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Spectrophotometer and Beer-Lambert Law

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Abstract. This study was designed to investigate the relationship between the concentration of aqueous copper (II) sulfate (CuSO4) solutions and their light absorbance, and to validate the Beer-Lambert Law for quantitative analysis. A series of standard solutions, with concentrations from 0.0131 M to 0.510 M, were prepared by serially diluting a stock solution. We measured the absorbance of each solution at a wavelength of 640 nm with a UV-visible spectrophotometer. The resulting data showed a strong linear relationship (y=2.2344x−0.0084, with an R2 value of 0.9996). Using this calibration, we determined the concentration of an unknown CuSO4 solution to be 0.453 M, differing from its theoretical concentration by a minimal error of just 0.658%. These results confirm that the Beer-Lambert Law is both applicable and accurate for this chemical system in the tested range.

Keywords: Beer-Lambert Law; Spectrophotometry; Copper(II) Sulfate; Quantitative Analysis.

1. Introduction

The vast range of colors we see in the world is due to the way various compounds uniquely absorb certain wavelengths of light. The colors we observe are simply the wavelengths that aren't absorbed by an object [1]. This absorption is controlled by the electrons within the compounds, which get energized when they absorb specific wavelengths. The exact wavelengths absorbed depend on the types of atoms in the compound and the nature of their bonds. Spectroscopy, which examines how atoms and molecules absorb, emit, or scatter electromagnetic radiation, helps scientists determine a substance's characteristics based on the light it absorbs [2].

In aqueous solutions, many transition metal ions form colored coordination complexes. For instance, the copper(II) ion forms a pale blue aqua complex, [Cu(H2O)6]2+ [3]. This distinct color arises because the complex absorbs light in the orange-red region of the visible spectrum (around 600-800 nm), and the transmitted light is perceived as its complementary color, blue [4]. The intensity of this color is directly related to the concentration of the ion in the solution.

The Beer-Lambert Law is a fundamental principle in spectroscopy that relates the concentration of a substance in a solution to the amount of light it absorbs. It can be used to show the relationship between absorbance (A) and concentration (c) [5].

$$A = \varepsilon l c \tag{1}$$

In this equation, ε is the molar absorptivity, a constant specific to the substance at a given wavelength; l is the path length of the light through the solution (typically the width of the cuvette, which is constant); and c is the molar concentration of the absorbing species.

Disappointingly, Beer-Lambert Law isn't always plausible. One limitation of the Beer-Lambert law is that it tends to break down at higher concentrations. This happens because molecules begin to interact with each other, causing deviations from the expected linear relationship [5]. Another drawback is that the law only applies accurately within a specific concentration range. Once one goes beyond that, especially with more complex substances, the law may no longer hold true [6].

Therefore, the primary aim of this experiment was to first construct a calibration curve by measuring the absorbance of five prepared copper(II) sulfate solutions of known concentrations. The second

objective was to then use this curve to determine the concentration of an unknown copper(II) sulfate solution, thereby testing the practical application of the Beer-Lambert Law.

2. Methods

2.1. Materials

The primary chemical used was copper(II) sulfate pentahydrate ($CuSO4 \cdot 5H2O$) solid. Key equipment included a UV-visible spectrophotometer, two cuvettes, a 250 mL volumetric flask, four 100 mL volumetric flasks, a 20 mL glass pipette with a pipette filler bulb, an electronic balance, beakers, a glass rod, and plastic pipettes.

2.2. Procedure

We began by preparing a 0.510 M stock solution (Solution A) of copper(II) sulfate. This involved dissolving approximately 31.90 g of $CuSO4 \cdot 5H2O$ in distilled water and diluting it to a final volume of 250 mL in a volumetric flask. From this stock solution, a series of four standard solutions (B, C, D, and E) were then prepared by serial dilution. For each new solution, 40 mL of the preceding, more concentrated solution was transferred into a 100 mL volumetric flask and diluted to the mark with distilled water.

We performed the absorbance measurements using a UV-visible spectrophotometer. The instrument was first calibrated to zero absorbance with a cuvette filled with distilled water (the blank). The absorbance of each standard solution (A-E) and an unknown solution (F) was then measured sequentially. For each measurement, the cuvette was first rinsed with a small amount of the solution to be tested. All measurements were conducted at a fixed wavelength of 640 nm. Although we did not perform a full spectral scan to find the exact maximum absorbance wavelength (λmax), the 640 nm wavelength was selected because it falls within the known absorption band for the $[Cu(H2O)6]2+$ complex and was expected to provide sufficient absorbance for reliable measurement [4].

3. Results

The concentrations of the standard solutions were calculated based on the initial mass and the dilution factors. The amount of $CuSO4 \cdot 5H2O$ for Solution A was calculated as:

$$n(\mathrm{CuSO_4 \cdot 5H_2O}) = \frac{m}{M} = \frac{31.9\mathrm{g}}{250\mathrm{g/mol}} = 0.128\mathrm{mol} \quad (2)$$

Then the concentration of solution A is:

$$c(\text{ Solution } A) = \frac{n}{V} = \frac{0.128\mathrm{mol}}{0.25L} = 0.510M \quad (3)$$

The concentrations of the subsequent serially diluted solutions were calculated accordingly. The measured absorbance for each standard solution is presented in Table 1.

Table 1. Concentration and Absorbance of Standard Solutions.

# Solution	Concentration/M	Absorbance
A	0.510	1.128
B	0.204	0.460
C	0.0816	0.163
D	0.0326	0.061
E	0.0131	0.026

A calibration curve was plotted using these data, showing absorbance as a function of concentration (Figure 1).

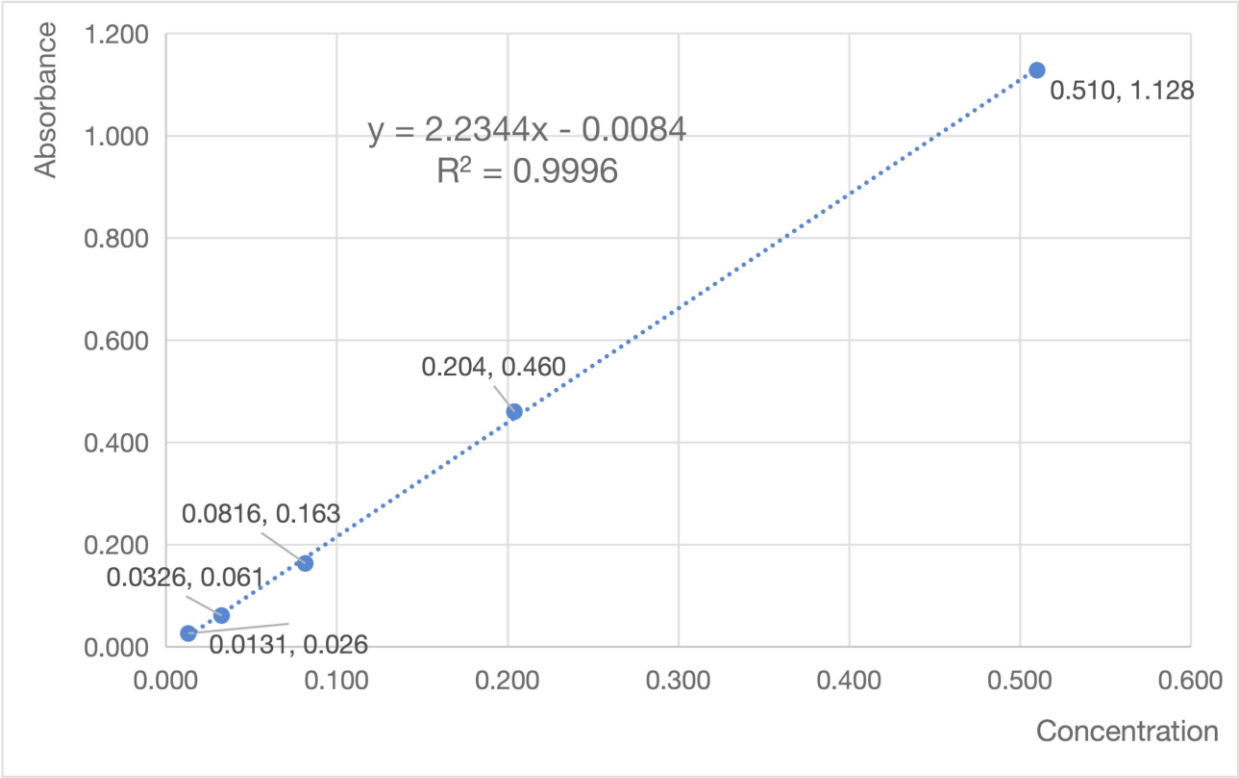

Figure 1. Calibration curve for standard CuSO4 solutions at 640 nm.

The data shows a strong linear relationship, with a linear regression equation of

$$y = 2.2344x - 0.0084 \tag{4}$$

and a coefficient of determination (R2) of 0.9996.

An unknown solution, Solution F, was prepared with a theoretical concentration of 0.456 M, based on dissolving 28.50 g of CuSO4 · 5H2O in 250 mL of water. The absorbance of this unknown solution was measured to be 1.004.

Using the linear regression equation from the calibration curve, the experimental concentration of Solution F was calculated:

$$c(\text{ Solution } F) = \frac{1.004+0.0084}{2.2344} = 0.453M \tag{5}$$

The percentage error between the theoretical and experimental concentration was then calculated:

$$\text{Percentage Error } = \frac{|\text{ Theoretical-Experimental }|}{\text{Theoretical}} \times 100\% = \frac{|0.456M-0.453M|}{0.456M} \times 100\% = 0.658\% \tag{6}$$

4. Discussion

Looking at the results, the experiment provides strong support for the validity of the Beer-Lambert Law for aqueous copper(II) sulfate solutions, at least within the concentration range we tested (0.0131 M to 0.510 M). The coefficient of determination, R2=0.9996, is very close to 1.0, which indicates an exceptionally strong linear fit between absorbance and concentration. Such a high degree of linearity suggests that, for our experiment, any interactions between solute molecules were likely minimal. These interactions are known to cause deviations from the law at higher concentrations [5], but they did not appear to be a significant factor here.

It is worth noting that the calibration curve had a small negative y-intercept (-0.0084). Ideally, the line should pass directly through the origin (0,0). This small deviation is most likely the result of minor systematic errors, perhaps from an imperfect blanking of the spectrophotometer or slight instrumental noise.

The percentage error for the unknown solution was found to be a very low 0.658%. This high level of accuracy is a good sign, validating both the experimental procedure and the utility of our calibration curve. When considering the source of this small error, a few possibilities come to mind. On the instrumental side, there could have been minor fluctuations in the light source. On the procedural side, errors could have been introduced through the inherent precision limits of the volumetric glassware, slight inaccuracies in pipetting, or even small temperature changes in the lab that might affect solution volumes. Additionally, it is important to remember that we used an approximate molar mass for $CuSO4 \cdot 5H2O$ (250 g/mol instead of the more precise 249.69 g/mol). This simplification itself would have introduced a small, consistent error across all our standard solution calculations.

Finally, we must acknowledge that the absorbance was measured at a fixed wavelength of 640 nm without first performing a scan to identify the wavelength of maximum absorbance (λmax). Standard practice favors using λmax because this is where the absorbance is strongest and the measurement is least sensitive to small wavelength calibration errors [2]. However, the strong linearity and high accuracy we achieved suggest that 640 nm was a sufficiently sensitive wavelength for this analysis, and the instrument's wavelength accuracy was stable throughout the measurements.

5. Conclusion

In summary, the experimental data revealed a clear and direct relationship between the absorbance and the concentration of the CuSO4 solutions, which strongly adheres to the Beer-Lambert Law. The experiment successfully generated a reliable calibration curve described by the function $y = 2.2344x - 0.0084$ (R2=0.9996). With the aid of this curve, we determined that the unknown concentration of solution F was 0.453 M, with a percentage error of only 0.658%. This result demonstrates that spectrophotometry is an effective and precise tool for this type of quantitative analysis.

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