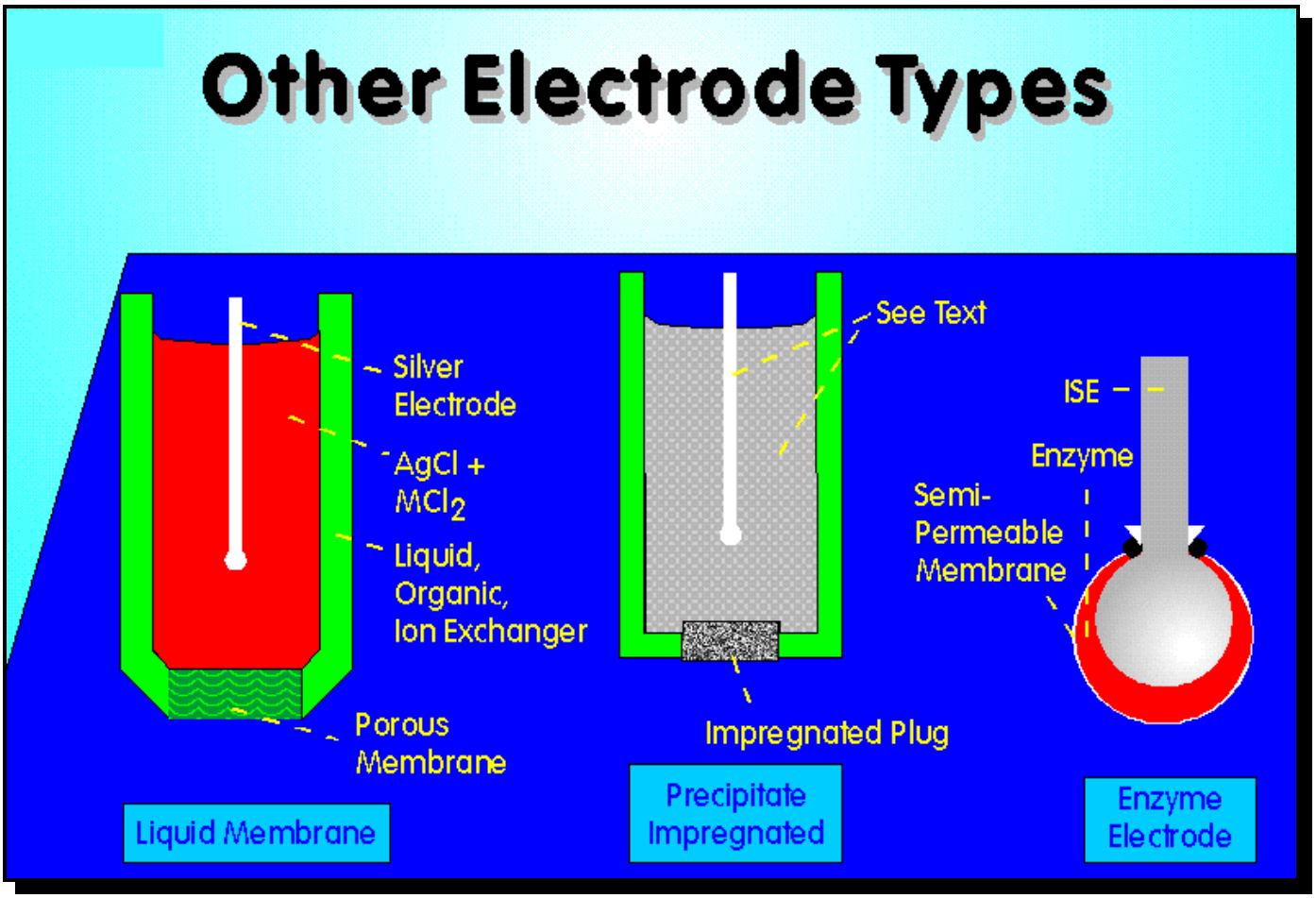

Figure 9

halide can be imbedded into a silicone rubber matrix, producing a halide sensitive membrane. For example, AgCl could be used for a Cl^- selective membrane; AgBr or AgI can be used for Br^- or I^- selective membranes, respectively. The internal solution must also contain a fixed concentration of the ion of interest (Cl^-, Br^-, I^-, etc.). Electrodes of this type are rugged and exhibit very good reproducibility.

Solid state electrodes are constructed in a manner very similar to the devices described above, but have a crystalline membrane in place of the precipitate-impregnated rubber matrix. An example of a crystalline type is LaF_3 (doped with a small amount of Europium to lower the electrical resistance of the crystal); this creates a fluoride (F^-) sensitive electrode. Electrical conduction through the crystal is achieved by "faults" in the crystalline lattice where fluoride atoms can move about. Since no other atom can enter the crystalline lattice due to size, shape and charge characteristics, it will only respond to changes in F^- activity across the membrane. As a result, solid state electrodes can truly be called, ion-specific electrodes as they are sensitive virtually no other species than that which can move through the lattice.

The response of LaF_3 electrode depends solely on the ratio of inside to outside F^- activities:

$$\frac{[F\ ]_{int}}{[F\ ]_{ext}}$$

Enzyme Electrodes

As shown on the far right of **Figure 9**, a whole new array of molecular-sensitive electrodes can be created by using a common cation-sensitive electrode (H^+, NH_4^+, etc.) and a "bag of enzymes". The "bag" must be a semipermeable membrane, allowing free diffusion of the molecule of interest, and it must contain an enzyme to break down that molecule into products that can be detected by the glass electrode inside the bag.

For example, urease can be immobilized in a layer of acrylamide gel around the bulb of an ammonium ion glass electrode, and held in place by a nylon netting or cellophane film. When placed in a test solution containing urea, it will diffuse through the outer support to the urease-gel layer, where it will be enzymatically broken down, yielding ammonium ions which are then sensed and measured as a proportional potential from the glass electrode.

This technique has the specificity that is characteristic for an enzyme assay (generally high). Alternatively, this could also be turned around where a substrate is immobilized on a glass electrode, and the corresponding enzyme is sensed by the electrode. All sorts of possibilities have been explored; from the simple urea-electrode described, to systems for detecting and quantitating complement.

Selectivity Constants

Since we have already alluded to the "selectivities" of various electrodes on a rather intuitive level, let's formally define SELECTIVITY CONSTANTS, which are used to determine the applicability of a particular electrode for a required task, and to compare electrodes. The selectivity constant (or coefficient) for an electrode is a measure of how it measures the activity of one ion, while in the presence of a second (interfering) ion. They are not absolute values, but comparisons of response. So, for a selectivity constant to make sense, you must know what ion is being measured and what the other competing ion is.

Algebraically, the relation is seen in equation 6 of **Figure 2**, where,

a	=	activity of the ion under test
a_i	=	activity of the interfering ion
k_i	=	selectivity constant of the test ion over the interfering ion
z	=	ionic charge of the ion under test
z_i	=	ionic charge of the interfering ion

The formula indicates that k_i represents a fraction of response attributable to a second ion. Selectivity constants can range from 0 (no interference) to very large values (selectivity for the interfering ion). A k_i of 1.000 means that the electrode exhibits equal response to each ion.

As an example, consider a Na^+ (a) electrode with a selectivity over K^+ (a_i) of 0.01. This indicates that the potential change, attributable to a given change in Na^+ concentration, could also be achieved by a 100 times larger change in K^+ concentration.

When expressed in the form of equation 6, **Figure 2**, smaller values of k_i indicate greater selectivity. Unfortunately, there are other ways of expressing k_i in a reciprocal format. In the previous example, the selectivity coefficient may be given as 100, instead of 0.01. For any specified selectivity coefficient, how does one evaluate the electrode? It must be taken in context. If a journal article talks about a potassium electrode with a k_i of 0.01 over sodium, then it must be assumed that it is 100 times more sensitive to the potassium ion. On the other hand, if a vendor catalog lists a potassium electrode with a selectivity of 100 over sodium, then it must be assumed to be comparable to the one described in the journal article.

The Big Picture

This paper focused on ion selective electrodes, which are predominantly examples of potentiometric methods of analysis. To be sure we see this in context, it is important to note that the realm of electrochemical methods, includes other important techniques, listed below:

Analysis Type	**Measured Quantity**
Potentiometry	Voltage (V) (zero current)
Coulometry	Product of Current and Time
Voltammetry / Polarography	Current (I) at an applied V
Conductometry	Conductance (1/R)

Most electrochemical methods can be classified into one of the above categories. In all cases, the Measured Quantity is related back to analyte concentration.

Many laboratorians who recall the old Büchler-Cotlove chloridometers should recognize this as a coulometric technique. With this instrument, current it held constant (providing a constant source of silver ions, Ag^+); when all the chloride ion has precipitated (AgCl), the excess Ag^+ causes an increase in the solution's conductivity, which signals the endpoint of this electrochemical titration. The chloride ion concentration will be proportional to time. The instrument is calibrated, in this case, by adjusting the Ag^+ generation rate, which, effectively, adjusts the titration time.

In a future presentation, we will examine more carefully some of these other techniques.

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Answers to the Problems

1) 1.3253 volts,

2) $[Mn^{+2}] = 0.0005$ M

... did you get these values?

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