**Spectrophotometry Simulation to Accompany PhET Beer’s Law**

**Part I: Introduction to Using the Spectrophotometer**

1. Click on the “Beer’s Law Simulation” PhET. This will take you to the PhET web simulator. (Google PhET Beer’s Law to get to the website).

2. Click on the right icon to start the “Beer’s Law” Simulation.

3. In the “Green Readout Box” on the top right of the screen, switch the reading from “Transmittance” to “Absorbance”.

4. Select the potassium permanganate (KMnO4) in the dropdown box at the bottom of the simulation window where you can select the substance (it is preset to “Drink Mix”).

5. The spectrophotometer will automatically preset the wavelength for maximum absorbance of the KMnO4 substance. Switch the dropdown box to each of the other substances. Write down the Maximum Absorbance Wavelength for each substance in the chart below.

6. Set the concentration of KMnO4 to 100 μM using the slider bar or arrows.

7. Click on the red button on the “Spectrophotometer Icon” in order to shine light on the sample. Adjust the width of the sample tube (cuvette) using the yellow arrow to get an absorbance value of 0.30 in the “Green Readout Box”.

8. For each substance, select a concentration of 100 mM or 100 μM (control the concentration using the slider below the dropdown box that sets the substance). Record the Absorbance in Column 2 in the chart below.

9. In the “Green Readout Box” on the top right of the screen, switch the reading from “Absorbance” to “Transmittance”. For each substance at 100 mM or 100 μM (control the concentration using the slider below the dropdown box that sets the substance), record the Transmittance **in decimal form** in Column 4 in the chart below.

10. In the Column 5 of the chart below, calculate the absorbance for each substance at 100 mM or 100 μM using the equation Absorbance = -log10(Transmittance). Verify that the calculated value matches the measured value in Column 3.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Column 1** | **Column 2** | **Column 3** | **Column 4** | **Column 5** |
| **Substance** | **Maximum Absorbance (wavelength)** | **Absorbance**  **(for [ ] = 100 mM or** μM**) – Note which!** | **Transmittance (decimal value for**  **[ ] = 100 mM or** μM**)** | **Calculated Absorbance Abs = -log10­(T)** |
| KMnO4 |  |  |  |  |
| Drink Mix |  |  |  |  |
| Co(NO3)2 |  |  |  |  |
| CoCl2 |  |  |  |  |
| K2Cr2O7 |  |  |  |  |
| K2CrO4 |  |  |  |  |
| NiCl2 |  |  |  |  |
| CuSO4 |  |  |  |  |

**Part 2: Effect of Path Length on Absorbance**

1. Set the dropdown box to potassium permanganate at 300 μM. (Tip: Remember that KMnO4 is purple 🡪 it shows up a lot!). In the “Green Readout Box” on the top right of the screen, switch the reading from “Transmittance” to “Absorbance”.

What is the absorbance reading of the 300 μM KMnO4? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

2. Try using the yellow arrow on the cuvette to increase the path length (width of the sample tube). What happens to the absorbance value if you increase the path length that the light shines through?

3. Try using the yellow arrow on the cuvette to decrease the path length. What happens to the absorbance value if you decrease the path length?

**Part 3: Absorbance Spectra (Absorbance vs. Wavelength)**

1. Set the dropdown box to potassium permanganate at 100 μM again. Make sure the “Green Readout Box” on the top right of the screen is set to read “Absorbance”. Reset the cuvette width so that the Absorbance reading is 0.40.

2. Go to the spectrophotometer icon on the left side and click the radio button from “preset” to “variable”. Try moving the slider on the “Spectrophotometer Icon” to different wavelengths and record the absorbance of the indicated wavelengths in the chart below. Press the red button on the “Spectrophotometer Icon” to get an Absorbance value in the “Green Readout Box”.

This chart below gives yields the absorbance spectrum for KMnO4­ and indicates which wavelengths are absorbed the most and the least by KMnO4.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Absorbance Spectrum for KMnO4 at 100 μM** | | | | | | | | | |
| **Wavelength** | 390 nm | 450 nm | 500 nm | 525 nm | 580 nm | 610 nm | 650 nm | 700 nm | 750 nm |
| **Absorbance** |  |  |  |  |  |  |  |  |  |

Which wavelength above is the peak absorbance for KMnO4? \_\_\_\_\_\_\_\_\_\_\_ Is it close to the preset value?

**Part 4: Generating a Reference Curve and Determining Concentration**

1. Set the dropdown box to potassium permanganate at 100 μM. Set the path length on the cuvette to get an absorbance value of 0.40

2. Go to the spectrophotometer icon on the left side and click the radio button from “variable” to “preset”. Press the red button on the “Spectrophotometer Icon” to get an Absorbance value in the “Green Readout Box”.

3. Change the concentration of the potassium permanganate by using the slider below the dropdown box used to set the substance. Record the absorbance of potassium permanganate for each of the concentrations given in the chart below.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Absorbance vs. Concentration for KMnO4** | | | | | | | | | |
| **Concentration (**μM) | 25 | 50 | 100 | 150 | 300 | 450 | 550 | 650 | 750 |
| **Absorbance** |  |  |  |  |  |  |  |  |  |

4. Plot a graph of absorbance vs. concentration for potassium permanganate using data in the chart above. Remember to label your axes and title the graph. Right clicking on the graph will bring up a menu that will allow you to edit the data and enter your X and Y values. Label your axes.

5. Find the equation for the line relating absorbance to concentration.

6. Find the concentrations of the following unknown KMnO4 samples. The absorbance for each sample is given:

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Absorbance for Unknown KMnO4 Samples** | | | | | | |
| **Absorbance** | 0.43 | 0.64 | 0.75 | 0.26 | 0.95 | 0.82 |
| **Concentration based on line equation** |  |  |  |  |  |  |
| **Concentration from PhET Simulation** (use the slider to find the absorbance and note the concentration) |  |  |  |  |  |  |