**Concentration Measurement of Hemoglobin Using Beer-Lambert Law**

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**Abstract:**

Light can be used to measure the concentration of a substance in a solution. Normal hemoglobin levels in blood is an important aspect to a healthy individual as hemoglobin is a protein which is responsible for transporting oxygen through the body (“Hemoglobin” n.d.). The concentration of hemoglobin in two unknown solutions were found by comparing the voltage of light that passed through the samples to the voltage of light through a sample of known concentration of hemoglobin. A linear relationship was discovered allowing transmission of light passing through concentrations to be related to the concentration of hemoglobin present.

**Introduction:**

Light can be helpful in determining characteristics of materials by studying how light interacts with them. The Beer-Lambert Law gives the equation , which provides transmittance or the ratio of intensity of light that passes through a material. This quantity is dependent on the concentration of the material, the optical path length, and the wavelength dependent absorption cross-section of the material. The use of these relationships can aid in determining the concentration of a material in a sample.

Hemoglobin is a protein in blood which gives it it’s red color (“Hemoglobin” n.d.). This protein is an important aspect to humans because it enables the transport of oxygen to different areas of the body, and aids in the exhalation of carbon dioxide (“Hemoglobin” n.d.). It is necessary to keep hemoglobin levels at a certain concentration in blood to ensure that enough oxygen is being transferred to parts of the body and that the viscosity of blood stays at a healthy flow (Barrell). Hemoglobin absorbs wavelengths other than red, and therefore will affect the intensity or voltage of light going through it, allowing light to be used to determine the concentration of hemoglobin in an unknown sample using the beer-Lambert equation. The Beer-Lambert equation can be manipulated to be solved using the ratio of voltage in and voltage out of the material the light is going through rather than intensity because the ratios of both will be equal, .

**Methods:**

We set up a laser to reflect off of two mirrors and shine through an iris and into a detector and recorded the voltage of the laser. We then put a sample of liquid containing a known concentration of hemoglobin between the iris and the detector, for the laser beam to pass through. Again, we recorded the voltage of the laser after it had passed through the liquid. We repeated this with two more samples of liquids containing unknown concentrations of hemoglobin. To get a larger range of data we repeated this process again but diluted each original sample by 20mL of water three times for a total of twelve voltages recorded, including the initial voltages recorded.

**Methodology:**

We solved for the constant of  in the Beer-Lambert equation of , σ being the wavelength absorption cross section, and b being the optical path length in meters, using the voltage recorded for the sample of known concentration, and manipulating the equation to  Then we solved for the concentration (c) of the unknown concentrations using the voltages recorded with the calculated value of from the known concentration using the manipulated Beer-Lambert equation of , as follows:

**(sample 1 known)**

=18.27g/dL **(sample 2 no dilution)**

When we diluted the samples, we used the same equations and methodology, however we had to find the new concentration of the solutions as the solutions were diluted. We solved for the moles of hemoglobin in the samples to determine the concentrations in the diluted versions of the known sample. We did this by converting concentration to g/mL and dividing the concentration of hemoglobin by the molecular weight of hemoglobin (65,000g/mol). We then proceeded to use the equation , where is the initial concentration in moles and is the initial volume while is thenew volume and is the new concentration in moles. Then we converted back to concentration in g/dL as shown below for dilution 1 of sample 1:

**(Sample 1)**

**(Sample 1 *C* Post dilution 1)**

**(sample 1 C post dilution 1 in g/dL)**

**Results:**

We used a 632.5nm wavelength laser with a minimum output power of 2mW which emitted an original voltage of 10.7 V. We set up a system shown below where the blue rectangle is the light source, the grey rectangles are the mirrors, the black oval is the iris, the grey oval is the detector and the cube is our blood sample:

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Original Voltage (Volts) | Concentration (g/dL) | Dilution 1 Voltage (Volts) | Concentration (g/dL) | Dilution 2 Voltage (Volts) | Concentration (g/dL) | Dilution 3 Voltage (Volts) | Concentration (g/dL) |
| Sample 1(known concentration) | 1.84 | 14.7 | 2.4 | 13.39 | 2.75 | 12.24 | 2.95 | 11.30 |
| Sample 2 (unknown) | 1.2 | 18.27 | 1.4 | 16.56 | 1.55 | 15.23 | 1.75 | 14.05 |
| Sample 3 (unknown) | 3.45 | 9.45 | 3.9 | 8.6 | 4.1 | 7.86 | 4.45 | 7.25 |

**Table 1.** Recorded Voltages of light through each sample and concentrations calculated originally and after each dilution.

**Graph 1.** Transmission of light ( through a sample versus hemoglobin concentration (g/dL)

**Discussion:**

We calculated the hemoglobin concentration in the original unknown samples, as 18.27g/dL for sample 2 and 9.45 g/dL for sample 3. We were also able to create a larger range of data by finding the transmittance through diluted samples. We created a graph with a linear regression line which presents an inverse relationship between transmittance and hemoglobin concentration. One source of error in our experiment could have been a ±.1V error for each voltage reading as a result of human error. There could also have been small error in the addition of exactly 20mL when diluting the samples in our experiment.

**Conclusion:**

We discovered the concentration of hemoglobin in two unknown samples and created a linear regression line relating the transmittance of light through a solution and the concentration of hemoglobin in the solution. This was completed by using the relationship presented in the Beer-Lambert Law between the concentration of a material and the transmittance of light passing through a material. The concentration of hemoglobin in the unknown samples was calculated using the constant of found from a sample of known concentration, and the transmittance of light through the sample placed in the Beer-Lambert equation. The regression curve was created by a greater range of data calculated through diluting our original three samples. Our results present a linear inverse relationship between transmittance of light and concentration of hemoglobin, as the concentration of hemoglobin increases, the transmittance of light through the sample decreases.

**References:**

Barrell, Amanda. “Hemoglobin Levels: Levels, Imbalances, Symptoms, and Risk Factors.” *Medical News Today*, MediLexicon International, [www.medicalnewstoday.com/articles/318050](http://www.medicalnewstoday.com/articles/318050).

“Hemoglobin.” *Patient Education on Blood, Urine, and Other Lab Tests*, labtestsonline.org/tests/hemoglobin.