ELEMENTARY ASPECTS OF IONIC EQUILIBRIA

(A self-instructional package)

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INTRODUCTION
Ions are of great importance in the function of all the cells of the body. For example, ions are vital in controlling the action of enzymes. Divalent cations (Ca\(^{2+}\), Mg\(^{2+}\)) are especially important in this regard. The concentration of Ca\(^{2+}\) regulates the onset and the intensity of muscle contraction. The distribution of Na\(^{+}\) and K\(^{+}\) across the cell membranes of muscle cells results in the resting membrane potential that is vital for the excitability of these cells. The movements of Na\(^{+}\) and K\(^{+}\) in response to changing membrane permeabilities causes the action potential. The purpose of this unit is to help you learn about ions in equilibrium across cell membranes and the use of the principles of equilibria. You will understand certain aspects of cellular function in these terms and this will provide a basis for your subsequent study of ion flows across cell membranes, resting membrane potentials, and action potentials.

OVERVIEW AND OBJECTIVES
This package will help you learn the meaning of ionic equilibrium. First you will study the concept of the "electrochemical potential" of an ion and how you can use this concept in understanding the energetics and the direction of ion movement. Next you will learn about the Nernst Equation that describes mathematically some consequences of chemical equilibrium. Finally you will consider the Gibbs-Donnan Equilibrium, a cellular equilibrium characterized by certain ions being in the equilibrium across the plasma membrane, while other ions are impermeant to the membrane.

Learning Objectives

I. You will learn the definition and some of the uses of the concept of the electrochemical potential of an ion.

A. You will write the equation that defines the electrochemical potential difference (\(\Delta \mu\)) of an ion across a membrane.
B. You will know from the sign of \(\Delta \mu\) whether energy is required for a given ionic flow or whether energy can be produced by that ionic flow.

II. You will learn the Nernst Equation that describes the equilibrium of an ion across a membrane.

A. You will write the Nernst Equation.
B. You will recognize different phrases that are synonymous with or that imply that the distribution of a species of ion on the two sides of a membrane satisfies the Nernst Equation.
C. You will use the Nernst Equation to calculate the electrical potential (voltage) difference across a membrane or the ratio of ionic concentrations on the two sides of a membrane for an ion in equilibrium across the membrane.

III. You will learn that the Gibbs-Donnan Equilibrium involves ionic species to which are impermeant to the membrane. You will be able to calculate the following properties of a Gibbs-Donnan Equilibrium: the final concentrations of permeant and impermeant ionic species involved on both sides of the membrane and the transmembrane electrical potential difference (voltage).
**PRACTICE CYCLE #1**

**INPUT 1:** Earlier you learned that if an uncharged molecule is distributed unequally across a membrane to which it is permeable, the substance will diffuse from the side where it is more concentrated to the side where it is less concentrated. In considering ions, however, you also need to know if there is an electrical potential (voltage) difference across the membrane. This is because an ion bears a charge and is therefore affected by an electrical field as well as a concentration difference.

Consider the situation as diagrammed below. There is no difference in the concentrations of NaCl on the two sides of the membrane. Thus no net flow of either Na⁺ or Cl⁻ will occur because of a concentration difference. Note, however, that there is a voltage difference (100 millivolts, abbreviated mV) between the two chambers, with side A being positive with respect to side B and side B negative with respect to side A. A positive ion (a cation like Na⁺) will be repelled from the positively charged chamber and attracted to the negatively charged chamber. A negative ion (an ion like Cl⁻) will be repelled from the negatively charged chamber and attracted to the positively charged chamber.

![Diagram](image)

**PRACTICE 1:** The diagram below shows two solutions separated by a membrane. There is an electrical potential (or voltage) difference between the two chambers the magnitude (60 mV) and direction (side A negative) of which are indicated in the diagram.

![Diagram](image)

1. In which direction will K⁺ tend to flow due to the concentration difference?
   ______A to B, ______B to A, _______ no net flow.

2. In which direction will K⁺ tend to flow due to the electrical potential difference?
   ______A to B, ______B to A, _______ no net flow.

3. In which direction will Cl⁻ tend to flow due to the electrical potential difference?
   ______A to B, ______B to A, _______ no net flow.
FEEDBACK 1:
1. Did you check no net flow for Question 1? That is right because in the absence of a concentration difference of K⁺ between chambers A and B, no net flow due to a concentration difference will occur.

2. Did you say that K⁺ will tend to flow from B to A due to the electrical potential difference? Good. This is because a positively charged ion will be repelled from the positively charged chamber and attracted to the negatively charged chamber.

3. The answer to Question 3 is A to B. Since Cl⁻ has opposite charge to K⁺ the force exerted on Cl⁻ by the electric field will be opposite in direction to that on K⁺. In other words, Cl⁻ will be repelled from the negatively charged chamber and attracted to the positively charged chamber.

PRACTICE CYCLE #2

INPUT 2: In considering ion flow in the presence of a concentration difference for that ion and an electrical potential difference, we need to consider both factors in deciding which way the ion will flow. If there is a concentration difference tending to cause K⁺ to flow from chamber A to B and also an electrical potential difference tending to cause K⁺ to flow from A to B, these tendencies will be additive and a flow of K⁺ from A to B will occur that is larger than that caused by the concentration difference alone or by the electrical potential difference alone. If, on the other hand, if the concentration difference of K⁺ tends to make K⁺ flow A to B, but the electrical potential difference tends to cause K⁺ to flow from B to A, then we have opposing tendencies. In order to know whether a net flow of K⁺ will occur, and if so, in which direction we need to be able to compare quantitatively the strength of the opposing tendencies (the "concentration force" versus the "electrical force").

PRACTICE 2: The diagrams below show two solutions separated by a membrane permeable to Na⁺. In each case, can you tell which direction Na⁺ will tend to flow?

1. ______ A to B,      ______ B to A,      ______ can't tell.
2. ______ A to B,      ______ B to A,      ______ can't tell.
**FEEDBACK 2:** Did you check A to B for example 1? Good. The concentration of Na\(^+\) being higher in A makes Na\(^+\) tend to flow from A to B. Note that the excess electronegativity in chamber B also tends to cause Na\(^+\) to flow from A to B. If you checked “can't tell” for example 2, you are right. As in the first example, the concentration difference tends to make Na\(^+\) flow from A to B, but now the electrical field is reversed, so that this force tends to cause Na\(^+\) to flow from B to A. Not knowing which tendency is larger, you can't tell which direction Na\(^+\) will flow.

**PRACTICE CYCLE #3**

**INPUT 3:** You can see from the last example that we are in need of a way to compare the tendency of an ion to flow due to a difference in concentration and its tendency to flow due to an electrical potential difference. Since electrical potential difference (\(\Delta E\)) is measured in volts and concentration difference (\(\Delta C\)) is measured in moles/liter, it is not clear how you can compare the two tendencies or forces. The fathers of physical chemistry defined a quantity known as the **electrochemical potential** of an ion (\(\mu\)) that will help you out of your dilemma. The expression for the difference in electrochemical potential (\(\Delta \mu\)) of an ion, say Na\(^+\), across a membrane is the difference between \(\mu\) on side A and \(\mu\) on side B

\[
\Delta \mu_{Na} = \mu_A - \mu_B = RT \ln \frac{[Na]_A}{[Na]_B} + zF(E_A - E_B)
\]

where \(R\) is the gas constant, \(T\) is the absolute temperature (in \(^\circ\)K), \(\ln\) is the natural logarithm (log to the base e), \([Na]_A\) and \([Na]_B\) are the Na\(^+\) concentrations on side A and B, respectively, \(z\) is the charge number for the ion (+1 for Na\(^+\), -1 for Cl\(^-\), +2 for Ca\(^{2+}\), etc.), \(F\) is Faraday’s number (96,500 coulombs/mole), and \(E\) is the electrical potential (for example in volts). The units of \(\Delta \mu\) are energy/mole, typically Cal/mole or joule/mole.

This last equation tells you exactly how to add up the tendency for Na\(^+\) to flow because of a concentration difference (this is the first term on the right hand side of the equation) and the tendency for Na\(^+\) to flow due to an electrical potential difference (the second term on the right hand side). For pedagogical purposes we will call the first term the **concentration force** and the second term the **electrical force**.

**Concentration force:** \(RT \ln \frac{[Na]_A}{[Na]_B}\)

**Electrical force:** \(zF(E_A - E_B)\)
PRACTICE 3:  
Which way will Na\(^+\) tend to flow if \(\Delta\mu\) is a positive number? If \(\Delta\mu\) is a negative number? If \(\Delta\mu\) is zero?

FEEDBACK 3:  
If \(\Delta\mu_{Na}\) is positive (this means \(\mu_{Na(A)} > \mu_{Na(B)}\)) the net tendency will be for Na\(^+\) to flow from A to B. If \(\Delta\mu_{Na}\) is negative, then \(\mu_{Na(A)} < \mu_{Na(B)}\) and Na\(^+\) will tend to flow from B to A in the net sense. If \(\Delta\mu\) is zero that means that the electrochemical potential of Na\(^+\) is the same on sides A and B, so there will be no net flow.

Net flow will spontaneously occur from where the electrochemical potential \(\mu\) is higher to where it is lower.

PRACTICE CYCLE #4

INPUT 4: To restate what you just learned RT\(\ln \frac{[Na]_A}{[Na]_B}\) represents the tendency for Na\(^+\) to move from A to B due to the concentration difference and \(zF(E_A - E_B)\) represents the tendency for Na\(^+\) to flow from A to B due to the electrical potential difference (voltage) across the membrane. If either term turns out to have a negative value, this tells you that it makes the ion tend to flow from B to A. Thus both the magnitude and direction of each "force" or tendency can be obtained.

If the "concentration force" and the "electrical force" are equal in magnitude, but opposite in sign, \(\Delta\mu = 0\). This means that the concentration force and the electrical force are equal and opposite, so that the net force on the ion is zero. \(\Delta\mu = 0\) is the definition of equilibrium and means the ion has no net tendency to flow in either direction.

PRACTICE 4: Given the situations described in the sketch below, let's determine the directions of the "concentration force" and the "electrical force".

\[z = +1\text{ for } K^+\text{. Since side A is electrically negative with respect to side B, } E_A - E_B \text{ must be a negative number. Plugging in the other values from the diagram we obtain:}\]

\[\Delta\mu_K = RT \ln \left(\frac{1}{0.1}\right) + F (-0.1). \text{ (Recall that } \ln x \text{ is positive if } x > 1. )\]
1. Use the signs of the terms in $\Delta \mu_K$ equation to tell which way the "concentration force" and the "electrical force" tend to make $K^+$ flow.

   a) "concentration force": ______A to B, ______B to A, _____no force.
   b) "electrical force": ______A to B, ______B to A, _____no force.

2. If you could calculate the magnitude of each term how would you decide the direction of net $K^+$ flow?

**FEEDBACK 4:**

1) Did you say a) "concentration force" is directed from A to B and b) "electrical force" is from B to A. That's great. Note that this result coincides with the result you obtained in Practice Cycle #2 by using more ordinary reasoning. If you missed this one, here is how you might have gotten it:

   a) "concentration force" = $RT \ln \frac{[Na]_A}{[Na]_B} = RT \ln(1/0.1) = RT \ln 10$. $R, T$, and $\ln(10)$ are all positive numbers. Thus the "concentration force" must be positive and thus denotes an A to B tendency.

   b) "electrical force" = $zFE_A - E_B = (+1)F(E_A - E_B)$. $F$ is positive, $E_A - E_B = -0.1$ volt, so that "electrical force" is negative and thus denotes a B to A tendency.

2. If you calculated the magnitude of each term, you could add the two terms algebraically to decide whether $\Delta \mu$ is positive (A to B flow), negative (B to A flow) or zero (no net flow).

**PRACTICE CYCLE #5**

**INPUT 5:** The last example showed us that in order to tell the direction of net ion movement we need to be able to calculate the magnitudes of the "concentration force" and the "electrical force", as well as their directions. If $\Delta \mu$ is positive, this means that $K^+$ will tend to flow spontaneously from A to B, if you want to force $K^+$ to flow the other direction you must do work on the $K^+$ ions. The units of $\Delta \mu$ are energy/mole and $\Delta \mu$ represents the minimum amount of work you must do to move 1 mole of $K^+$ ions against a tendency to flow from A to B spontaneously. If we allow $K^+$ to flow according to the forces acting on it and it flows from A to B (since $\Delta \mu_K$ is positive) it is theoretically possible to harness the energy lost by the $K^+$ ions in flowing from higher $\mu$ to lower $\mu$ to do some other sort of work. The maximum amount of work that can be done by one mole of $K^+$ flowing down its electrochemical potential difference is $\Delta \mu$. 
**PRACTICE 5:** In the example shown below, $\Delta \mu$ of Li$^+$ (the difference in electrochemical potential of Li$^+$ on side A minus that on side B) equals -3923 joule/mole.

In which direction will Li$^+$ tend to flow?

Is energy required to cause Li$^+$ to flow from B to A?

Can Li$^+$ flowing from B to A do work? How much work?

Is work required to make Li$^+$ flow from A to B? How much work?

**FEEDBACK 5:**

$\Delta \mu_{Li} = \mu_{Li}(A) - \mu_{Li}(B)$ is negative. This means that $\Delta \mu_{Li(B)}$ is larger than $\Delta \mu_{Li(A)}$. So Li$^+$ will flow spontaneously from B to A. No energy input is required for flow from B to A. In flowing down its electrochemical potential difference, Li$^+$ releases energy that is theoretically available to do work. The maximum amount of work that can be done is 3923 joules per mole of Li$^+$ that flows. It takes energy to move Li$^+$ from A to B. The minimum amount of work required to cause 1 mole of Li$^+$ to flow from A to B is 3923 joules for each mole of Li$^+$ that flows.

**PRACTICE CYCLE #6**

**INPUT 6:** Remember I promised you would learn something about ionic equilibrium? With what you just learned as background, this will be easy. For an ion to be in equilibrium across a membrane, $\Delta \mu$ across the membrane must be zero. This will happen when the "concentration force" and the "electrical force" on the ion are equal and opposite, so that the net force on the ion is zero. When this is true, we say that the ion is in equilibrium. Under such conditions there will be no net flow of the ion.

$\Delta \mu = 0 = RT \ln \frac{C_A}{C_B} + zF(E_A - E_B)$, which leads to (algebra):

$E_A - E_B = -\frac{RT}{zF} \ln \frac{C_A}{C_B} = \frac{RT}{zF} \ln \frac{C_B}{C_A}$ (Recall that $-\ln x/y = \ln y/x$)

This is the Nernst Equation, named after the great German physical chemist Nernst. Please note that it is neither more nor less than a statement of electrochemical equilibrium ($\Delta \mu = 0$). This equation holds only if equilibrium exists for the ion in question.
PRACTICE 6:

Statement: The distribution of X- across the membrane "satisfies" or is consistent with the Nernst Equation. Please check the statements below which are synonymous with or implied by that statement.

1._________we do not know which direction X- will flow.
2._________X- will show no net flow.
3._________\( \Delta \mu_x \) is positive.
4._________\( \Delta \mu_x \) is negative.
5._________\( \Delta \mu_x = 0 \).
6._______There is no transmembrane voltage.
7._______There is no "concentration force" on X-.
8._______The "concentration force" is equal and opposite to the "electrical force" on X-.
9._______X- will flow from high \( \mu \) to low \( \mu \).
10._______The flow of X- will obey Fick's First Law.

FEEDBACK: Did you check 2, 5, and 8? Good. \( \Delta \mu_x = 0 \) (answer #5) is the definition of equilibrium. Since the statement said the Nernst Equation is satisfied, you know you are dealing with an equilibrium situation. An ion at equilibrium has no net force on it: so that "concentration force" + "electrical force" = 0 as you said for answer #8. With no net force there will be no net flow (answer #2). The other answers are either ruled out by the correctness of #2, #5, and #8, or have nothing to do with the original statement.

Good. Now, you will get to see what the Nernst Equation is really good for.

PRACTICE CYCLE #7

INPUT 7: Since the Nernst Equation, you just told me, is a statement meaning that the "concentration force" is equal and opposite to the "electrical force", you can use the Nernst Equation as written: 

\[
E_A - E_B = \frac{RT}{zF} \ln \frac{C_B}{C_A} = \frac{2.303RT}{zF} \log \frac{C_B}{C_A}
\]

to calculate the value that the electrical potential difference \( E_A - E_B \) must have to balance the concentration force due to the
difference between $C_B$ and $C_A$. Now we will also learn that if we know $E_A - E_B$, we can also calculate the concentration ratio $C_B/C_A$ that will set up a "concentration force" just equal and opposite to the "electrical force" created by $E_A - E_B$.

To make calculations a bit easier, you should know that $2.303RT/F$ at physiologically-relevant temperatures equals approximately $60 \text{ mV}$ (0.06 volt). So at room temperature

$$E_A - E_B = \frac{60 \text{ mV}}{z} \log_{10} \frac{C_B}{C_A} = -\frac{60}{z} \log_{10} \frac{C_A}{C_B}$$

Let's consider two examples. In the situation diagrammed just below, what potential difference must exist between A and B in order that Na$^+$ will be in equilibrium (the "electrical force" must be equal and opposite to the "concentration force").

Since equilibrium is to exist, the Nernst Equation applies

$$E_A - E_B = \frac{60 \text{ mV}}{z} \log_{10} \frac{C_{Na(B)}}{C_{Na(A)}} = \frac{60 \text{ mV}}{z} \log_{10} \frac{0.1}{1} = 60 \text{ mV} \log_{10}(0.1) = -60 \text{ mV} \log(10) = -60 \text{ mV}$$

This says that $E_A - E_B$ required to balance the "concentration force" is minus 60 mV. Thus A is negative relative to B. Check this out with reference to the diagram below. The "concentration force" tends to cause Na$^+$ to flow from A to B, so if equilibrium is to exist the "electrical force" must cause Na$^+$ to flow from B to A. If A is negative relative to B, Na$^+$ will be attracted to A and repelled by B, so the flow due to the "electrical force" will be from B to A. Using the Nernst Equation you get the correct sign for $E_A - E_B$, (if you do the algebra right). But you can also obtain the correct sign for $E_A - E_B$ from the sort of reasoning we just applied.
Now let’s use the Nernst Equation to find the equilibrium concentration ratio of an ion that is required to balance a given “electrical force”. In the example below, what will be the equilibrium concentration of \( \text{Cl}^- \) in chamber B?

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 M</td>
<td>NaCl</td>
<td>? M NaCl</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>60 mV</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Solving the Nernst Equation for \( \log \left[ \frac{[\text{Cl}^-]_B}{[\text{Cl}^-]_A} \right] \), we obtain

\[
\log \left( \frac{[\text{Cl}^-]_B}{[\text{Cl}^-]_A} \right) = \frac{z_{\text{Cl}^-}(E_A - E_B)}{60 \text{ mV}} = \frac{-1(-60 \text{ mV})}{60 \text{ mV}} = +1
\]

taking the antilog of both sides we get

\[
\frac{[\text{Cl}^-]_B}{[\text{Cl}^-]_A} = 10
\]

Since \([\text{Cl}^-]_A = 1\), then \([\text{Cl}^-]_B = 10 \text{ M}\)

Let’s see if this makes sense. Since side A is negative with respect to B and since \( \text{Cl}^- \) bears a negative charge, the "electrical force" will tend to cause \( \text{Cl}^- \) to flow from A to B. Thus for equilibrium to exist the "concentration force" must tend to cause \( \text{Cl}^- \) to flow from B to A. That is \([\text{Cl}^-]_B \) must be greater than \([\text{Cl}^-]_A\), as we found. By now you probably have got the message that a 60 mV potential balances a 10-fold concentration difference of an univalent ion, and vice versa, provided the "electrical force" and "concentration force" are in opposite directions.

**PRACTICE 7:** Refer to the sketches below:

1) Calculate \( E_A - E_B \) if \( \text{Cl}^- \) is in equilibrium across the membrane.

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 M</td>
<td>KCl</td>
<td>0.1 M KCl</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>? mV</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

2) Compute \( \text{Ca}^{2+} \) concentration on side B if \( \text{Ca}^{2+} \) is in equilibrium across the membrane.

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 M</td>
<td>CaSO(_4)</td>
<td>? M CaSO(_4)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>30 mV</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
FEEDBACK 7: Did you get +60 mV for (1) and 0.1M for (2)? Good. If not, here's how:

1) \( E_A - E_B = \frac{60 \text{mV}}{z} \log \frac{[\text{Cl}^-]_B}{[\text{Cl}^-]_A} = \frac{60 \text{mV}}{-1} \log \frac{0.1}{1} = 60 \text{mV} \)

(Since this is positive, A is positive with respect to B.)

2) \( \log \frac{[\text{Ca}^{2+}]_B}{[\text{Ca}^{2+}]_A} = \frac{z}{60} (E_A - E_B) = \frac{2}{60} (-30) = -1 \) so \( \frac{[\text{Ca}^{2+}]_B}{[\text{Ca}^{2+}]_A} = 0.1 \). Since \([\text{Ca}^{2+}]_A = 1\text{M}, then \([\text{Ca}^{2+}]_B = 0.1 \text{M}. \)

Note that since the valence of \( \text{Ca}^{2+} \) is 2, it is affected twice as strongly by a given electrical potential difference as is an univalent ion. Thus for a divalent ion, only 30 mV is required to balance a 10-fold concentration difference (rather than the 60 mV required to balance a 10-fold difference of an univalent ion).

PRACTICE CYCLE #8

INPUT 8: With what you have learned so far, you are in a position to learn about the Gibbs-Donnan Equilibrium. This is a special sort of ionic equilibrium that obtains when some of the ions involved cannot cross the membrane separating A from B because the membrane is impermeable to that ion, but the membrane is permeable to water and the other ions in the system. (The cytosol of all cells contains an excess of impermeant anions: proteins, nucleic acids, metabolic intermediates, etc.) Consider the situation shown below. The membrane has is permeable to \( \text{K}^+ \), \( \text{Cl}^- \), and water, but impermeable to \( \text{X}^- \). There is no electrical force just yet. \( \text{X}^- \) cannot flow, \( \text{K}^+ \) has equal concentration in A and B and thus won't flow. \( \text{Cl}^- \) has greater concentration in B than A and thus will \( \text{Cl}^- \) flow from B to A. This will transfer negative charges from B to A, so that a transmembrane potential (A negative with respect to B) will be set up. \( \text{Cl}^- \) will flow until this electrical potential results in an "electrical force" that is opposite in direction and equal in magnitude to the "concentration force".

**INITIAL SITUATION**

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 M</td>
<td>( \text{K}^+ )</td>
<td>0.1 M</td>
</tr>
<tr>
<td>0.1 M</td>
<td>( \text{X}^- )</td>
<td>0.1 M</td>
</tr>
<tr>
<td>0 mV</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**PRACTICE #8:**

1. Will the flow of Cl\(^-\) ever stop?  ________ yes,  ________ no.
2. When?  (What will characterize the condition under which net Cl\(^-\) flow will stop, if it will stop?).

**FEEDBACK #8:** Did you answer: 1) yes and 2) when EA - EB = (60mV/-1)log\(\frac{C_{Cl(B)}}{C_{Cl(A)}}\) (or some equivalent statement). 1) Since as Cl\(^-\) flows, a countervailing "electrical force" builds up and the "concentration force" progressively diminishes, the forces will ultimately balance. 2) When the "electrical force" is equal and opposite to the "concentration force", the flow of Cl\(^-\) will stop. This is expressed by the Nernst Equation:  

\[
E_A - E_B = \frac{60mV}{-1} - \log \frac{[Cl^-]_B}{[Cl^-]_A}
\]

**PRACTICE CYCLE #9**

**INPUT 9:** Remember that the EA - EB (A negative) built up as Cl\(^-\) flowed from B to A in the last example. Consider what that voltage will do to K\(^+\). It will create an "electrical force" to move K\(^+\) from B to A and there is initially no "concentration force" to oppose this "electrical force". Note that as K\(^+\) flows, an oppositely-directed "concentration force" will build up. So that it appears that K\(^+\) must ultimately stop flowing too. There is one more principle that will help us deal with this situation: The **Principle of Electroneutrality**. This principle says we cannot separate charges across the membrane. However many Cl\(^-\) ions flow from B to A, the same number of K\(^+\) ions must flow from B to A as well. (In reality the numbers of Cl\(^-\) ions that flow will be infinitesimally larger than the number of K\(^+\) ions that move, but the difference will be chemically insignificant.)

Now you can use the knowledge that both must ultimately attain equilibrium to learn more about this situation. If both K\(^+\) and Cl\(^-\) must attain equilibrium both of these ions must satisfy the Nernst Equation or equivalently \(\Delta \mu = 0\) for each ion.

For K\(^+\) at equilibrium:  

\[
E_A - E_B = \frac{60mV}{+1} - \log \frac{[K^+]_B}{[K^+]_A}
\]

For Cl\(^-\) at equilibrium:  

\[
E_A - E_B = \frac{60mV}{-1} - \log \frac{[Cl^-]_B}{[Cl^-]_A}
\]

**PRACTICE 9:** Use the fact that both Cl\(^-\) and K\(^+\) attain equilibrium, so that both must satisfy the Nernst Equation to derive a relationship among the concentrations of Cl\(^-\) and K\(^+\) in A and B at equilibrium.

Here's how:  

\[ K^+ \text{ satisfies the Nernst Equation} \implies E_A - E_B = \frac{60mV}{1} \log \frac{[K^+]_B}{[K^+]_A} \]

\[ Cl^- \text{ satisfies the Nernst Equation} \implies E_A - E_B = \frac{60mV}{-1} \log \frac{[Cl^-]_B}{[Cl^-]_A} \]

Now there can only be one value of \(E_A-E_B\) at equilibrium. Thus these two expressions must be equal:

So that  

\[ \frac{60mV}{1} \log \frac{[K^+]_B}{[K^+]_A} = \frac{60mV}{-1} \log \frac{[Cl^-]_B}{[Cl^-]_A} \]

Cross-multiplying gives \([K^+]_A[Cl^-]_A = [K^+]_B[Cl^-]_B\)

This is called the *Donnan Relation* or the *Gibbs-Donnan Relation*.

PRACTICE CYCLE #10

INPUT: You can now use the Donnan Relation plus Electroneutrality Principle to really define the *equilibrium* situation that will obtain given the initial situation shown in the previous figure. If you assume that A and B have equal volumes and that the flow of Cl\(^-\) from B to A reduces the concentration of Cl\(^-\) in B by Z, then the concentration of Cl\(^-\) in A must increase by Z. Electroneutrality says the same amount of K\(^+\) must flow from B to A so that the changes in concentration of K\(^+\) in B and A are also equal to Z. See the diagram below. Now, you also know that these equilibrium concentrations of K\(^+\) and Cl\(^-\) must also obey the Donnan Relation.

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>(K^+ = 0.1 + Z)</td>
<td>(K^+ = 0.1 - Z)</td>
</tr>
<tr>
<td>(Cl^- = Z)</td>
<td>(Cl^- = 0.1 - Z)</td>
</tr>
<tr>
<td>(X^- = 0.1)</td>
<td>(X^- = 0)</td>
</tr>
</tbody>
</table>

PRACTICE 10: Set up the Donnan Relation and find out the final equilibrium concentrations of K\(^+\) and Cl\(^-\) in A and B. (Z  = ?)
**FEEDBACK 10:** Did you get the Donnan Relation to look like:

\[ \left[ K^+ \right]_A [Cl^-]_A = \left[ K^+ \right]_B [Cl^-]_B = (0.1 + Z) (Z) = (0.1 - Z) (0.1 - Z)? \]

If you multiply this out and solve for Z, you get \( Z = 0.033 \ldots \). Using this value for Z and the previous diagram we obtain:

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>( K^+ )</td>
<td>0.133...M</td>
<td>0.066...M</td>
</tr>
<tr>
<td>( Cl^- )</td>
<td>0.033...M</td>
<td>0.066...M</td>
</tr>
<tr>
<td>( X^- )</td>
<td>0.1 M</td>
<td>0</td>
</tr>
</tbody>
</table>

Thus using the two things that you learned, namely the Donnan Relation and Eletroneutrality Principle, you were able to find the equilibrium concentrations of all the ions involved in this particular Gibbs-Donnan situation.

You should note one more aspect of the Gibbs-Donnan "Equilibrium". While the permeable ions have come to equilibrium, water has not. Note in the last figure that the total concentration of permeant ions on side A is 0.1666... compared with only 0.1333.. in B. Besides, there is 0.1 M \( X^- \) on side A and none on side B. Thus the total concentration of osmotically active solutes is a good deal greater on side A than on side B. Water will tend to flow from B to A unless it is restrained, for example by making chamber A and the membrane rigid or by applying a pressure to chamber A sufficient to just counterbalance the osmotic imbalance. Plant cells and certain bacteria deal with the osmotic imbalance due to the Donnan equilibrium by enclosing themselves in rigid cell walls. Animal cells must actively extrude certain ions (most notably \( Na^+ \)) to achieve osmotic balance.

**PRACTICE CYCLE FOR OVERALL OBJECTIVE**

**INPUT:** Understanding the Gibbs-Donnan Equilibrium involves most of the principles we have learned. If you can put all of this together at once, you have really mastered the basics of ionic equilibria. In the practice below try as much as possible to work with first principles in mind (to solidify your grasp of these principles).

**PRACTICE:** The membrane is permeable to everything but \( X^- \). We have just filled A and B with the solutions shown. Chamber A (and the membrane) are rigid. In the diagram, there has not been sufficient time for ions to flow or for a membrane potential difference to develop. With time, ions will flow and a membrane potential will develop.

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>( Li^- )</td>
<td>1 M LiX</td>
<td>1 M LiNO(_3)</td>
</tr>
</tbody>
</table>

1) Calculate the **final** concentrations of \( Li^+ \), \( X^- \), and \( NO_3^- \) in both chambers.
2) Calculate the **steady-state** (final) value of \( E_A - E_B \).
FEEDBACK:

1) Did you obtain these results?

\[
\begin{array}{c|cc}
 & A & B \\
\hline
\text{[Li}^+\text{]} & 1.333... & 0.666... \\
\text{[NO}_3^-\text{]} & 0.333 & 0.666... \\
\text{[X}^-\text{]} & 1.0 & 0 \\
\end{array}
\]

Great.

2) Did you get \( E_A - E_B = -18 \text{mV} \)? Fine. If you got all of these, you have fulfilled most of the learning objectives of this self-instructional package.

1) Here's showing you how: Let \( y \) be the \( \text{NO}_3^- \) concentration change. Then using the procedure we used in the previous cycle and the Donnan Relation, we obtain

\[
\left[\text{Li}^+\right]_A\left[\text{NO}_3^-\right]_A = \left[\text{Li}^+\right]_B\left[\text{NO}_3^-\right]_B
\]

\[
(1+ y) (y) = (1- y)^2 \implies y = 0.3333
\]

This allows us to compute the final ion concentrations on side A and side B:

\[
\begin{array}{c|cc}
 & A & B \\
\hline
\text{[Li}^+\text{]} & 1 + y = 1.333... & 1 - y = 0.666... \\
\text{[NO}_3^-\text{]} & y = 0.333... & 1 - y = 0.666... \\
\text{[X}^-\text{]} & 1 & 0 \\
\end{array}
\]

2) Both \( \text{NO}_3^- \) and \( \text{Li}^+ \) satisfy the Nernst Equation, so you may use either to get \( E_A - E_B \):

\[
E_A-E_B = (60\text{mV}/+1) \log(0.666../1.333..)= -60\text{mV} \log 2 = -18\text{mV}
\]

POST-TEST

1. Write down the equation that defines "the electrochemical potential difference of the ion \( X^- \)" across a membrane. Label the "concentration force" and the "electrical force".

2. Chambers A and B are separated by a membrane permeable to \( \text{Na}^+ \) and \( \text{Cl}^- \). The chambers

\[
\begin{array}{c|cc|cc}
 & A & & B & \\
\hline
\text{1 M NaCl} & - & + & 0.1 \text{ M NaCl} & + \\
\text{60 mV} & 0.06 \text{ V} & \\
\end{array}
\]
contain the concentration of Na⁺ and Cl⁻ shown and Eₐ - Eₚ is as indicated below. What is Δμₜₙ across the membrane? In which direction will Na⁺ tend to flow? In which direction will Cl⁻ tend to flow?

3. Given the situation in problem 2, is energy required to cause Cl⁻ to move from B to A or can energy be obtained when Cl⁻ flows from B to A?

4. Na⁺ is in equilibrium across a membrane. The electrochemical potential of Na⁺ on one side is 2700 joule/mole. Calculate the electrochemical potential of Na⁺ on the other side.

5. Write the Nernst Equation.

6. Check the statements below that follow from the statement: "X⁻ satisfies the Nernst Equation"
   ____ a. X⁻ may or may not be in equilibrium.
   ____ b. X⁻ is in equilibrium.
   ____ c. there is no net force on X⁻.
   ____ d. μₓ on one side = μₓ on the other.
   ____ e. The "electrical force" on X⁻ is equal and opposite to the "concentration force".
   ____ f. there is neither a concentration gradient X⁻, nor is there a voltage across membrane.
   ____ g. no net flow of X⁻ will occur.
   ____ h. X⁻ may tend to flow in the net sense in either direction spontaneously.
   ____ i. The flow of X⁻ will follow Fick's First Law.
   ____ j. X⁻ will flow down its electrochemical potential gradient.

7. Chambers A and B are separated by a membrane, as shown below. If K⁺ is in equilibrium across the membrane, what is the value of Eₐ - Eₚ (get correct sign). Which side is positive?

8. K⁺ is in equilibrium across the membrane of the cell shown below. If Cl⁻ is also in equilibrium, what is the intracellular chloride concentration?
9. A membrane permeable to K\(^+\), Cl\(^-\) and H\(_2\)O separates chambers A and B. The membrane is impermeable to X\(^-\). We place solutions of the indicated composition in A and B. Chamber A and the membrane are rigid.

At equilibrium what will be
a. The concentrations of all ionic species in A and B?
b. The transmembrane voltage, \(E_A - E_B\)?
c. Will a hydrostatic pressure exist in A at equilibrium that exceeds the pressure in B?

**POST TEST ANSWERS**

1. \(\Delta \mu_{Na} = \mu^A_X - \mu^B_X = RT \ln \left( \frac{[X]^A}{[X]^B} \right) + zF(E_A - E_B) \) = "conc. force" + "elec. force"

2. \(\Delta \mu_{Na} = 0\). There is no tendency for Na\(^+\) to flow in either direction. Cl\(^-\) will tend to flow from A to B.

3. _____ X _____ energy required, ________ no energy required.

4. 2700 joule/mole.

5. \(E_A - E_B = -\frac{RT}{zF} \ln \frac{C_A}{C_B} = RT \ln \frac{C_B}{C_A}\)

6. b, c, d, e, and g are implied by the statement.

7. \(E_A - E_B = -60 \text{ mV} \); Side B is positive.

8. 12.86 mM intracellular [Cl\(^-\)]. (Use the Donnan Relationship to get the answer.)
9.

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>a.</td>
<td>K⁺</td>
<td>0.666…..</td>
</tr>
<tr>
<td></td>
<td>Cl⁻</td>
<td>0.166…..</td>
</tr>
<tr>
<td></td>
<td>X⁻</td>
<td>0.5</td>
</tr>
</tbody>
</table>

b. $E_A - E_B = -18\text{mV}$ (be sure to include sign)

c. Yes.