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Experimental Background

Bovine Serum Albumin (BSA) is a protein that circulates in the blood of cows. Purified BSA can be used with Biuret solution in serial dilutions to generate a **Standard Curve**. The standard curve will illustrate the relationship between concentration (the **dependent variable**) and absorbance at 540 nm (the **independent variable**). We can then use this curve to estimate the concentration of unknown samples.

1. On a graph, do you remember which axis is the dependent and which is the independent variable?
2. In the table below, can you identify which samples are the **negative controls** and which are the **positive controls**?
3. What is the prediction of the absorbance or color intensity of the different tubes?

Dilute BSA Standards

1. Label 9 tubes A-I
2. Combine the components of the table below to generate appropriate concentration of solutions

A sample dilution series of protein can be used as a series of reference standards for the estimation of protein concentration when the initial protein concentration is known of the references.

3. The instructor will begin to set-up the unknowns for distribution
    1. Connect SpectroVis Plus to computer via USB
    2. Launch the **Vernier Spectral Analysis** program or Chrome App
    3. Select “By Concentration (Beer’s Law)”

4. Unit will warm up the lamp
5. After warm-up has completed, place a blank cuvette into the SpectroVis and select “Calibrate”
6. Select Wavelength to 540nm and press “Done”
7. In the data table, click on “…” and change units to “mg/ml”

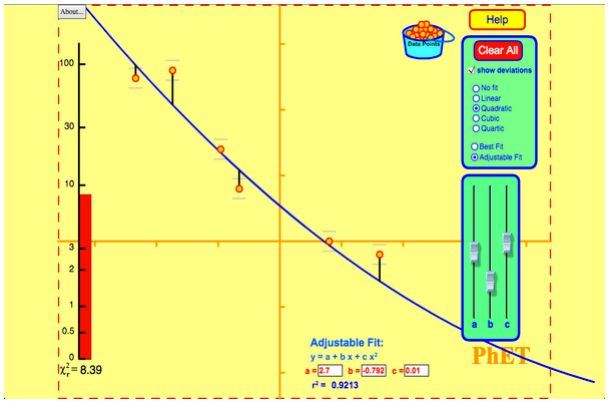
4. Place sample in the SpectroVis and press “Collect”
    1. After the absorbance value has stabilized, press “Keep”
    2. Enter the concentration of the sample solution
    3. Press “Keep Point”
5. Replace with a new sample solution and start from step 4 (“Collect”) for each new solution
6. Do NOT press “Keep” when reading the absorbance of the unknown solutions.
        1. simply record those absorbance values manually
7. Sequentially read each sample at the stored wavelength ($\mathbf{A}_{\mathbf{540nm}}$)and record values in table below

1. Plot each BSA dilution in plot.ly as a scatterplot (Log-on using Facebook/Google/Twitter credentials for free)
2. Generate best-fit line for these standards with the equation of the line
3. Use the equation of the line to estimate the concentration of the unknown sample.

Curve Fitting

Review: https://openlab.citytech.cuny.edu/bio-oer/biology-basics/quantitative-skills/

Run the simulation below to understand how you can use the standard dilution series to estimate your sample concentrations.



Click on image above to begin simulation on curve fitting

Scatterplot Tutorial

You can watch this tutorial at 1.25X and pause when needed.

Tags: quantitative reasoning