



## Lesson #8

### Graphing the Standard Curve

#### Objectives:

- The learner will demonstrate analysis of colorimetric assay results by analyzing them using a computer graphing program.
- The learner will demonstrate application of standard curves by finding other areas of science that can utilize standard curves in analysis procedures.

#### National Science Education Standards:

SAI 1, ST 2, HNS 2

#### Benchmarks:

1C, 2A, 2B, 2C, 3A, 8D, 8E, 9A, 9B, 9C, 9E, 12B, 12C, 12D

#### Materials:

data sheets  
computer graphing program

#### Background:

Understanding the use and application of standard curves is important for students. It is a skill that will help them to understand how science is done. The colorimetric assay (Lesson #7) measures the concentration of  $\text{Fe}^{2+}$  in the samples taken from the bottle. When using the spectrophotometer, the absorbance of the material being tested is measured. Because you are looking for the concentration, you must figure out how to convert from the absorbance to the concentration. Remember you know the concentration of a set of standards which has been prepared and you can find their absorbances.

#### Preparation:

The procedures for this activity are written using Microsoft Excel™, a common computer graphing program in many schools. If you will be using a different graphing program with your students it may be necessary to give students a different set of procedures based on that particular program.

Students will be using the data collected in Lesson #7 to graph a standard curve of absorbance vs. concentration. Then they will use the curve to calculate the concentration of iron in their samples. If you do not have data from your own columns, you can use the sample data sheet provided. The sheet includes data for 3 bottles over a 3 week time period (4 tests, 0, 7, 14, & 21 days). You may decide to assign groups a bottle number so that there is more than one set of answers. Because the samples in the bottles show change over time, you may want to have students look at data from the same bottle over the three week period to note any changes.

#### Warm-Up:

Tell students, “There is an apple tree in an orchard. Some of the apples have fallen to the ground while others are still on the tree. 15 % of the apples are on the ground and 85% of the



apples are still on the tree. If there are 45 apples on the ground, how many apples are there in total on the ground and in the tree?" Using this word problem, guide students through the process of finding the total number of apples. Discuss the background information on the student labs. Talk about how a set of ratios were set up to calculate the total number of apples. Talk about how a set of numbers can be represented by a point on a graph. When many of these sets are plotted, a line can be fit to the graph. All of the points along that line have the same ratio. This is displayed as an equation. Their data fits onto this line as well. If a student finds the absorbance along the vertical side and follows it across until coming to the line, a point could be placed there. Following that point down towards to horizontal line, the student would find the concentration. The concentration for that point would be the concentration of the sample in question.

Give students about 5 minutes to work on their hypothesis after this discussion.

### **Procedures:**

Students will be following the procedures on the student lab sheet to graph the standard curve and find the equation of that standard curve. These procedures are written to be used with Microsoft Excel™. If you will be using a different graphing program, you will need to modify the instructions to fit that program.

### **Variations and Follow-Up Activities:**

Many computer graphing programs, such as Excel™, offer special functions allowing the user to make calculations on the spreadsheet. Including instructions on using these functions is an excellent way to enhance this activity, providing for even more links between science and the use of technology. For information on these specific functions, see your school computer coordinator or the instruction manual for your specific computer program. In Excel™, the symbol = is the beginning of all equations. See the attached "Teacher Tutorial" for more details on this option.

As a follow-up activity, students can look into researching the other applications for these kinds of graphing programs. Without such programs it can be very time consuming to analyze data. Have students ask parents about their use of computers and graphing programs in the workplace. Some students may have a parent, guardian, friend, or neighbor who can give some them some information on how this type of technology is useful.

### **Assessments:**

Students can be assessed while they are working on this activity. Assessing a students' understanding of the mathematical concepts behind this activity can be done while students are working on the warm-up activity. This can be done through the use of questioning techniques as well as grading the students' work. It may be helpful to ask students to explain the warm up activity to other students and check each others understanding. It is important to assess this understanding before continuing with the lesson, as students who are unsure will be confused as to why they are graphing the concentrations and absorbances.

An assessment of graphing skills and mathematical skills as well as scientific understanding of the concepts being presented can be obtained through the student lab report. It has been designed to allow students the opportunity to show all of these skills.



## Directions for Formulas in Excel™ (Teacher Tutorial)

When using Excel™ it is possible to put formulas into the cells to automatically make calculations for you. Follow these steps to make a formula in Excel™. Once you feel comfortable with the process, you can do this activity with your students. For the purposes of this teacher tutorial, we will use some of the data that was gathered from the colorimetric assays. A sample spreadsheet is shown.

1. Open an Excel™ spreadsheet.
2. For this exercise we will be using the equation  $Y=12.565X-0.0746$  (This was the equation from one of my standard curves.)
3. In cell A1, type **Absorbance (nm)**. In cell B1, type **Concentration (mM)**.
4. In the cells underneath, type in the absorbance of each of the samples tested. In this tutorial we will use the following:

Cell A2: 0.007

Cell A3: 0.037

Cell A4: 0.432

Cell A5: 1.350

1. Once these have been entered, move the cursor to cell B2. You will be writing your equation in this cell. Type  **$=(A2*12.565)-0.0746$**  This means that you want to take the number in cell A2 and multiply it by 12.565 and then subtract 0.0746 from that product. Hit the **Enter** key. You should see a number appear in the cell. Check to be sure that number is correct.
2. Highlight cells B2-B5. Click on **Edit** and then **Fill** and then **Down**. This will tell the computer to use the same equation for the rest of the cells, but it will substitute the correct number for A2 in the equation. This means that instead of always multiplying by A2, the computer will multiply by whichever cell is the one in the A column in that row (i.e., in cell B3, the equation will multiply by A3, in B4 it will multiply by A4).

A	B	C	D	E	F	G
1	Absorbance (nm)	Concentration (mM)				
2	0.007	0.013356				
3	0.037	0.390306				
4	0.432	5.35348				
5	1.35	16.88815				
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						



## Sample Data

### Graphing Data

#### Standard Curve

Concentration (mM)	Absorbance (nm)
0.000	0.000
0.150	0.004
0.300	0.012
0.600	0.027
1.250	0.055
2.500	0.096
5.000	0.348
10.000	0.815
20.000	1.540

#### Sample Data for Bottles

##### Bottle #1

Time	Port A	Port B	Port C	Port D
Day 0	0.014	0.020	0.018	0.020
Day 7	0.000	0.014	0.027	0.794
Day 14	0.000	0.038	0.067	0.774
Day 21	0.003	0.009	0.048	0.448

##### Bottle #2

Time	Port A	Port B	Port C	Port D
Day 0	0.005	0.015	0.029	0.012
Day 7	0.000	0.035	0.049	0.802
Day 14	0.003	0.037	0.432	1.350
Day 21	0.004	0.021	0.341	1.320

##### Bottle #3

Time	Port A	Port B	Port C	Port D
Day 0	0.034	0.021	0.053	0.030
Day 7	0.002	0.026	0.017	0.580
Day 14	0.007	0.070	0.113	0.664
Day 21	0.005	0.044	0.036	0.200



### **Observations:**

I found that the standard curve was difficult for my students to understand. After graphing and finding the equation of the line, the students were better equipped to understand where the equation came from and why it was needed. Discussing the standard curve was just not practical before creating it. I just had to tell my students to follow the directions and make the graph and that we would discuss it later. That seemed to work for my students, but you may need to modify that strategy for your students.

My students used the sample data and we compared it among groups because we did not perform the colorimetric assay using the ferrozine reagent. The sample data along with the pictures that accompany this lesson work well for those who will not be performing the colorimetric assay with the students.

This lesson is another one in which students need to focus on the big idea. Here the big idea is that we will take numbers that we don't understand and put them into a form which we can use to compare them to other samples. If students can understand this, then they are understanding some of the basic ideas of science and analyzing scientific data.



Name \_\_\_\_\_

Date \_\_\_\_\_

### Graphing the Standard Curve

#### Problem:

The spectrophotometer measures the absorbance of the ferrozine /  $\text{Fe}^{2+}$  complex in the sample from the Winogradsky bottle. How can you find the concentration of those samples if you know both the concentration and absorbance of a set of standards.

#### Background:

The colorimetric assay activity measures the concentration of  $\text{Fe}^{2+}$  in the samples taken from the bottle. When using the spectrophotometer, the absorbance of the material being tested is measured. Because you are looking for the concentration, you must figure out how to convert from the absorbance to the concentration. Remember, you know the concentration of a set of standards which has been prepared and you can find their absorbances.

**Hypothesis:** If \_\_\_\_\_,

then \_\_\_\_\_,

because \_\_\_\_\_

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#### Materials:

data sheets

computer graphing program

#### Procedures:

1. Begin by opening your graphing program.
2. In cell A1 write **absorbance**.
3. In cell B1 write **concentration**.
4. Type the data into the two columns.
5. Highlight all of the cells which you have put information into.
6. Click on **Insert** and then click on **Chart**.



7. Click on the **XY (Scatter)** option. Check to see that the picture that is highlighted does not have any lines connecting the points.
8. Click on **Next**.
9. Check to see that the points are on the sample graph. Click **Next**.
10. Type **Standard Curve** in the blank under chart title.
11. Type **Absorbance (nm)** in the blank under Value (X) Axis.
12. Type **Concentration (mM)** in the blank under Value (Y) Axis.
13. Click on **Next**.
14. Click on the circle next to **As New Sheet**.
15. Click on **Finish**.
16. Click on the name of the graph. A box will come up. Click on the **Font** tab. Find the word **underline** and click on the pull down box to highlight **single**. Click on **OK**. This will underline the title of your graph.
17. Click on **Chart** and then on **Add Trendline**.
18. Click on the **Type** tab. Be sure the picture for the **Linear** Trendline is highlighted. Click on the **Options** tab and click the box to the left of **Display Equation on Chart**. This will place a check in this box. Click on **OK**.
19. You will see the equation for the line at the top of the Graph. This is the equation you will need to calculate the concentrations from the absorbances you recorded on the spectrophotometer. Copy down this equation.

### Results and Analysis

1. The equation for the line is \_\_\_\_\_
2. Find the concentration for each of your sample absorbances. Show all of your work. (Hint: your absorbance is the "X" in this equation.) You may want to attach a separate sheet.

### Conclusions

Use the following to help you write a detailed conclusion on a separate sheet and attach it to this lab.

- Were you able to support your hypothesis? Why or why not?
- Why is a standard curve needed in finding the concentrations of  $\text{Fe}^{2+}$ ?
- How is creating a standard curve similar to a ratio? (Hint:  $\text{abs./conc} = \text{abs./conc.}$  )
- In what other areas of science could a standard curve be used?