Experiment HK-2: Kidney Diffusion and Conductivity

Background

Osmosis is the movement of solvent, such as water, through a semipermeable membrane from a less concentrated solution into a more concentrated solution. Diffusion is the movement of a solute in the same manner from high to low across a concentration gradient. A semipermeable membrane allows either the solvent, small molecules or ions to pass through. The cell membrane forms the barrier that separates the internal environment of the cell from the external environment. The membrane maintains the balance of fluid, molecules or ions gained and lost by the cell. Any fluctuations in the osmotic and ionic conditions in the cell are minimized, and reactions can proceed normally.

Animals living in the ocean are exposed to an external environment that has minimal fluctuations in osmotic and ionic conditions. The relatively constant conditions in the external environment of the ocean, coupled with the similarity of the internal environment of the organism, to the chemical composition of seawater, makes the maintenance of the internal environment of these animals relatively simple.

Animals living in freshwater are exposed to an environment with low concentrations of ions and a high concentration of water. In this type of environment, cells lose ions and take on water. Animals have developed mechanisms that remove water and retain ions to prevent cells from swelling and bursting.

In the air, humans are exposed to a very harsh environment with a low concentration of water. Without proper levels of water, cells can dehydrate and shrink. In this environment, humans and other terrestrial vertebrates have developed mechanisms that conserve water and remove excess ions. The vertebrate kidney is a good example of an organ that is an important component in a feedback mechanism that regulates the retention or release of water and ions from the body. The mammalian kidney plays a major role in waste excretion and the balance of water and electrolytes. This role in osmoregulation will be examined in this lab.

Changes in the osmotic state of bodily fluids occur daily as we work, play, eat, drink and sleep. These alterations are minimized through feedback control mechanisms which allow osmoregulatory organs, like the kidney, to adjust their activity and maintain the stability of the internal environment. A stable internal environment buffers the body's tissues against the variations and extremes of the external environment.

The control of water retention by the human kidney is a well known example of one of these feedback regulations. This system involves both neural and endocrine mechanisms (Figure HK-2-B1).

You can trace the process with the following example. The body perspires to release heat and cool off, but it also releases water as evaporation occurs on the surface of the skin. The lost water is replenished from adjacent tissues and ultimately from the circulatory system. Unless the water lost from the circulatory system is replaced quickly enough by water that is ingested, the concentration of blood solutes will increase. The hypothalamus responds to the increased concentration of blood solutes. The impulses from the hypothalamus are carried down to the pituitary gland. The hormone, ADH (Anti-diuretic Hormone), is released from the pituitary to the surrounding circulatory system. The ADH travels through the circulatory system to its target tissue, the epithelial walls of the collecting ducts in the kidney.

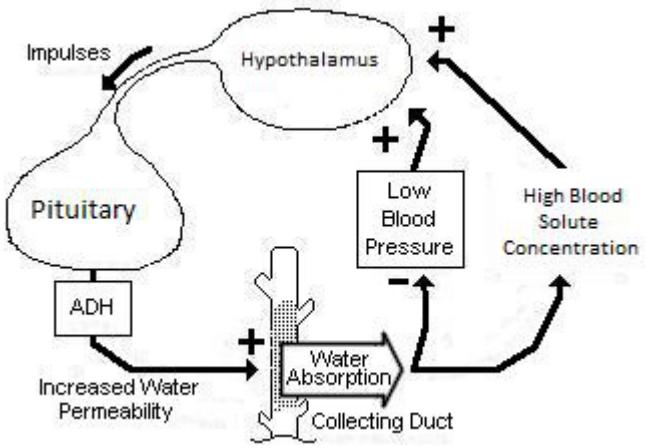

Figure HK-2-B1: Feedback regulation of blood concentration by action of ADH.

The walls of the collecting ducts become more permeable to water as the blood level of ADH increases. More water is drawn out of the collecting duct into the surrounding fluid. So, as ADH increases the permeability of the collecting ducts and more water is drawn out of the urine as it passes down the duct toward the renal pelvis, more water is conserved by the body; urine more concentrated in solutes is created.

The water conserved by the collecting ducts reenters the circulatory system through the capillary network that surrounds the entire tubular system of the kidney. The blood concentration decreases as the solutes in the blood are diluted by the reabsorbed water. The lower blood concentration is sensed by cells in the hypothalamus and it slows its reaction, causing the secretion of ADH to be reduced. The permeability of the collecting duct walls to water is reduced until another increase in the blood solute concentration is detected. (Figure HK-2-B2)

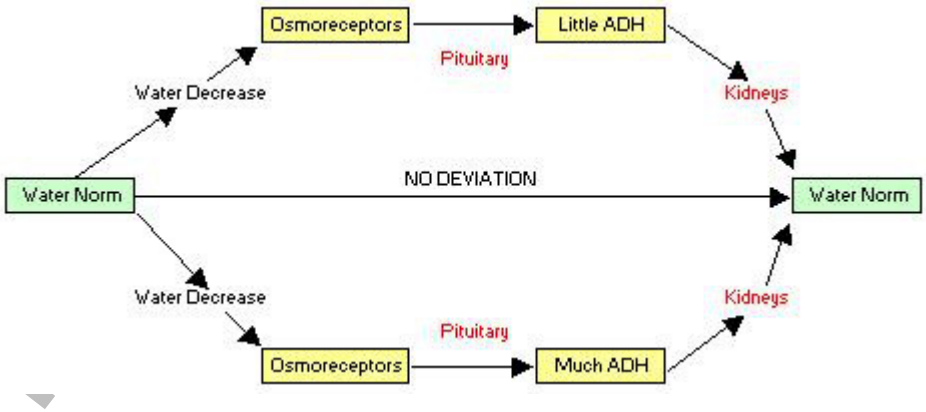

Figure HK-2-B2: Kidney osmoregulation.

Characteristics of Urine

Transparency: Normal fresh urine samples are transparent. Old samples of urine may be cloudy due to the presence of bacteria growing after the samples were collected. Fresh urine samples that are cloudy may be due to urinary tract infections (bacteria growing inside the urethra) or may indicate the presence of blood cells, pus or fat.

Color: The color of urine depends in part on its concentration. Pale, dilute urine may be the result of drinking large volumes of liquids, but it may indicate diabetes. Dark, concentrated urine may be the result of dehydration or of fever. A smoky-red color may indicate the presence of red blood cells, which may be due to damaged kidneys.

Odor: The normal odor of urine may be altered by several factors. A foul odor in fresh urine can indicate the presence of bacteria. A fruity odor indicates the presence of ketones. Ketones are a product of the breakdown of fat, which can occur due to diabetes, starvation or alcohol intoxication.

Sugar Content: Sugar may be present in your urine after eating a meal rich in carbohydrates or during periods of stress. However, a consistent finding of sugar in urine may indicate diabetes.

Protein Content: Protein in urine indicates an abnormal condition known as proteinuria. This condition may result from disease or damage to the kidneys.

pH: Kidneys are the body's most effective regulators of blood pH. Because more acids than bases usually enter blood, more acids than basses are usually excreted by the kidneys. Average pH of urine 6.0 (slightly acidic), but can range from 4.6 to 8.0. Diet and medications can affect pH, but a high pH may indicate kidney failure, vomiting, or a urinary tract infection whereas a low pH may be due to diabetes, diarrhea, or starvation.

Specific Gravity: The concentration of urine is measured by its specific gravity, ranging from 1.0006 to 1.035. The higher the number, the more concentrated the sample. High specific gravity can cause precipitation of solutes and formation of kidney stones.

Performing the Lab

Chemical analysis for this lab can be performed one of three ways.

1) Using pre-made, synthetic urine. Recipes for different types of urine samples for testing are included in the Appendix for this lab.

2) Using real urine. If choosing to use real urine for this experiment, students should be prepared to handle their own sample. Gloves should be provided and proper disposal methods for urine should be adhered to.

It is suggested that each person be given a protocol of what to drink and eat prior to coming to lab, to test the conductivity of their samples.

3) Using water with various solutes added.

Observational analysis of urine – Students will look at and smell each urine sample for characteristics as listed above.

In this experiment, students will test the conductivity of a variety of urine samples using a conductivity meter attached to an iWorx data acquisition unit.

Additional exercises may include relating conductivity to temperature, measuring the conductivity of samples based on diet, correlating conductivity to pH, and observational data about the urine samples.

Experiment HK-2: Kidney Diffusion and Conductivity

Equipment Required

PC or Mac Computer

IXTA data acquisition unit

USB cable

IXTA power supply

CM-100 conductivity meter

100 ml beakers, 250 ml beakers, and a 500 ml beaker (for waste)

Wash bottle

Deionized water

2 cm wide dialysis tubing and String

Gloves

Conductivity standard solutions (see Appendix)

Urine or urine testing solutions (see Appendix)

IXTA Setup

1. Place the IXTA on the bench, close to the computer.
2. Check Figure T-1-1 in the Tutorial Chapter for the location of the USB port and the power socket on the IXTA.
3. Check Figure T-1-2 in the Tutorial Chapter for a picture of the IXTA power supply.
4. Use the USB cable to connect the computer to the USB port on the rear panel of the IXTA.
5. Plug the power supply for the IXTA into the electrical outlet. Insert the plug on the end of the power supply cable into the socket on the rear of the IXTA. Use the power switch to turn on the unit. Confirm that the power light is on.

Start the Software

1. Click on the LabScribe shortcut on the computer's desktop to open the program. If a shortcut is not available, click on the Windows Start menu, move the cursor to All Programs and then to the listing for iWorx. Select LabScribe from the iWorx submenu. The LabScribe Main window will appear as the program opens.
2. On the Main window, pull down the Settings menu and select Load Group.
3. Locate the settings folder that contains the settings group, IPLMv6Complete.iwxgrp. Select this group and click Open.

4. Pull down the Settings menu again. Select the Kidney Diffusion and Conductivity settings file from Human Kidney.
5. After a short time, LabScribe will appear on the computer screen as configured by the Kidney Diffusion and Conductivity settings.
6. Once the settings file has been loaded, click the **Experiment** button on the toolbar to open any of the following documents:
    - Appendix
    - Background
    - Labs
    - Setup (opens automatically)

Conductivity Meter Setup

1. Locate the CM-100 conductivity meter in the iWorx kit or the add-on kit (Figure HK-2-S1).

Figure HK-2-S1: The CM-100 conductivity meter.

2. Plug the DIN8 connector of the CM-100 conductivity meter into the Channel A5 on the IXTA (Figure HK-2-S2).

Figure HK-2-S2: The CM-100 conductivity meter connected to the IXTA.

Calibration of the Conductivity Meter

Note: *For calibration purposes, set the conductivity selector switch to the 0-20,000 uS position. Recipes for solutions are in the Appendix for this lab.*

1. Rinse the CM-100 conductivity meter with deionized water while holding the electrode over a 500 ml beaker.
2. Place the tip of the conductivity meter in a 250 ml beaker containing enough room temperature deionized water to submerge the tip. Keep the electrode in deionized water for at least five minutes.
3. Prepare two 100 ml beakers, each filled with 50 ml of the conductivity standard solutions used for calibration. The solutions should be at room temperature. One beaker is filled with a solution of 2000 uS NaCl and the other is filled with a 10,000 uS NaCl solution. Each beaker should be filled with enough solution to cover the tip of the conductivity meter.
4. Place the beaker containing the 2000 uS solution next to the meter. Remove the meter from the deionized water and blot any extra drops of water. Carefully lower the tip of the electrode into the beaker of NaCl so that the tip does not hit the bottom.
5. Click Record on the LabScribe Main window to begin recording. After a few seconds, the trace will reach a stable baseline toward the top of the recording channel. Type the words "Calibration-2000" in the Mark box to the right of the Mark button. Click the Mark button to mark the stable baseline of the recording. This will mark the output of the CM-100 conductivity meter in this standard solution. Continue recording while changing the beakers of solutions.
6. Remove the conductivity meter from the beaker of 2000 uS NaCl solution. Hold the meter over the beaker used for collecting deionized waste water and rinse it well with deionized water. Blot any extra drops of water.
7. Place the beaker of 10,000 uS NaCl solution close to the meter. Carefully lower the conductivity meter into the beaker so it does not forcefully hit bottom.
8. As you continue to record, the trace will reach a stable baseline. Type the words "Calibration-10,000" in the Mark box to the right of the Mark button. Click the Mark button to mark the stable baseline of the recording. This marks the output of the CM-100 conductivity meter in this standard solution.
9. The recording will look like the recording shown in Figure HK-2-S3.
10. Click Stop to halt the recording.
11. Select Save As in the File menu, type a name for the file. Choose a destination on the computer in which to save the file, like your lab group folder). Designate the file type as *.iwxdata. Click on the Save button to save the data file.
12. Remove the conductivity meter from the beaker of solution. Hold the meter over the beaker used for collecting deionized waste water, and rinse it well with deionized water from a wash bottle. Blot any extra drops of water and place the meter in another beaker of deionized water.

Units Conversion

1. Scroll to the beginning of the calibration data for the CM-100 conductivity meter.
2. Use the Display Time icons to adjust the Display Time of the Main window to show the data collected at 2000 uS and 10,000 uS on the Main window at the same time. The required data can also be selected by:
    - Placing the cursors on either side of data required and,
    - Clicking the Zoom between Cursors button on the LabScribe toolbar to expand the segment of data to the width of the Main window.
3. Click the 2-Cursor icon (Figure HK-2-S4) so that two cursors appear on the Main window. Place one cursor on the flat section of data collected when the CM-100 conductivity meter was in the 2000 uS solution and the second cursor on the flat section of data collected when the meter was in the 10,000 uS solution.
4. To convert the voltages at the positions of the cursors to conductivity values in uS, use the Simple Units Conversion dialogue window (Figure HK-2-S5). To access this dialogue window, click on the arrow to the left of the channel title, Conductivity, to open the channel menu. Select Units from the channel menu, and select Simple from the Units submenu.

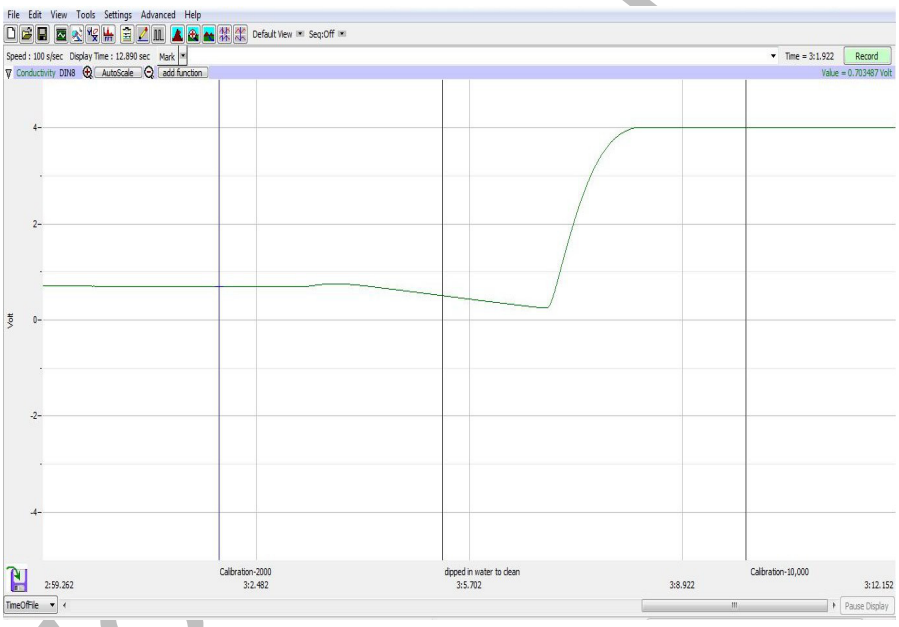

Figure HK-2-S3: Conductivity calibration data recorded showing the positions of the cursors for changing the recorded voltage to conductivity in uS.

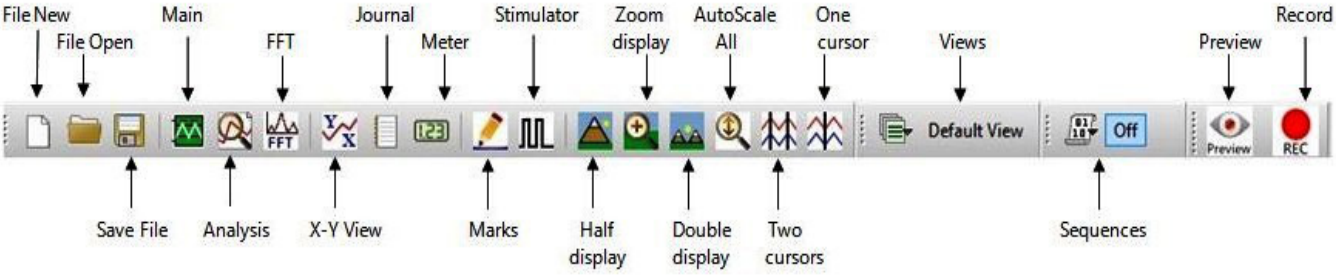

Figure HK-2-S4: The LabScribe toolbar.

5. On the units conversion window, make sure 2 point calibration is selected in the pull-down menu in the upper-left corner of the window. Put a check mark in the box next to Apply units to all blocks.
    - Notice that the voltages from the positions of the cursors are automatically entered into the value equations.
    - Enter the two calibration solutions used in the calibration recording in the corresponding boxes on the right side of the conversion equations. Enter the name of the units, uS, in box below these values.
    - Click on the OK button in the lower right corner of the window to activate the units conversion.

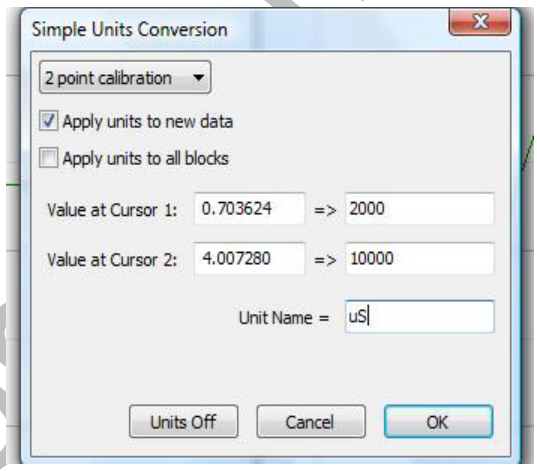

Figure HK-2-S5: The Simple Units Conversion dialog window for conductivity conversion.

Preparation of the Dialysis Tubes

1. Obtain three pieces of two centimeter-wide dialysis tubing that are fifteen centimeters long. Soak the tubing in deionized water to soften them.
2. Tightly knot one end of each dialysis tubing to form a sac. The other end will be tied with string after filling.

3. Fill one of the sacs about two-thirds full with "normal" urine. Carefully twist the bag closed, trying to remove all air bubbles. Tie the end tightly with string to prevent any leakage.
4. Rinse the outside of the dialysis sac containing the urine sample with tap water to remove any that may have gotten on the outside.
5. Stand the completed dialysis sac in a clean, dry 250 ml beaker. Label the beaker.
6. Repeat Steps 3, 4, and 5 to make two more sacs, each filled with a different urine sample (or from a different subject). Put each dialysis sac in its own clean, dry, labeled 250 ml beaker.

NOTES

- If using synthetic urine, be sure that each group has a sample of "normal" urine and two different samples of "abnormal" urine. Label the beakers appropriately.
- If using actual, student urine samples – students should handle their own samples.

  Each student should have a list of what they ate and drank prior to coming to lab, so comparisons can be made about diet and diffusion. Label the beakers Subject 1, 2 and 3 respectively.

Experiment HK-2: Kidney Diffusion and Conductivity

Before beginning the testing, observe each urine sample for transparency, color, and odor. Create a table, or put notes in the Journal about your observations.

Exercise 1: Diffusion and the Conductivity of Urine Electrolytes.

Aim: To determine the conductivity of different electrolytes in urine.

Procedure:

Note: *Always begin testing samples with the conductivity selector switch in the 0-200 uS range. While recording the conductivity of each test sample, toggle the switch to the 0-20,000 uS position. This is especially important for measuring solutions with higher conductivities.*

1. Fill a 250 ml beaker with 100ml deionized water.
2. Make sure the conductivity selector switch is in the 0-200 uS position.
3. With the CM-100 conductivity meter in the beaker of deionized water, click Record on the LabScribe Main window to begin recording.
4. When the recording on the Conductivity channel reaches a stable baseline, type DI Water in the Mark box to the right of the Mark button. Press the Enter key on the keyboard to mark the recording.
5. Type Urine Sample in the Mark box to the right of the Mark button.
6. Place the "normal" urine (or urine from Subject 1) into the deionized water.
7. Press the Enter key on the keyboard to mark the recording when you place the dialysis tubing in the beaker. The recording should look like Figure HK-2-L1.

Note: *You may need to toggle the conductivity selector switch to the 0-20,000 uS range to get an accurate reading of conductivity.*

8. Record for 10 minutes, marking the recording at 30 second intervals by clicking the F1 key on the keyboard.
9. Click Stop to halt the recording.
10. Select Save in the File menu and name your data file. Save it to the proper location as stated by your professor.
11. Remove the conductivity meter from the beaker. Hold the meter over the 500 ml beaker used for collecting waste deionized water, and rinse it with a wash bottle. Blot any drops of DI water from the meter and place the meter in a beaker containing fresh DI water. The meter will be used in the next section of the exercise.

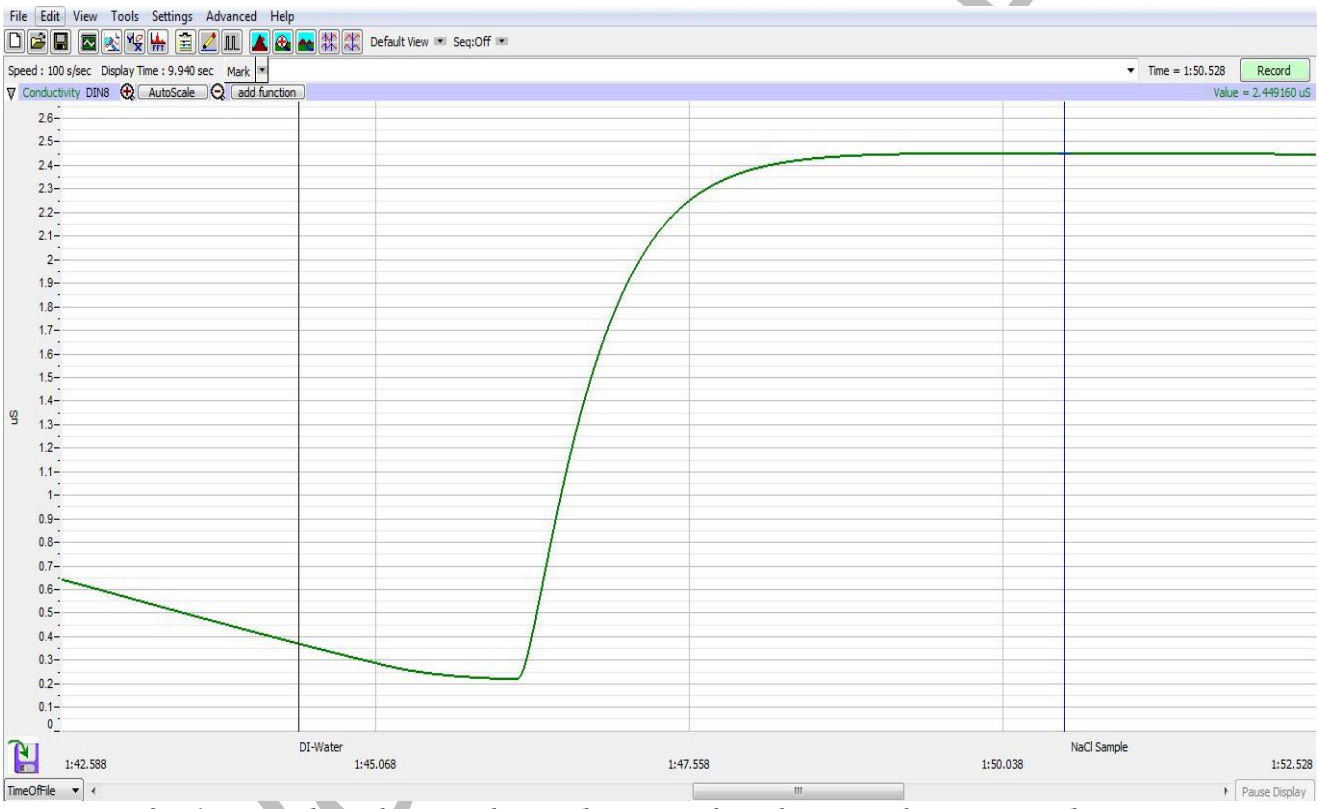

Figure HK-2-L1: Recording showing the conductivity of a solution in the Main window.

12. Repeat steps 1 through 11 using the "abnormal" urine samples or samples from Subjects 2 and 3.

Note: *Be sure to mark the recording appropriately for each of these solutions for ease of data analysis.*

13. Place the CM-100 conductivity probe in a beaker of fresh deionized water during data analysis.

Data Analysis

1. Scroll to the section of data for deionized water and the urine sample that were recorded at the beginning of Exercise 1.
2. Use the Display Time icons on the LabScribe toolbar to position this section of recording on the Main window. The required data can also be selected by:
    - Placing the cursors on either side of the section of data needed. Place one cursor on the stable conductivity value recorded from pure deionized water. Place the second cursor on the stable conductivity value recorded after moving the meter to the urine sample.
    - Clicking the Zoom between Cursors button on the LabScribe toolbar to expand the segment of data to the width of the Main window.
3. Look at the Function Table that is above the uppermost channel displayed in the Analysis window. The mathematical functions that are listed should include Title, Value1, and Value2. The values for these parameters from each channel are seen in the table across the top margin of each channel.
4. Once the cursors are placed in the correct positions for determining the conductivity, the values can be recorded in the on-line notebook of LabScribe by typing the names and values directly into the Journal.
5. The functions in the channel pull-down menus of the Analysis window can also be used to enter the names and values of the parameters from the recording to the Journal. To use these functions:
    - Place the cursors at the locations used to measure the conductivity from the Conductivity channel.
    - Transfer the name of the mathematical function used to determine the conductivity to the Journal using the Add Title to Journal function in the Conductivity channel pull-down menu.
    - Transfer the value for the conductivity to the Journal using the Add Ch. Data to Journal function in the Conductivity channel pull-down menu.
6. Place one cursor on the stable conductivity value of the pure deionized water. Place the second cursor on a stable point in the recording of the urine sample right after it was added to the beaker.
7. Measure the values for the following parameters from the Conductivity channel for the region of data selected:
    - Conductivity-DI Water, which is Value1 on the Conductivity channel.
    - Conductivity-Urine, which is Value2 on the Conductivity channel.
8. Record the values for these parameters in the Journal using one of the procedures described in Step 5, and on Table HK-2-L1.
9. Click the down arrow next to the Mark box to choose the next section of data.
    - Highlight and click on the next Mark on the list – F1. This mark will be the one 30 seconds after the start of recording.

- This will automatically move the recording to the section of data with that Mark.

10. Place left hand cursor on this mark and record the information for Value1 (there will be no data for Value2).
11. Repeat step 10, moving to the next F1 mark on the list (60 seconds).
12. Repeat steps 9 and 10 for every 30 seconds of the 10 minutes of data collected, recording conductivity values in uS on the data table.

Discard the contents of each beaker and dialysis sac as directed by your instructor into the waste container.

Note: *Remove the conductivity meter from the beaker and rinse it with a wash bottle. Blot any drops of DI water from the meter and place the meter in the beaker containing fresh DI water. Make sure the CM-100 is clean and dry prior to storage.*

Data Tables

Table HK-2-L1: Conductivity of Different Urine Samples

Conductivity Values over Time for Three Urine Samples			
Time (sec)	Normal Urine - uS (Subject 1)	Abnormal Sample 1 - uS (Subject 2)	Abnormal Sample 2 - uS (Subject 3)
0			
30			
60			
90			
120			
150			
180			
210			
240			
270			
300			
330			

360			
390			
420			
450			
480			
510			
540			
600			

Questions

1. Which urine sample had the highest conductivity? The lowest?
2. What is the main contributor to the difference in conductivity values between the samples tested?
3. Did any samples show no change in conductivity (thus no diffusion)? If so, which samples were these and why was there no diffusion? Think of the properties of the molecules and ions that can diffuse.
4. Did the subject's diet, prior to testing the urine, effect the conductivity values (diffusion of substances)? If so, what did you notice?
5. For each of the urine samples, what did you observe about transparency, color and odor? If asked to test for specific gravity, what was the specific gravity for each sample?
6. What does your observational analysis tell you about each urine sample? Look at information about urinalysis in the Background document.