

CALCULATING CONCENTRATIONS USING BEER-LAMBERT'S LAW AND LINEAR REGRESSION

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Abstract: *The article demonstrates an indirect method for determining the concentration of a substance in a solution by applying Beer-Lambert's law and linear regression.*

In chemistry and biochemistry, quantitative analysis of chemical concentrations is a fundamental task that often cannot be implemented through direct weighing. Therefore, we used Beer-Lambert's law and regression techniques to construct a calibration curve that models the linear relationship between absorbance and concentration based on spectrophotometric data. After that, the resulting linear regression model is then used to predict the unknown concentration given its measured absorbance.

Keywords: *Beer-Lambert's law, linear regression, absorbance, spectrophotometry, spectroscopy, concentration, calibration curve*

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1. INTRODUCTION

Concentration determination plays a crucial role in chemistry, biochemistry, environmental science, and medicine. In many cases, direct methods are impractical or even impossible, especially when dealing with highly dilute solutions, light-sensitive substances, or samples in limited quantities (Akash, n.d.; StudyCorgi, 2023). For these situations, one of the most widely used indirect methods is spectrophotometry - using color intensity to compute the concentration of a solution. In particular, Beer-Lambert's law states a linear relationship between the solution's absorbance and its concentration, assuming constant path length and molar absorptivity (O'Haver, 2015). Previous research has applied this relationship in combination with statistical methods - particularly linear regression - to generate calibration curves from standard solutions and subsequently determine unknown concentrations (Dekking, 2005; Bluman, 2012).

While the theoretical basis and experimental procedure are well established, fewer studies have focused on integrating multiple computational tools to illustrate and enhance the teaching of Beer-Lambert's law. In particular, the combined use of GeoGebra, Python, and R for visualizing, analyzing, and validating spectrophotometric data has not been extensively discussed in the literature. This article explains how Beer-Lambert's law and linear regression work together in practice and demonstrates a practical workflow to implement this method using spectrophotometric data with Geogebra, Python and R.

1.1 Beer-Lambert's law

In figure 1, the spectrophotometer is used to determine how much light is absorbed by a colored chemical dissolved in the solution. The monochromator takes the light from the light source and splits it into different colors or wavelengths. Then, the aperture acts as a color filter or wavelength selector. The absorbance $A = -\log_{10} \left(\frac{I}{I_0} \right) = -\log_{10} T$ where I_0 is the intensity of the original incident light, I is the intensity of the light that passes through the

sample cuvette and $T = \frac{I}{I_0}$ is the transmittance. The spectrometer measures T , then calculates A .

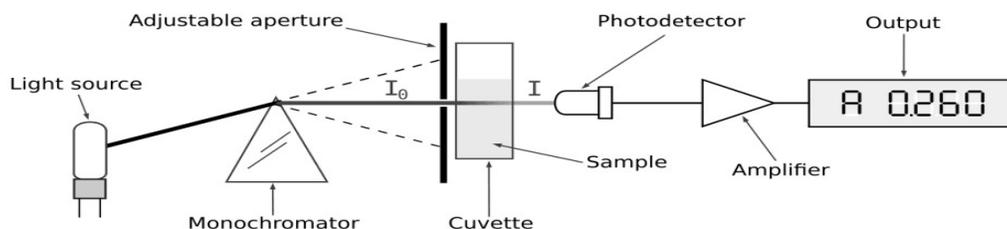


Figure 1: Spectrophotometer components

According to the Beer-Lambert's law, $A = \epsilon \cdot c \cdot l$ where $\epsilon [M^{-1}cm^{-1}]$ is the molar absorptivity or molar extinction coefficient, $c [M]$ is the concentration of the substance, and $l [cm]$ is the path length of the cuvette. When ϵ and l are constant, this equation indicates a linear relationship between absorbance and concentration.

The phET interactive simulation in figure 2 can help us visualize and interact with the components of Beer's law.

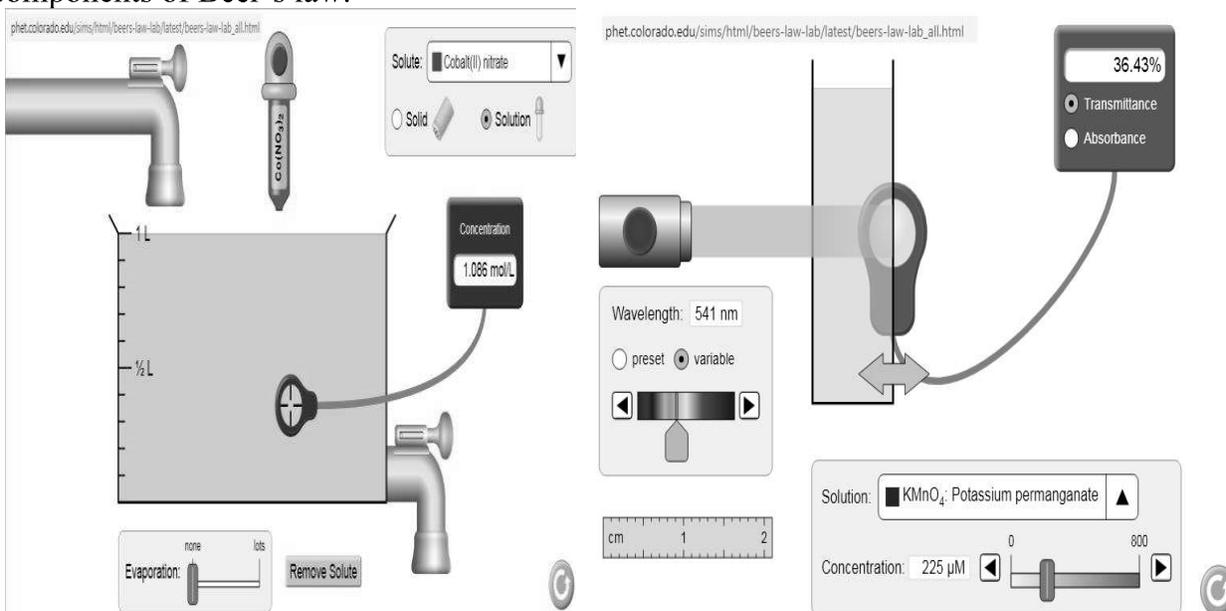


Figure 2. The PhET Beer's law simulation

1.2 Linear Regression

Suppose two random variables under our study are X and Y . For a sample of size n , we obtain data points $\{(x_i, y_i)\}_{i=1}^n$. We set $\Sigma x = \sum_{i=1}^n x_i$, $\Sigma y = \sum_{i=1}^n y_i$, $\Sigma xy = \sum_{i=1}^n x_i y_i$,

$$\Sigma x^2 = \sum_{i=1}^n x_i^2, \Sigma y^2 = \sum_{i=1}^n y_i^2, \bar{x} = \frac{\Sigma x}{n}, \bar{y} = \frac{\Sigma y}{n}, s_{xy} = \Sigma xy - \frac{\Sigma x \cdot \Sigma y}{n} = \Sigma xy - n \cdot \bar{x} \cdot \bar{y},$$

$$s_{xx} = \Sigma x^2 - n \cdot \bar{x}^2, \quad s_{yy} = \Sigma y^2 - n \cdot \bar{y}^2$$

Spearman's rank correlation coefficient

$$r = \frac{n \cdot (\sum_{i=1}^n x_i \cdot y_i) - (\sum_{i=1}^n x_i) \cdot (\sum_{i=1}^n y_i)}{\sqrt{[n(\sum_{i=1}^n x_i^2) - (\sum_{i=1}^n x_i)^2] \cdot [n(\sum_{i=1}^n y_i^2) - (\sum_{i=1}^n y_i)^2]}} = \frac{s_{xy}}{\sqrt{s_{xx} \cdot s_{yy}}} \in [-1, 1]$$

Based on the Beer-Lambert's law, for spectrophotometric data of absorbance and concentrations, r would be close to 1 since there is a strong positive linear relationship between the variables.

Assume that the desired linear regression line is $\hat{y} = \beta_1 \cdot x + \beta_0$ then the sum of squared residuals is $SSE = \sum_{i=1}^n (y_i - \hat{y}_i)^2 = \sum_{i=1}^n (y_i - \beta_1 \cdot x_i - \beta_0)^2$.

Using the least squares method, the minimum of SSE is found when

$$\frac{\partial SSE}{\partial \beta_1} = \frac{\partial SSE}{\partial \beta_0} = 0$$

$$\Rightarrow \sum_{i=1}^n (y_i - \beta_1 \cdot x_i - \beta_0) \cdot x_i = 0, \sum_{i=1}^n (y_i - \beta_1 \cdot x_i - \beta_0) = 0$$

$$\Rightarrow \beta_0 = \bar{y} - \beta_1 \cdot \bar{x}, \beta_1 = \frac{s_{xy}}{s_{xx}} \quad (\text{see [2], [5]})$$

The coefficient of determination $R^2 = 1 - \frac{SSE}{s_{yy}}$ measures the goodness of fit of the linear model.

For spectrophotometric data, the slope β_1 of the calibration curve corresponds to the product of the molar absorptivity and the path length: $\beta_1 = \epsilon \cdot l$. Since in most experiments, the path length l is fixed (typically $l = 1 \text{ cm}$), the slope β_1 gives an empirical estimate of how strongly the solute absorbs light at the chosen wavelength. The steeper slope means higher sensitivity, that is, small changes in concentration result in large changes in absorbance. Ideally, the y-intercept β_0 should be 0 (i.e., when the cuvette has only distilled water, the absorbance should be 0). In reality, β_0 reflects the instrumental noise or residual absorbance from solvents, cuvettes, or impurities.

2. COMPUTING CONCENTRATIONS

2.1. Experimental setup and method

2.1.1. Wavelength selection and justification

The molar absorptivity ϵ is wavelength - dependent. For the same substance, different wavelengths of light are absorbed differently (see Figure 3). To maximize sensitivity and linearity in our calibration curve, we would carry out the following steps:

- Step 1: Plot absorbance vs wavelength
- Step 2: Identify the peak absorbance wavelength λ_{max}
- Step 3: Avoid nearby wavelengths where the compound does not absorb strongly.

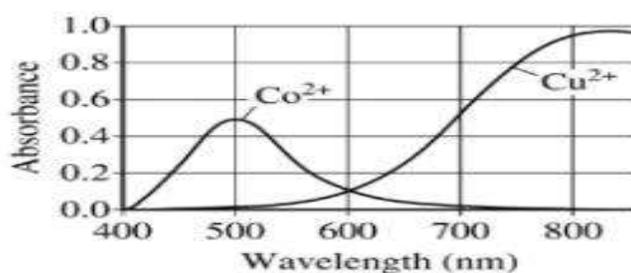


Figure 3. Set frequency in the spectrophotometer

In figure 3, in a solution that contains both Co^{2+} and Cu^{2+} , we only want to determine the concentration of Co^{2+} . Based on the graph, Co^{2+} ions absorb over a wide range of different wavelengths and its relatively high absorbance value is at 500 nm with very little interference from Cu^{2+} . Therefore, we set frequency $\lambda_{max} = 500 \text{ nm}$ in the spectrophotometer.

2.1.2. Data analysis and calibration curve

We follow these steps to determine the concentration of an unknown solution using Beer-Lambert's law:

- Step 1: Prepare a series of standard solutions with known concentrations
- Step 2: Measure the absorbance of each standard using spectrophotometer
- Step 3: Create a scatter plot of absorbance vs concentration
- Step 4: Find the best-fit line to the data
- Step 5: Measure the absorbance of the unknown solution
- Step 6: Use the regression model to predict the unknown concentration

Using the pHET Beer's law simulation, we can calculate concentrations following the process: simulate → record → model → predict.

2.2. Numerical results

We present the following worked example to illustrate the practical application of Beer-Lambert's law and linear regression in computing concentrations. ([1], [3], [4])

Given the absorbance and concentration data for $NiCl_2$ in figure 4, we can determine the concentration of an unknown solution $NiCl_2$ (aq) that has a measured absorbance of 0.5005.

2.2.1. Wavelength selection

Wavelength (nm)	A	Wavelength (nm)	A
780	0.21	480	0.07
760	0.22	460	0.26
740	0.21	440	0.51
720	0.19	420	0.39
700	0.19	400	0.11
680	0.16	380	0.03
660	0.11	425	0.47
640	0.07	430	0.52
620	0.04	435	0.53
600	0.02	440	0.51
580	0.01	445	0.46
560	0.01	450	0.4
520	0.01	455	0.33
500	0.03	413	0.27

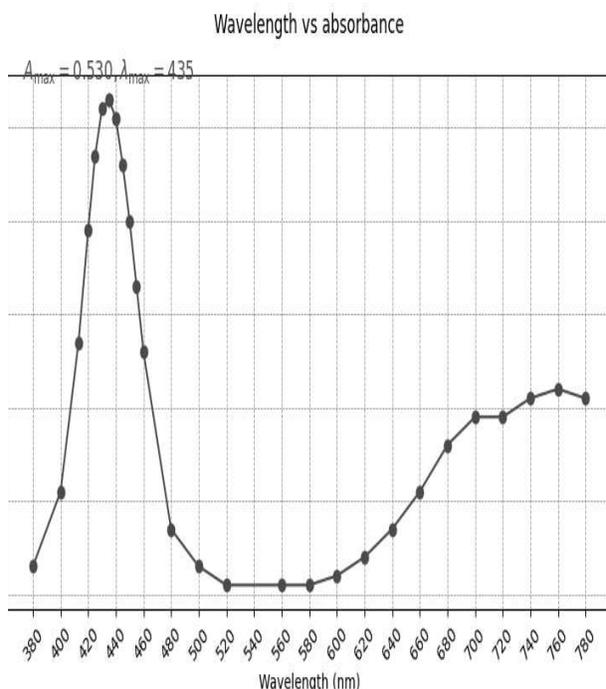


Figure 4: Wavelength (nm) vs absorbance (files: NiCl2.csv, NiCl2_wavelength.py)
The wavelength of maximum absorption $\lambda_{max} = 435 \text{ nm}$.

2.2.2. Constructing a calibration curve

c (mM)	A
0	0
25	0.13
50	0.27
75	0.4

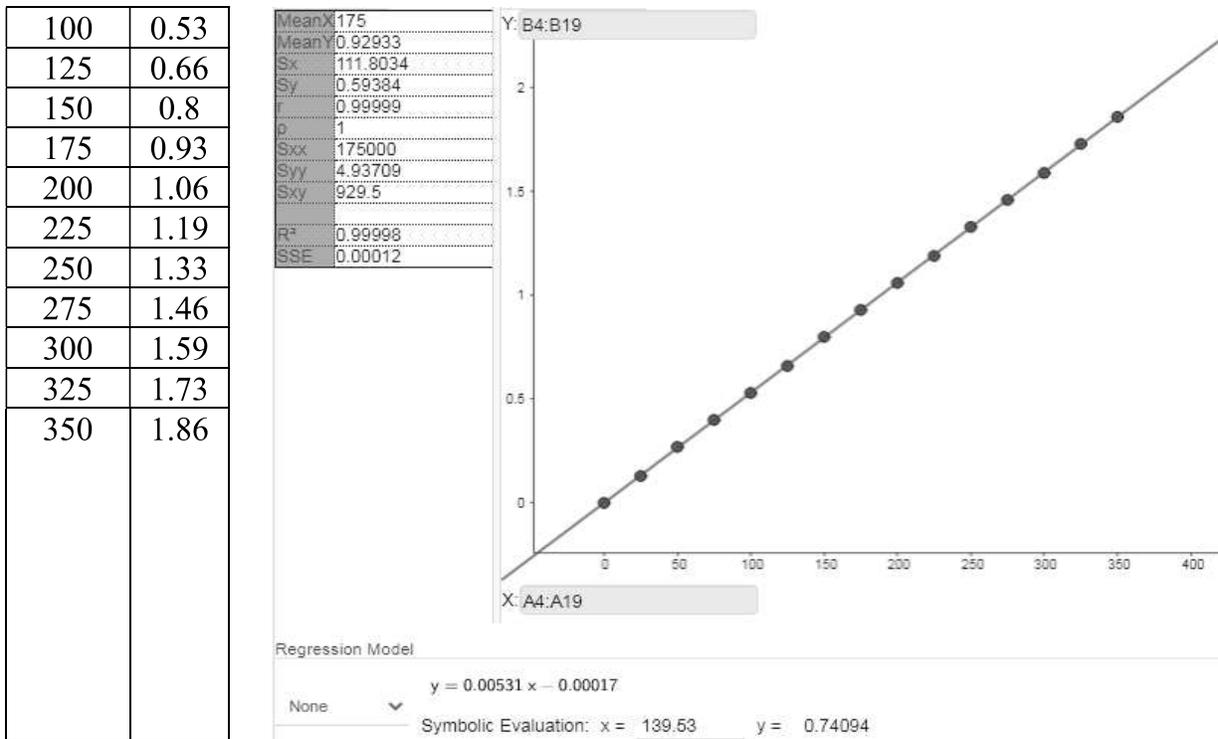


Figure 5: Absorbance of $NiCl_2$ at $\lambda_{max} = 435 \text{ nm}$ (GGB code: [axsvyp5s](#)) On the left of figure 5, we have prepared a series of standard solutions with known concentrations and the absorbance of each standard was measured using spectrophotometer. Plotting the absorbance (y-axis) versus the concentration (x-axis), we see that data points tend to cluster closely around a straight line. This indicates absorbance is directly proportional to concentration, consistent with Beer-Lambert's law.

Mean of concentration $\bar{x} = 175$, mean of absorbance $\bar{y} = 0.9293$,
 $s_{xx} = 175000$, $s_{yy} = 4.93709$, $s_{xy} = 929.5$
 $\Rightarrow \beta_1 = \frac{s_{xy}}{s_{xx}} \approx 0.00531$, $\beta_0 = \bar{y} - \beta_1 \cdot \bar{x} \approx -0.00017$ is negligible.

The linear regression model is $\hat{y} = 0.00531 \cdot x - 0.00017$.

Pearson's rank correlation coefficient $r = \frac{s_{xy}}{\sqrt{s_{xx} \cdot s_{yy}}} \approx 0.99999$ is very close to 1.

This suggests that there is a strong positive linear relationship between the variables.

$SSE = \sum_{i=1}^n (y_i - \hat{y}_i)^2 = 0.00012$ is really small.

The coefficient of determination $R^2 = 1 - \frac{SSE}{s_{yy}} \approx 0.99998$ shows that this linear regression model fits our data well. In figure 6, residuals appeared randomly distributed, confirming no model bias.

$A = 0.5005 \Rightarrow c \approx \frac{A}{\beta_1} = 94.2561 \text{ (mM)}$. Thus, given the measured absorbance of 0.5005, the concentration of an unknown solution $NiCl_2$ is approximately equal to 94.2561 (mM).

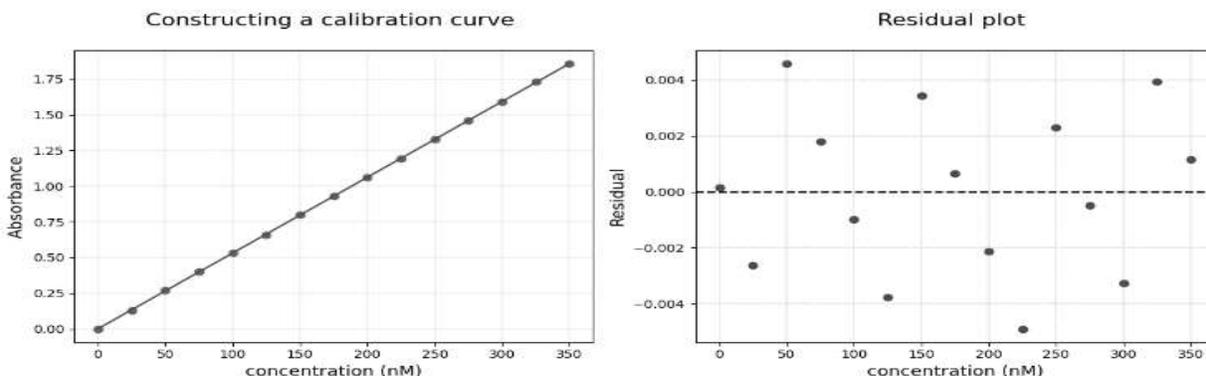


Figure 6. Residual plot

2.2.3. Python code

• **NiCl₂ wavelength.py** # wavelength selection

```
import pandas as pd
import numpy as np
import matplotlib.pyplot as plt
df = pd.read_csv("NiCl2.csv").sort_values(by="Wavelength (nm)")

max_idx = df['Absorbance'].idxmax()
lambda_max = df.loc[max_idx, 'Wavelength (nm)']
absorbance_max = df.loc[max_idx, 'Absorbance']

plt.figure(figsize=(8, 5))
plt.plot(df["Wavelength (nm)"], df["Absorbance"],
         marker='o', markersize=6, color='g', label="Data")
plt.scatter(lambda_max, absorbance_max,
           color='red', zorder=5, label=f"λmax= {lambda_max} nm")
plt.annotate(rf"$A_{\mathrm{{max}}}$ = {absorbance_max:.3f}, $"
            + rf"$\lambda_{\mathrm{{max}}}$ = {lambda_max}$",
            (lambda_max, absorbance_max), textcoords="offset points",
            xytext=(0, 10), ha='center', fontsize='12', color='red')

plt.xticks(np.arange(df["Wavelength (nm)"].min(),
                    df["Wavelength (nm)"].max() + 1, 20), rotation=45)
plt.title("Wavelength vs absorbance\n")
plt.xlabel("Wavelength (nm)")
plt.ylabel("Absorbance")
plt.grid(True, color='gray', linestyle='--', linewidth=0.5)
plt.tight_layout()
plt.show()
NiCl2_concentration.py      # construct the calibration curve
import pandas as pd
import numpy as np
import matplotlib.pyplot as plt
spec_data = pd.read_csv("spectrophotometric_data.csv").sort_values(by='c (mM)')
n = len(spec_data['A'])
mean_x = np.mean(spec_data['c (mM)'])
```

```

mean_y = np.mean(spec_data['A'])
s_xy = np.sum(spec_data['c (mM)'] * spec_data['A']) - n * mean_x * mean_y
s_xx = np.sum(spec_data['c (mM)'] ** 2) - n * mean_x ** 2
s_yy = np.sum(spec_data['A'] ** 2) - n * mean_y ** 2
slope = s_xy / s_xx
y_intercept = mean_y - slope * mean_x
corr_coefficient = s_xy / np.sqrt(s_xx * s_yy)
y_pred = slope * spec_data['c (mM)'] + y_intercept
residuals = spec_data['A'] - y_pred
sse = np.sum((spec_data['A'] - y_pred) ** 2)
r_squared = 1 - sse/s_yy
fig, (ax1, ax2) = plt.subplots(1, 2, figsize=(12, 5))

ax1.scatter(spec_data['c (mM)'], spec_data['A'], marker='o')
ax1.plot(spec_data['c (mM)'], slope * spec_data['c (mM)'] + y_intercept, color='r')
ax1.set_xlabel("concentration (nM)", fontsize=13)
ax1.set_ylabel("Absorbance", fontsize=13)
ax1.grid(True, color='gray', linestyle='--', linewidth=0.2)
ax1.set_title("Constructing a calibration curve \n", fontsize=16)
ax2.scatter(spec_data['c (mM)'], residuals, color='g')
ax2.axhline(0, color='black', linestyle='--')
ax2.grid(True, color='gray', linestyle='--', linewidth=0.3)
ax2.set_xlabel("concentration (nM)", fontsize=13)
ax2.set_ylabel("Residual", fontsize=13)
ax2.set_title("Residual plot \n", fontsize=16)
plt.tight_layout()
plt.show()

```

3. CONCLUSION

In this article, we demonstrated how Beer-Lambert's law, combined with linear regression techniques can be used to determine the concentration of an unknown solution based on spectrophotometric data. The regression model not only confirms the strong linear relationship between absorbance and concentration, but also allows us to make reliable predictions for unknown concentrations of the same substance. Moreover, through our computational example, we also highlighted the use of Python to effectively and efficiently deal with large spectrophotometric datasets. Overall, this indirect approach provides a powerful, reliable method for concentration determination.

REFERENCE

1. Akash, M. S. H. (n.d.). Introduction to spectrophotometric techniques. In *Essentials of pharmaceutical analysis* (pp. 19–27). Springer.
2. Bluman, A. G. (2012). *Elementary statistics: A step by step approach* (8th ed., pp. 534–572). McGraw-Hill.
3. StudyCorgi. (2023, April 18). Laboratory report on visible spectrophotometry of nickel (II) chloride. *StudyCorgi*. <https://studycorgi.com/laboratory-report-on-visible-spectrophotometry-of-nickel-ii-chloride/>

4. O'Haver, T. (2015). Interactive computer models for analytical chemistry instruction. *University of Maryland*. <https://terpconnect.umd.edu/~toh/models/BeersLaw.html>
5. Dekking, M. (2005). *A modern introduction to probability and statistics: Understanding why and how*. Springer. <https://doi.org/10.1007/1-84628-168-7>.

SỬ DỤNG ĐỊNH LUẬT BEER-LAMBERT VÀ HỒI QUY TUYẾN TÍNH ĐỂ XÁC ĐỊNH NỒNG ĐỘ CỦA CHẤT HÓA HỌC

Tóm tắt: Bài báo này đưa ra phương pháp gián tiếp để xác định nồng độ một chất hóa học trong dung dịch bằng cách áp dụng định luật Beer-Lambert's law và mô hình hồi quy tuyến tính.

Trong hóa học và hóa sinh, việc phân tích định lượng nồng độ các chất hóa học là một nhiệm vụ quan trọng, then chốt nhưng đôi khi không thể tiến hành định lượng trực tiếp. Do đó, trong bài báo này, tác giả áp dụng định luật Beer-Lambert's law và kỹ thuật hồi quy tuyến tính để xây dựng đường chuẩn từ dữ liệu thực nghiệm đo bởi máy đo ảnh phổ. Từ quan hệ tuyến tính giữa tính hấp thụ ánh sáng và nồng độ của chất, ta có thể dự đoán nồng độ của chất khi biết độ hấp thụ ánh sáng của nó.

Từ khóa: Định luật Beer-Lambert, hồi quy tuyến tính, tính hấp thụ ánh sáng, quang phổ, máy đo ảnh phổ, nồng độ, đường chuẩn.