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How to Calculate and Measure Solution Concentration using UV-Vis Spectrum Analysis: Supporting Measurement in the Chemical Decomposition, Photocatalysis, Phytoremediation, and Adsorption Process

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ABSTRACT

UV-visible (UV-Vis) spectroscopy is a powerful instrument for qualitative investigation and quantitative detection of pollutants in water. UV-Vis spectrophotometry is an analytical method using the concept of transmission of light in UV and Visible wavenumber. Generally, compounds can be identified using UV-Vis Spectrophotometry, based on the concept of light absorption, specifically for compounds with a chromophore group and an auxochrome group. Although the utilization of UV-Vis spectrum analysis has been well-documented, no information regarding detailed step-by-step measurement for examining detailed quantitative analysis, particularly in determining the concentration of an analyte in an aqueous solution sample. Here, this study explores the idea and application of UV-Vis technology in water quality detection, including guidelines for determining the concentration of the sample in an aqueous solution. To support the analysis, we also added practical examples for understanding concentration during the organic decomposition. This paper is intended to be useful for researchers and students in understanding UV-Vis spectrophotometry when analyzing chemical composition during chemical decomposition, photocatalysis, phytoremediation, and adsorption analysis.

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1. INTRODUCTION

In recent years, the degradation of various types of organic and inorganic pollutants in water using photocatalytic and adsorption methods have been studied extensively (Yu & Huang, 2023; Behera *et al*., 2021; Gautam *et al*., 2020; Tufail *et al*., 2021; Hu *et al*., 2020). Photocatalytic and adsorption methods have been studied extensively due to their relatively high activity, biological and chemical stability, low cost, simplicity, and non-toxic (Behera *et al*., 2021; Sanakousar *et al*., 2022).

The determination and trace of organic and inorganic content in water are interesting. For example, some inorganic compounds in water are important and some are toxic (Saravanan *et al*., 2021). Metals such as zinc, manganese, copper, chromium, and iron-cobalt are essential elements for humans, animals, and plants. On the other hand, lead, cadmium, nickel, arsenic, and mercury are toxic even at low levels (Mehrandish *et al*., 2019). Then, the organic content causes a lack of dissolved oxygen which can cause death in aquatic biota due to lack of oxygen (Mahaffey *et al*., 2020). Therefore, it is necessary to estimate the organic and inorganic content in the solution, particularly in the aqueous sample.

Many analytical techniques exist in the literature for being utilized in the measurement and traces of heavy metals and organic compounds in mixed solutions, especially in the aqueous solution. The classification of methods for the determination of water quality is shown in **Figure 1**. Chemical, biological, and physical methods are currently used to determine water quality parameters. Chemical oxygen demand (COD), heavy metal content, nitrate nitrogen (NO_3-N), dissolved organic carbon (DOC), and turbidity are the most important water quality parameters. Titration analysis and electrochemical analysis are the two most common chemical methods for determining pollutant content in the laboratory.

However, these methods need some instruments with large, complicated, and expensive apparatuses as well as a requirement of a high amount of reagents that sometimes create issues in the production of secondary pollution as a byproduct. Further, the results are somewhat not of a real-time process. Biological methods primarily include enrichment analysis and biosensor technology; however, detection accuracy and sensitivity are significantly lower than in other methods. Physical methods primarily include hyperspectral remote sensing technology and molecular spectroscopy technology (Olaniran *et al*., 2013; Guo *et al*., 2020).

Among the above methods, spectrophotometry is a well-recognized method for identifying substances. It conducts quantitative determinations using the emission or absorption spectra of substances. It has been widely used in the field of rapid water quality determination in recent years.

Recently, due to the advantages of high precision, high detection efficiency, non-destructive sampling, environmental protection, low cost, and portability, ultraviolet-visible (UV-Vis) technology has been developed into an advantage and an effective tool for detecting pollutants in aqueous environments (Guo *et al*., 2020; Pratiwi & Nandiyanto, 2021). Some researchers have attempted to summarize the application of UV-VIS spectroscopy for determining the chemical structure of compounds previously. Some researchers also deepened UV-Vis spectroscopy for determining the chemical structure of compounds. However, discussion in the field of water quality detection, research objects, and research methods is still limited.

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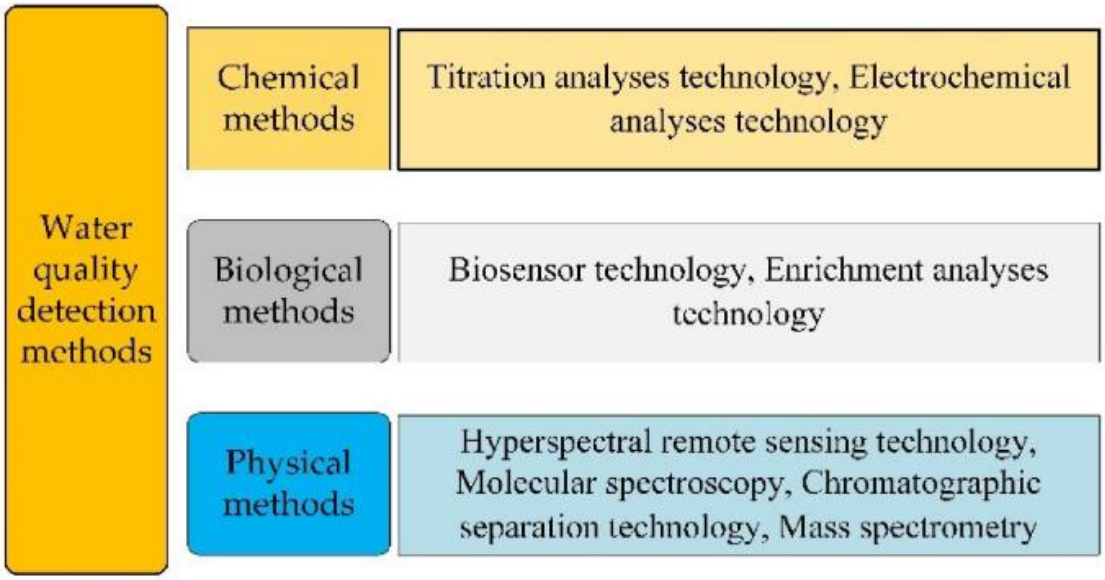

Figure 1. Methods for Determining Water Quality (adopted from Guo *et al*., 2020).

Therefore, this study introduces the theoretical basis for determining various parameters of water quality by UV-Vis spectroscopy and outlines the complete spectral data analysis process, including data pre-processing, characteristics of wavelength extraction, and absorbance formation. All explanations were introduced step by step to make clear and easily understand researchers and students for understanding solution concentration in aqueous solution, especially when analyzing chemical composition during chemical decomposition, photocatalysis, phytoremediation, and adsorption analysis.

2. BASIC PRINCIPLES

2.1. Theoretical of UV-VIS Spectrophotometer in Water Treatment

UV-Vis spectrophotometer is a spectrophotometric technique in the area of ultraviolet and visible light. This tool is used to measure the absorption of light ultra-violet and visible light in a sample. This type of spectrophotometer is more widely used in analyzing a material quantitatively by measuring the light absorbance value of a sample based on a specified wavelength (Albert *et al*., 2012). This absorbance value is used in determining the amount of concentration (for quantitative analysis) and type of component contained in a sample (for qualitative analysis) (Bardik *et al*., 2020). Light absorbed by a substance is different from the light captured by the human eye. Visible light (or light seen in our daily life) is called complementary colors. For example, a substance will be orange if it absorbs blue from the visible light spectrum and a substance will be black if it absorbs all colors contained in the visible light spectrum. For more details, the absorbed colors and complementary colors are shown in **Table 1** (Akash *et al*., 2020).

Based on the Lambert-Beer law, the working principle of the UV-Vis spectrophotometer is that polychromatic light from a light source will be converted into monochromatic light using a monochromator. The light is partially absorbed by the cell in the sample and some light will be passed through the photocell based on a certain wavelength. The amount of light that passes through this will be counted by the detector. The absorbance value of the light that is passed will be proportional to the concentration of the solution in the cuvette (Guo et al., 2020).

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Table 1. The Absorbed Colors and Complementary Colors.

Wavelength (nm)	Absorbed colors	Complementary colors (visible colors)
390-450	Violet	Yellow
450-495	Blue	Orange
495-570	Green	Red
570-590	Yellow	Violet
590-620	Orange	Blue
620-750	Red	Green

In detecting the concentration of analyte in the aqueous solution, UV-Vis spectroscopy is based on the fact that at certain wavelengths, pollutant molecules in water can absorb UV-Vis light. Light at certain wavelengths is absorbed due to the movement of electrons from the ground state to the excited state, which reduces the amount of light transmitted. Pollutants primarily absorb light in the UV-Vis range. As a result, we can detect the concentration of pollutants in water using the Lambert-Beer law as a theoretical foundation. Organic matter and turbidity are effectively adsorbed in the 380-750 nm wavelength range (Guo *et al*., 2020). The range of absorption spectra and the substance characteristics of the various substances are shown in **Table 2**.

2.2. Lambert-Beer Law

According to Lambert, absorption is directly proportional to the thickness of the irradiated cell, while according to Beer, absorption is directly proportional to concentration. These two statements are put together in the Lambert-Beer Law, namely, absorption is directly proportional to the concentration and thickness of the cell. This is because if the cell increases, the absorption will increase. If the concentration increases, the number of molecules through which the transmission beam of light will increase. Indeed, the absorption also increases. The Lambert-Beer Law must comply with the requirements in its use. The terms of the Lambert-Beer Law, namely the amount of concentration used is not extreme (the sample used is not highly concentrated), the sample to be analyzed must not dissociate, associate, or react with solvents that will create other products. Or, the color formed must be stable. The absorption of light by the specified substance and the light measured is monochromatic light (Akash *et al*, 2020). In short, the Lambert-Beer Law, namely the concentration of the solution being analyzed is directly proportional to the amount of light (absorbance) absorbed by the substance contained in the solution. The wavelength used in this tool ranges from 200 to 700 nm. The color absorbed by a compound or element is complementary. A schematic diagram of the Lambert-Beer law is shown in **Figure 2**.

Based on **Figure 2**, the outgoing light (I) correlates to solution turbidity and depends on the intensity of the incident light (I_0). K is the molar absorption coefficient related to the nature of the absorbing substance and the wavelength λ of the incident light. a is the concentration of the absorbing substance in mol/L. l is the thickness of the absorbing layer in cm.

Table 2. The Range of Absorption Spectra and the Substance Characteristics of the Various Substances.

Wavelength Range (nm)	Material Characteristics
200-220	Nitrate, nitrite
220-250	Conjugated diene, unsaturated aldehyde, unsaturated ketone
250-750	Organic matter and turbidity

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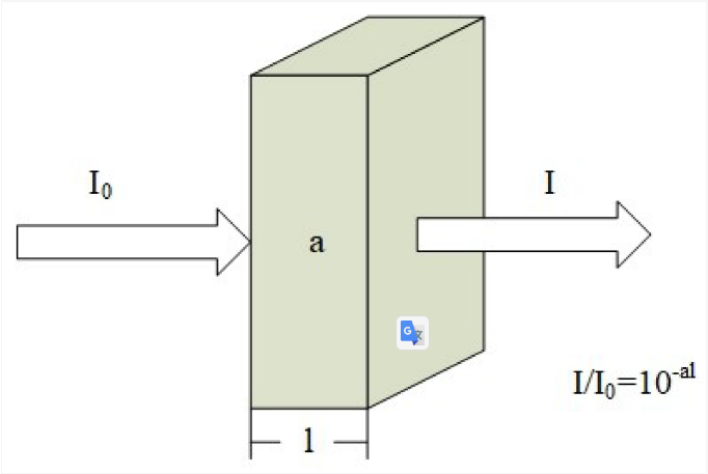

Figure 2. Schematic Diagram of the Lambert-Beer Law.

Absorbance is the amount of light or energy absorbed by the particles in the solution (the relative amount of light absorbed by the sample). The transmittance is the portion of the light that is transmitted (the relative amount of light that passes through the sample). The relationship between absorbance and transmittance is inversely proportional. The higher transmittance results in the lower the absorbance obtained, and vice versa. This is found in the Lambert-Beer Law, that is, if light passes through a solution without experiencing absorption, the absorption will be zero. If all light is absorbed, the transmittance is zero and the absorbance is infinity. The absorbed light is measured as absorbance (*A*), while the scattered light is measured as transmittance (*T*). All light phenomena are expressed by Lambert-Beer's law (Aljamali, 2015; Akash *et al*., 2020). Based on Lambert-Beer's law, the formula used to calculate the amount of light scattered is shown in Eq. (1) and Eq. (2).

$$T = \frac{I_t}{I_0} \quad (1)$$

Or

$$\%T = \frac{I_t}{I_o} \times 100\% \quad (2)$$

and absorbance is expressed by Eq. (3).

$$A = -\log T = -log\frac{I_t}{I_o} \quad (3)$$

The formula derived from the Beer's Law can be written by Eq. (4).

$$A = a \times l \times c \; or \; A = \varepsilon \times l \times c \quad (4)$$

By rearranging Eq. (4), the Lambert-Beer Law makes it possible to determine the concentration of a sample from a measured absorbance value. Molar absorptivity constant (ε) and path length (l) are known. The concentration of c can be calculated from the absorbance of A using Eq. (5).

$$c = \frac{A}{\varepsilon \times l} \quad (5)$$

where A is absorbance, l is the thickness of the cuvette is also generally 1 cm, c is the concentration of the solution measured. ε is the molar absorptivity constant (if the concentration of the solution measured is in molar terms) or the molar absorptivity constant (if the concentration of the solution measured is in mg/L terms).

The absorbance must be within the linear range of the instrument for optimal measurement results following the Lambert-Beer's Law. The optimal measurement range (i.e. the measurement range over the absorbance) is directly proportional to the concentration. It is given as $0.3 < A < 2.5$ (Mousa *et al*., 2017).

Therefore, it is advisable to avoid very high absorbance values ($A > 2.5$) as well as very low absorbance values ($A < 0.3$), which may result in a non-linear behavior of the calibration line. This is illustrated in **Figure 3**, where the measured values of above $A = 2.5$ and below $A = 0.3$ (red dotted line) deviate from the theoretical calibration line (green):

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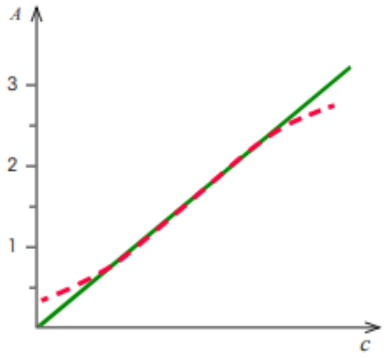

Figure 3. Non-Linearity: The Red Measured Values Outside the Linear Range Deviate from the Green Theoretical Calibration Line.

2.3. Measurement Principle

A UV/VIS spectrophotometer compares the intensity of light passing through a sample solution in a cuvette to the intensity of light before it passes through the sample. A light source, a sample holder, a dispersive device (e.g., a monochromator), and a suitable detector are the main components of a V/VIS spectrophotometer (Akash *et al.*, 2020). Measurement principle in UV/VIS spectroscopy shown in **Figure 4.** The working principle of a spectrophotometer is based on blank measurement and sample determination. The following steps are:

(i) Blank (a measure of the intensity of light transmitted through the solvent). Blank measurement is needed for the sample concentration determination. There are steps for blank measurement:
- The solvent (e.g. water or alcohol) is added into a suitable, transparent, and not absorbing container (a cuvette).
- A light beam emitted by the light source passes through the cuvette with the solvent.
- The intensity of the transmitted light at different wavelengths is then measured by a detector positioned after the cuvette with the solvent and recorded.

(ii) Sample determination. There are steps for sample determination:
- A sample dissolved in the solvent is added to the cuvette.
- A light beam emitted by the light source passes through the cuvette with the sample.
- When passing through the cuvette, the light is partially absorbed by the sample molecules in the solution.
- The transmitted light is then measured by the detector.
- The light intensity change at different wavelengths is calculated by dividing the transmitted intensity of the sample solution by the corresponding values of the blank. This ratio is finally stored by a recorder.

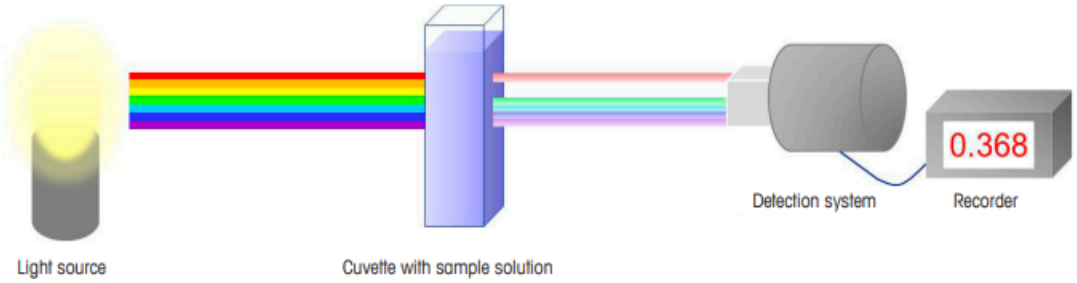

Figure 4. Measurement Principle in UV/VIS Spectroscopy.

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2.4. Important Aspects to be Considered During the Measurements

The aspects that need to be considered in spectrophotometer measurements, namely the sample used must be colored. If the solution to be analyzed is colorless, the sample must first be colored. It is intended that the sample can be analyzed based on its color-forming substances (Yang *et al*., 2017). The wavelength used also needs to be considered. The level of error in the measurement will be less if the maximum wavelength is used. This is because, at the maximum wavelength, the level of sensitivity of a sample will be maximum. In addition, by using the maximum wavelength, the absorbance curve obtained will comply with the Lambert-Beer law. In addition to these two aspects, the calibration of the wavelength and absorbance needs attention. A spectrophotometer is used to measure the intensity of light. Each sample used has a different light absorption depending on the compound formed. Therefore, it is necessary to calibrate to obtain more accurate results (Guo *et al*., 2020).

2.5. Step-by-Step Quantitative Analysis using UV-VIS using Standard Curves

One of the most common quantitative methods of analysis in water treatment is the determination of an analyte's concentration based on the absorption of ultraviolet or visible radiation that accumulates in water. One reason is that many organic and inorganic compounds have a strong electromagnetic spectrum absorption band in the UV/visible region (such as organic and inorganic compounds). Furthermore, analytes that do not absorb or weakly absorb UV/Vis radiation can frequently be chemically combined with species that do absorb ultraviolet and visible light. Quantitative analysis steps such as determining the concentration of analytes in a sample are generally described as follows:

(i) Step 1: Preparation of standard solutions

A standard solution is any chemical solution that has a precisely known concentration. Similarly, a solution with known concentration needs to be standardized. To prepare a standard solution, we dissolve a known mass of solute and then dilute it to the correct volume of solution. Standard solution concentration is usually expressed in terms of molarity (M) or moles per liter (mol/L). Usually, the solution is diluted with distilled water or another solvent (such as methanol, ethanol, etc.) to the limit and homogenized until a standard solution of 100 ppm is obtained. Not all substances are suitable solutes for standard solutions. The reagent must be stable, pure, and preferably of high molecular weight.

(ii) Step 2: Determination of the maximum wavelength

Before calculating the sample concentration using a UV-Vis spectrophotometer, we determine the maximum wavelength with the aim to be able to provide maximum sensitivity of samples containing analytes. Detailed determination of the maximum wavelength is carried out as follows:

- The spectro instrument is set to quantity mode and the wavelength is set.
- The wavelength is set and adjusted to the color of the complementary adsorbate solution to be measured.
- Before measuring the absorbance of the standard adsorbate solution, the blank solution is measured first at a predetermined wavelength. The blank solution is a solution that does not contain analytes or a solution without a sample. It is usually only solute or 0% of the analyte.
- Then, the absorption of approximately 3 mL of standard solution was measured using a UV-Vis spectrophotometer at maximum wavelength. Usually, the cuvette contains about 3 mL.
- After that, the absorbance value of each measured wavelength is recorded and tabulated (see **Table 3)**.

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Table 3. Example of Tabulated Wavelength vs Absorbance.

Wavelength (nm)	Absorbance
200	0.125
250	0.273
300	0.294
And so on	And so on

- Finally, the determination of the maximum wavelength is taken from the measured wavelength to produce maximum absorbance.

(iii) Step 3: Determination of the calibration curve

Making a calibration curve is used to find a linear regression equation. Thus, it can be used to find sample levels of measured absorbance. To create a detailed calibration curve, the steps are as follows:

- Making a calibration curve is done by preparing a series of standard solutions with a certain concentration. As previously explained, the calibration curve aims to find a linear regression equation. This linear regression equation is determined from the relationship between the analyte concentration series and the absorbance of the analyte. A series of standard solutions with a certain concentration is prepared by the dilution process of standard solutions. For example, working standards are made by making a series of six solutions. Each solution has a concentration of 2, 3, 4, 5, and 6 ppm. Each set of standard solutions is made by taking as much as 2; 3; 4; 5; and 6 mL of standard solution respectively, then transferred to a 100-mL volumetric flask and dissolved with distilled water to the limit and homogenized. To obtain a series of standard solutions with concentrations of 2, 3, 4, 5, and 6 ppm from a 100-ppm standard solution, it is calculated using the dilution formula as in Eq. (6).

$$V_1 M_1 = V_2 M_2 \quad (6)$$

where V_1 is the volume of stock solution taken, M_1 is the concentration of the diluted solution, V_2 is the volume of the diluted solution, and M_2 is the concentration of the solution resulting from the dilution. Detailed calculations for the manufacture of serial concentrations of standard solutions are as follows:

$V_1 M_1 = V_2 M_2$

$V_1 100\ ppm = 100\ \times 2$

$V_1 = 2\ mL$

Thus, to make a 2-ppm standard solution series with a volume of 100 mL, 100-ppm stock solution is taken as much as 2 mL.

- A series of standard solutions with a certain concentration has been prepared. Then, the absorbance is measured at the wavelength determined in the previous step in determining the maximum wavelength. Finally, the absorbance values of each series of standard solutions are recorded.
- After the absorbance values of a series of standard solutions are obtained, the absorbance values are plotted to become a curve of the relationship between the X and Y-axis curve. The series of analyte levels is plotted on the Y-axis and the absorbance of the analyte is plotted on the X-axis. **Figure 5** is a calibration curve determined by relating the series of analyte levels to the absorbance of the analyte. **Figure 5** has an intercept value (a) = 0.062 and a slope value (b) = 0.017 with a correlation value (r) = 0.998. Therefore, the linear equation is y = 0.062x – 0.017. The resulting absorbance data is quite good because all series contents from the smallest to the largest values have an absorbance value of 0.2 – 0.8, while the correlation value obtained is 0.998. This curve is very good because a good correlation value is close to 1.

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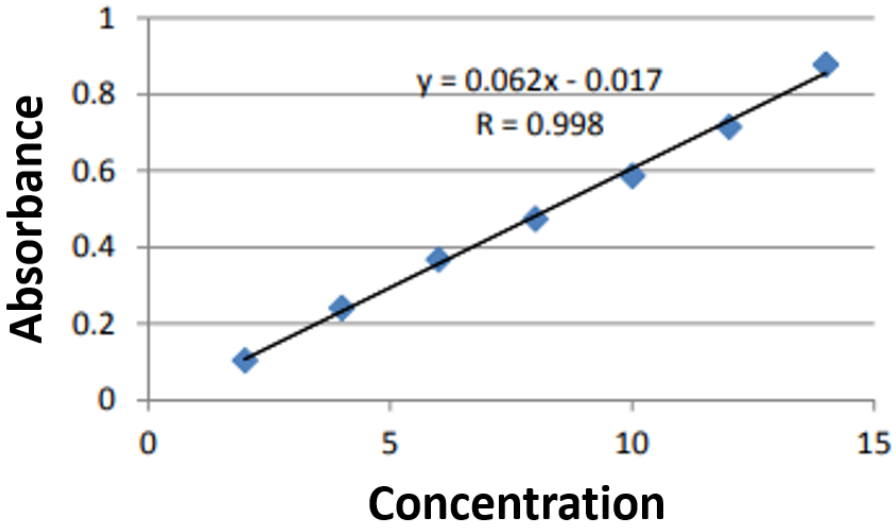

Figure 5. Example of The Calibration Curve.

(iv) Step 4: Determination of Sample Concentration using Calibration Curve

To determine the concentration of the analyte in the sample, the details of the steps are as follows:

- Before measuring the absorbance of the sample, the blank solution is measured first at a predetermined wavelength. The blank solution is a solution that does not contain analytes or a solution without a sample. For example, in the case of analyzing the concentration of curcumin in the aqueous solution, because curcumin is dissolved in pure water, the blank solution is pure water.
- The sample (or example a water sample containing organic or inorganic contaminants) is put into a cuvette and its absorbance is measured at the maximum wavelength determined in the maximum wavelength determination step (step 2).
- After the absorbance value is obtained, the absorbance value is recorded. The absorbance value will correspond to the concentration of the sample according to the Lambert-Beer law. For example, measuring a sample yields an absorbance value of 0.269.
- Next, the calculation of the concentration of the analyte in the sample is carried out using the linear equation that was previously obtained from the calibration curve determination step (step 3). The sample absorbance data is then substituted into the linear equation (y = mx + c) which has been obtained from the calibration curve based on the absorbance of the standard solution series. Assuming the sample absorbance data is the y variable, the x value is obtained as the concentration value. Based on the linear equation obtained from the calibration curve (step 3), the detailed sample concentration is calculated as follows:

$$y = 0.062x - 0.017$$

$$0.269 = 0.062x - 0.017$$

$$x = \frac{0.269 + 0.017}{0.062} = 4.61\ ppm$$

Thus, the analyte concentration in the sample is 4.61 ppm.

(v) Step 5: Determination of Sample Concentration using The Initial Concentration Peak

This method can be used as a shortcut for analyzing the concentration without additional calibration process. However, we should understand the initial concentration of solution.

To determine the concentration of the analyte in the sample using this method, the details of the steps are as follows:

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- Before measuring the absorbance of the sample, the blank solution is measured first at a predetermined wavelength. The blank solution is a solution that does not contain analytes or a solution without a sample. For example, in the case of analyzing the concentration of curcumin in the aqueous solution, because curcumin is dissolved in pure water, the blank solution is pure water.
- The initial sample (or example a water sample containing organic or inorganic contaminants) is put into a cuvette and its absorbance is measured at the maximum wavelength determined in the maximum wavelength determination step (step 2). The concentration using this initial concentration is a mandatory. The curve from initial concentration will be used as an initial standard for the next calculation.
- Other samples (or example a water sample containing organic or inorganic contaminants) are put into one by one into a cuvette and their absorbance is measured at the maximum wavelength determined in the maximum wavelength determination step (step 2).
- After the absorbance value is obtained, the absorbance value is recorded. The absorbance value will correspond to the concentration of the sample according to the Lambert-Beer law. For example, measuring a sample yields an absorbance value of 0.269.
- Next, the calculation of the concentration of the analyte in the sample is carried out by comparing to the initial concentration. The calculation using Eq. (7).

$$Concentration\ X = \frac{Absorbance\ X}{Absorbance\ Initial}\ x\ Concentration\ Initial \quad (7)$$

3. METHOD

To understand how to carry out quantitative analysis with UV-VIS instruments, this study is equipped with examples of how to determine the concentration of pollutant samples accumulated in water in water treatment applications. A detailed step-by-step approach for quantitative analysis in determining the concentration of a sample of a pollutant in the aqueous solution is described in this study.

4. RESULTS AND DISCUSSION

4.1. Experimental Results

One example of an organic dye used as a model for a pollutant in the case raised in this study is curcumin. In short, the water treatment process is carried out by reducing the concentration of dyes as a contaminant derived from curcumin which accumulates in water through a series of water treatment processes (e.g., photocatalyst, phytoremediation, or adsorption). Also, in this study, water treatment to reduce or remove pollutants such as organic dyes are demonstrated.

The success of the water treatment process is determined by how many pollutants accumulate in the water that can be removed or reduced. Thus, the initial (before treatment) and final (after treatment) concentrations of pollutant samples are compared to evaluate the success of the water treatment process. The water treatment process is said to be successful if the final pollutant sample concentration is lower than the initial pollutant sample concentration. Therefore, the determination of the concentration of pollutant samples that exist in the sample was carried out before and after the treatment was carried out.

However, here, to analyze the success of the process, the determination of the sample concentration is not only carried out before and after the treatment but the determination of the sample concentration is carried out at each time interval. Determination of the concentration of a sample of a solvent in an aqueous solution is based on the Lambert-Beer law of solids. According to the Lambert-Beer law, the

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amount of spectral absorption has a strong correlation with the concentration of water quality parameters. Thus, high-precision prediction models between water quality spectral data and water quality parameters can be created (Li *et al*., 2018).

4.2. Calculation Procedures (Step-by-Step Process): Example 1

In determining the concentration of the sample by UV-Vis spectroscopy, the experiment "determination of the concentration of curcumin accumulated in water after the water treatment process (i.e., photocatalysis or adsorption processes)" is exemplified step by step.

The steps for determining the concentration of curcumin dye solution with UV-Vis are divided into 2 stages, namely the pre-treatment stage and the post-treatment stage. In the pre-treatment stage, three steps must be carried out, namely preparing a standard solution (step 1), determining the wavelength (step 2), and preparing a calibration curve (step 3). Meanwhile, the post-treatment stage only includes one step, namely determining the sample concentration (step 4).

To determine the concentration of organic dyes (such as curcumin) as pollutants accumulated in water using a UV-Vis instrument, the steps are as follows:

(i) Step 1: Preparation of standard solution

To prepare a standard solution, dissolve a known mass of solute diluted and homogenized to the correct volume of solution. To prepare a 100-ppm standard solution, as a model organic dye, curcumin powder was used. A 100-ppm curcumin standard solution was prepared by dissolving and homogenizing 5 grams of curcumin powder with 600 mL of purified water. After being homogeneous, the 100-ppm curcumin solution was filtered through a vacuum filter to separate the insoluble curcumin residue. The filtrate resulting from the curcumin solution was a standard curcumin solution with a concentration of 100 ppm.

(ii) Step 2: Determination of the maximum wavelength

The steps for determining the maximum wavelength are as follows:

- The spectro instrument is set to quantity mode and the wavelength is set.
- The wavelength is set and adjusted to the color of the complementary adsorbate solution to be measured. Based on Table 1, because the curcumin solution has a yellow-to-orange complementary color, the color that will be absorbed is violet to blue. Therefore, the maximum wavelength is set at 390-495 nm.
- Then, standard adsorbate solutions with concentrations of 100-ppm were then measured for absorbance using a UV-Vis spectrophotometer at λ = 390-495 nm. The principle of determining the maximum wavelength is by scanning the absorbance value in the desired wavelength range. For example, here, the desired wavelength range is 390-495 nm. Thus, the maximum wavelength scanning process is carried out in the desired wavelength range, namely 390-495 nm.
- After that, the absorbance value of each interval wavelength is measured (starting from 390-495), recorded, and tabulated as exemplified in **Table 3**.
- After scanning the wavelength in the desired range, several absorption peaks will appear as shown in **Figure 6**. Finally, the determination of the maximum wavelength is taken from the measured wavelength to produce maximum absorbance. Here, the selected maximum wavelength of the curcumin solution is 395 nm due to has higher absorbance value. Because the maximum wavelength is known, the absorbance measurement for the standard curve (in step 3) and concentration (in step 4) is measured at this maximum wavelength, which is 390 nm.

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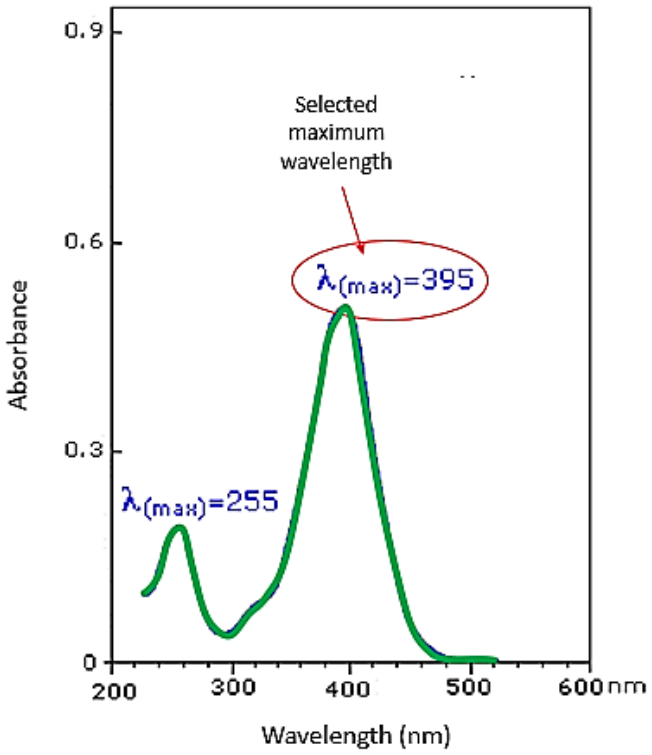

Figure 6. Some of The Absorption Peaks Result from Scanning the Wavelengths.

(vi) Step 3: Determination of the calibration curve

Before determining the calibration curve, there are several steps:

- The first step that must be taken is to prepare a series of standard solutions with certain concentration variations. A series of standard solutions are prepared by determining the series of standard solutions to be made from 100-ppm curcumin standard solution. For example, the concentration series of standard solutions to be made are 20, 30, 40, 60, and 80 ppm. After that, a series of standard solutions with concentrations of 20, 30, 40, 60, and 80 ppm were prepared by diluting 100 ppm curcumin standard solution. The dilution formula follows Eq. (1). An example of detailed calculations for making a standard solution with a concentration of 20 ppm with a volume of 10 mL from a standard solution of 100 ppm with dilution is as follows:

  $V_1 M_1 = V_2 M_2$

  $V_1 100\ ppm = 10 \times 20$

  $V_1 = 2\ mL$

  Thus, to obtain a series of standard solutions with a concentration of 20 ppm with a volume of 10 mL, the standard 100-ppm curcumin solution that must be taken is 2 mL. Then, the same method was adopted for the preparation of other series of standard solutions with concentrations of 30, 40, 60, and 80 ppm. The dilution method for preparing a standard adsorbate solution with a concentration of 20 ppm is also analogous to preparing a standard adsorbate solution with a concentration of 30, 40, 60, and 80 ppm by taking 3.0; 4.0; 0.6; and 0.8 mL of the 100-ppm curcumin standard solution, respectively.
- After a series of standard solutions were prepared, the next step was to measure the absorbance with the UV-VIS instrument at the maximum wavelength. The absorbance results are then recorded and tabulated for each value of the concentration of the solution. Absorbance data from a series of variations in the concentration of

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standard curcumin solutions are listed in **Table 4**.

- The concentration and absorbance data from a series of standard solutions were then converted into a calibration curve by plotting the X-axis as concentration and Y-axis as absorbance. The standard curve of the solution obtained is shown in **Figure 8**. From **Figure 8**, the linear equation obtained is y = 0.0114x + 0.0189 with an intercept value (*a*) = 0.0114 and a slope value (*b*) = 0.0189 with a correlation value (R^2) = 0.9794.

(iii) Step 4: Determination of Sample Concentration

After steps 1-3 (pre-treatment stage) have been carried out, the next step is to determine the concentration of the curcumin solution sample (pra-treatment stage). Determination of the concentration of curcumin solution samples can be done to determine the initial and final concentrations of curcumin solution samples that accumulate in the water. To determine the initial concentration of the curcumin solution accumulated in water, the absorbance was measured before the water treatment process (i.e., photocatalysis or adsorption processes). Meanwhile, to determine the final concentration of curcumin solution accumulated in water, the absorbance was measured after the water treatment process (i.e., photocatalysis or adsorption processes) was carried out. The hope is that through the water treatment process, the dye or pollutant compounds can be reduced or removed from the water. Therefore, to prove that the water treatment process is successful, the initial and final concentrations of the sample solution must be determined and compared. If the final concentration of the sample is less than the initial concentration of the sample, then the water treatment process is successful because based on determining the concentration of the sample, the pollutants accumulated in the water can be reduced or eliminated (Nandiyanto *et al*., 2020).

Table 4. Absorbance and Concentration Data of Series Curcumin Standard Solution.

Concentration (ppm)	Absorbance
20	0.210
30	0.348
40	0.538
60	0.707
80	0.903

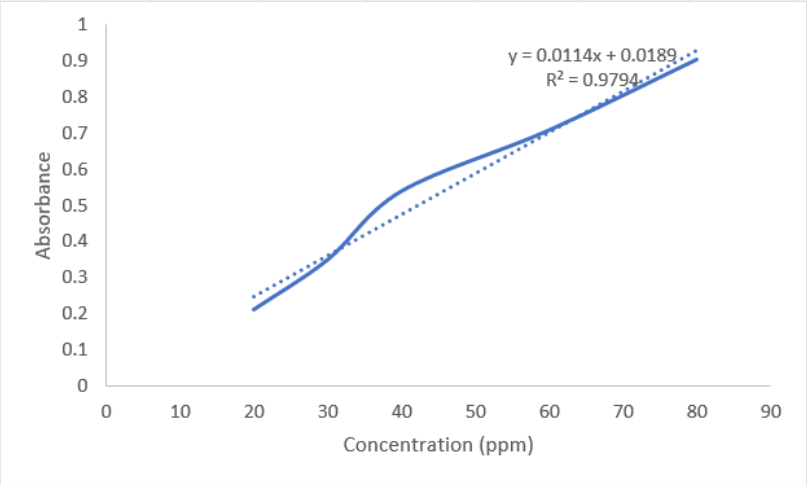

Figure 8. Calibration Curve of a Series of Standard Curcumin Solutions from Photocatalyst Applications.

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To measure the concentration of the sample, both initial and final concentrations, the blank solution is first measured at a predetermined wavelength. After that, the absorbance of the samples before and after the water treatment was measured one by one by inserting the sample solution into the cuvette and then measuring it with a UV-Vis instrument. According to the Lambert-Beer law, the absorbance value will correspond to the concentration of the sample. The absorbance measurement results for the initial and final solutions obtained values of 0.150 and 0.051 respectively. Furthermore, the calculation of the concentration of the analyte in the sample is carried out by substituting the absorbance value in the linear equation previously obtained from step 3. Here, the absorbance data of the sample after the treatment process at each time interval is exemplified and presented in **Table 5**. The detailed determination of the concentration is exemplified for the calculation of the initial and final concentrations of the sample solution by substituting the absorbance value into the linear equation which has been obtained from the calibration curve which can be calculated as follows:

- Calculation of the determination of the initial concentration

  $y = 0.0114x + 0.0189$

  $0.912 = 0.0114x + 0.0189$

  $x = \frac{0.912 - 0.0189}{0.0114} = 78.342\ ppm$

  Thus, the initial concentration of the sample is 78.342 ppm.
- Calculation of the determination of the final concentration

  $y = 0.0114x + 0.0189$

  $0.761 = 0.0114x + 0.0189$

  $x = \frac{0.761 - 0.0189}{0.0114} = 65.096\ ppm$

  Thus, the final concentration of the sample is 65.096 ppm. Examples of the calculation results presented in **Table 5**.

(vii) Step 5: Determination of Sample Concentration using The Initial Concentration Peak

This method can be used as a shortcut for analyzing the concentration without additional calibration process. The initial sample (or example a water sample containing organic or inorganic contaminants) is put into a cuvette and its absorbance is measured at the maximum wavelength determined in the maximum wavelength determination step (step 2). The concentration using this initial concentration is a mandatory. The curve from initial concentration will be used as an initial standard for the next calculation.

After the absorbance values are obtained, the absorbances will be compared to the initial concentration. For example, when we understand the initial concentration is 78 ppm (absorbance = 0.912), measuring a sample with an absorbance value of 0.877 can result concentration of 75 ppm. This value can be obtained by dividing 0.877 with 0.912 and multiplying with 78 ppm. Detailed calculation results are shown in **Table 5**. Detailed calculation is in the following:

$$Concentration\ X = \frac{Absorbance\ X}{Absorbance\ Initial} x\ Concentration\ Initial$$

$$Concentration\ X = \frac{0.877}{0.912} x\ 78\ ppm = 75\ ppm$$

Table 5. Example 1 of Absorbance Data of Pollutant Samples After Treatment at Each Time Interval.

Time (minutes)	Absorbance	Concentration based on Calibration (ppm)	Concentration based on Initial Concentration (ppm)
0	0.912	78.342	78.000
30	0.877	75.272	75.007
60	0.779	66.675	66.625
90	0.766	65.535	65.513
120	0.761	65.096	65.086

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4.3. Calculation Procedures (Step-by-Step Process): Example 2

Determination of the concentration for each time interval is also calculated in the same way as in the calculation of determining the concentration of the initial (before the treatment, 0 minutes) and final (after the process, 120 minutes) samples. Based on this step, the initial and final concentrations of the dye sample solution accumulated in water have been identified. The results showed that the final concentration of the sample solution decreased after the water treatment process. Based on the results described, the success of the process is characterized by a reduced concentration of dye contaminants accumulated in the water after the treatment process which is in line with the decrease in the absorbance value and color intensity of the solution at each time interval, respectively as evidenced by **Figures 9(a)** and **(b)**, corresponding to UV Vis spectra and photograph image for the decolorization, respectively. Using the above procedures, we can convert the absorbance into concentration in **Table 6**.

In this case, we cannot do regression. But we understand the initial concentration (at time of 0 min) is 100 ppm. Thus, we can convert the concentration at various times from absorbance. For example, when the reaction is 12 minutes and the absorbance is 0.7, the concentration can be obtained by dividing 0.7 with 0.8 and multiplying with 100 ppm (see equation (7)). The result will be 88 ppm. Detailed calculation is in the following:

$$Concentration\ X = \frac{Absorbance\ X}{Absorbance\ Initial} x\ Concentration\ Initial$$

$$Concentration\ X = \frac{0.7}{0.8} x\ 100\ ppm = 88\ ppm$$

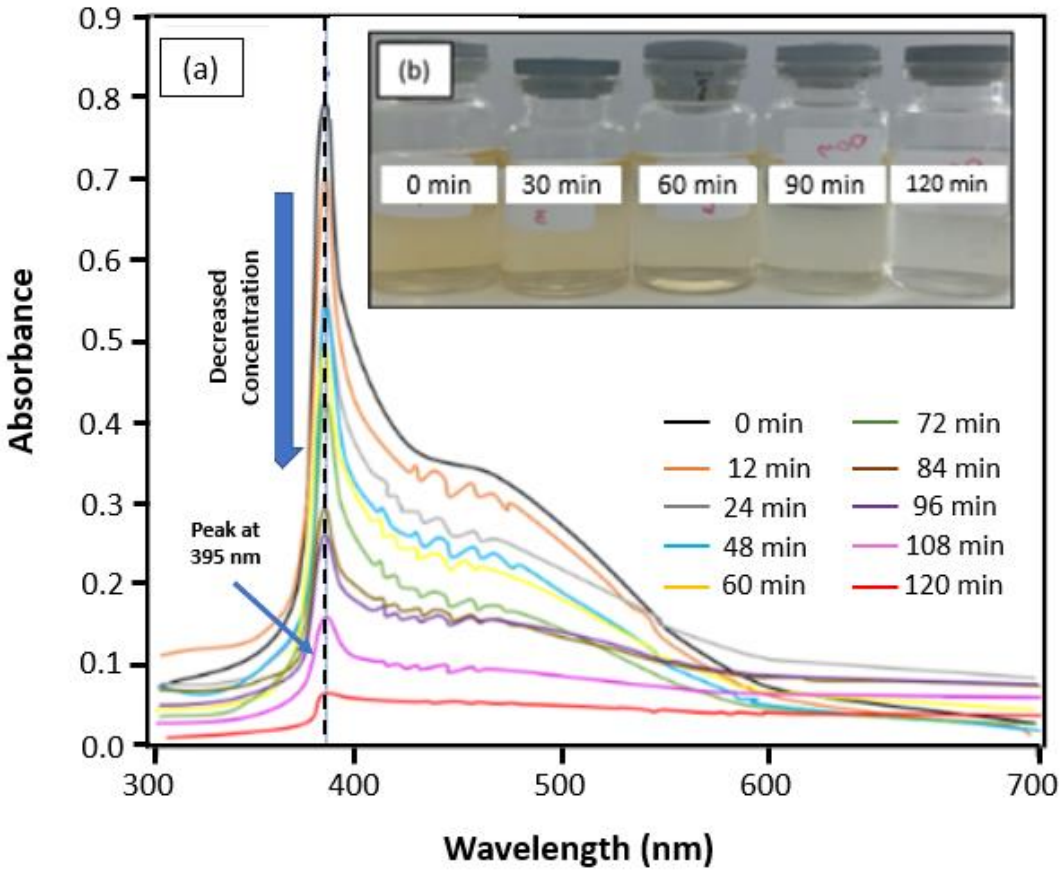

Figure 9. Evidence of the success of the curcumin solution process shown by the decrease in the absorbance value (a) and the physical appearance of the intensity of organic dyes in aqueous solution (b) (adopted from Sukmafitri *et al*., 2019).

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Table 6. Example 2 of absorbance data of samples

Time (Minutes)	Absorbance	Concentration Results Based on Initial Concentration (ppm)
0	0.79	100
12	0.70	88
48	0.55	69
84	0.29	36
120	0.05	6

5. CONCLUSION

This paper shows the steps for quantitative analysis using UV-VIS instruments, in particular the steps for determining sample concentrations using UV-VIS instruments are explained in detail. With this research, we believe that this paper can be used as a basis for understanding the determination of sample concentration in aqueous solutions using UV-VIS, especially when analyzing chemical composition during chemical decomposition, photocatalysis, phytoremediation, and adsorption analysis.

6. AUTHORS' NOTE

The authors declare that there is no conflict of interest regarding the publication of this article. Authors confirmed that the paper was free of plagiarism.

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