CHAPTER I
INTRODUCTION

1.1 Background

The process of analyzing a chemical is expected to provide accurate analysis results. The analysis process using instruments can make the measurement results more accurate. One of the quantitative analyzers using instruments is spectrophotometry where this analysis is carried out based on the transmittance or absorbance of the solution to light at a certain wavelength. Absorbance is the ratio of the intensity of the absorbed light to the intensity of the incident light. Then for transmittance itself is the fraction of incident light at a certain wavelength that passes through the sample. The absorbed light is measured as absorbance and the light released is measured as transmittance so that it can be concluded that the relationship between absorbance and transmittance is inversely proportional.

Accurate analysis results regarding the level of a substance are needed for various industries. For example in the drinking water industry, where drinking water sources must meet drinking water quality standards. One of the drinking water quality parameters is nitrite and nitrate content. Drinking water sources that contain nitrite and nitrate exceeding quality standards can endanger human health, especially pregnant women and babies. Based on the dangers posed by nitrite and nitrate contamination in drinking water, it is necessary to monitor the quality of drinking water so that the nitrite and nitrate content does not exceed the quality standards. The nitrite analysis method that is often used is spectrophotometry because it has good sensitivity and can be done with a simple spectrophotometer. Besides being used to measure nitrite and nitrate levels of water so that its quality is suitable for daily consumption, this spectrophotometric method can also be used to measure iron levels as a parameter of drinking water feasibility as well. Iron levels found in groundwater are generally in the form of Fe (II) because it has not been mixed with oxygen from the atmosphere. According to the Indonesian Minister of Health Regulation no. 32 of 2017, the maximum threshold of iron in drinking

water is 1 mg/L. The test method using Visible Spectrophotometry with a wavelength of 510 nm can be used to measure these iron levels with the help of phenanthroline reagent which makes the color of the test solution red-orange.

1.2 Practical Purpose

1. Determine the standard curve of the relationship of anthocyanin concentration vs absorbance by spectrophotometer spectrophotometric method.
2. Able to determine the optimum wavelength at the concentration of standard solution in terms of absorbance value.
3. Determine the concentration of SO42- ions in solution turbidimetrically by spectrophotometry.

1.3 Practical Benefits

1. Students are able to perform accurate quantitative analysis of a chemical substance using an instrument, in this case a spectrophotometer.
2. Students are able to understand the step process on the instrument used until the desired results are obtained.

CHAPTER II
LITERATURE REVIEW

2.1 Definition of Spectrophotometry

Spectrophotometry is a way of quantitative analysis based on the transmittance or absorbance of a solution to light at certain wavelengths using a spectrophotometer instrument. When a light containing the entire spectrum of wavelengths passes through a medium, such as colored glass or a solution that transmits light of a certain wavelength and absorbs other light, the medium seems to be colored. This color corresponds to the wavelength that is transmitted and is referred to as the complementary color.

The wavelength used is the optimum wavelength, which is the wavelength that is absorbed most by the compound / mixture being analyzed. There are several reasons why you should use the optimum wavelength, namely at the optimum wavelength the sensitivity is maximum because the change in absorbance for each unit of concentration is the greatest. Around the optimum wavelength, the shape of the absorbance curve is flat and under these conditions the Lambert- Beer law will be fulfilled. If repeated measurements are made, the error caused by resetting the wavelength will be very small, when the optimum wavelength is used. Table 2.1 Relationship between absorbed energy and molecular motion.

Table 2.1 Relationship between absorbed energy and molecular motion

Molecular Movement	Absorbed light	Energy
Rotation	Microwave,	Low
Vibration	Infrared	Medium
Electron transition	Visible, Ultraviolet	High

The electromagnetic spectrum range such as infrared, visible light, ultraviolet or X-ray can be used to interact with substances. The tool used in this practicum can also be called a colorimeter, because it can measure the absorption of light in the visible light spectrum. The scheme of the light

absorption process by a sample solution can be seen in Figure 2.1.

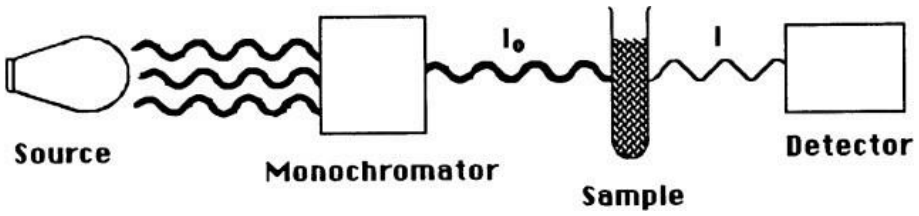

Figure 2.1 Absorption of light by sample solution

2.2 Equipment for Spectrophotometry

A very important component of a spectrophotometer, which is schematically shown in the figure below:

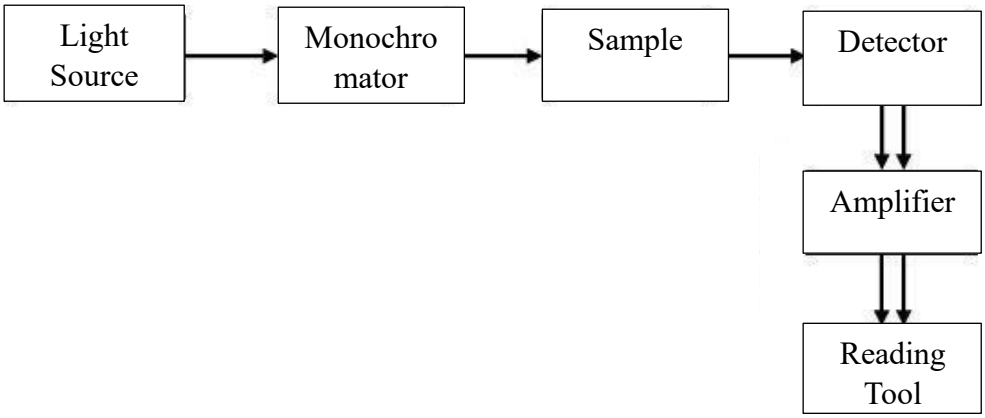

Figure 2.2 Principle of spectrophotometer

1. A continuous source of light energy covering the region of the spectrum in which the instrument is designed to operate. The light source used is usually a lamp. The lamp used is of course different based on the type of spectrophotometry used.
2. A monochromator, which is a device for isolating a narrow band of wavelengths from the wide spectrum emitted by the light source (100% monochromaticity is not achieved, of course). A monochromator consists of:
    - The entrance slit for determining the narrow beam of radiation from the source.
    - A collimation lens for collecting light.

- Prisms are for scattering light into specific wavelengths.A focusing lens to capture the dispersed light and sharpen the light to enter the cuvet through the exit slit.
- Exit slit to let the corrected wavelength of light into the sample cuvet

3. A container for the sample. The container used for the sample is a cuvet. The cuvet has two sides, the transparent side and the opaque side.
4. A detector, which is a transducer that converts light energy into an electrical signal. The detector should be sensitive and have a fast response over a large enough wavelength range. In addition, the electrical signal generated by the detector should be directly proportional to the emitted intensity.
5. An amplifier and associated circuitry that makes the electrical signal adequate for reading.
6. A reading system in which the magnitude of electrical signals is demonstrated.

Table 2.2 Light and dye uptake

λ (nm)	Forwarded Color	Absorbed Color/ Complementary Color
400 – 435	Violet	Yellow Green
435 – 480	Blue	Yellow
480 – 490	Aquamarine	Orange
490 – 500	Blue Green	Red
500 – 560	Green	Purple
560 – 580	Yellow Green	Violet
580 – 595	Yellow	Blue
595 – 610	Orange	Aquamarine
610 - 750	Red	Blue Green

2.3 Types of Spectrophotometry and Mechanism of Action

2.3.1 Visible Spectrophotometry

In this spectrophotometry, what is used as energy is visible light with λ 380-750 nm. The way this spectrophotometry works is that the sample to be analyzed must have color. Therefore, colorless samples must first be colored with specific reagents that will give color to the compound.

2.3.2 UV Spectrophotometry

UV spectrophotometry is based on the interaction of the sample with UV light which has a λ of 190-380 nm. UV light arena cannot be detected by our eyes, so compounds that can absorb this light are sometimes compounds that have no color, clear, and transparent. Therefore, colorless samples do not need to be made colored by adding certain reagents. But keep in mind that cloudy samples must first be made clear by filtration or centrifugation.

2.3.3 UV-Vis Spectrophotometry

It is a combination of visual and UV spectrophotometry because it uses two different light sources. So it can be used for both colored and colorless samples. UV-Vis spectrophotometry can identify molecules in solid or liquid samples, determine the concentration of certain molecules in solution, identify absorbance or transmittance through liquids or solids of various wavelengths, identify the reflectance properties of a surface or measure the color of a material, and study chemical reactions or biological processes.

2.3.4 IR (Infrared) Spectrophotometry

This spectrophotometry is based on the absorption of λ infrared. Infrared light is divided into near, mid, and far infrared. Infrared in spectrophotometry is far infrared and mid-infrared which has a wavelength of approximately 2.5-1000 μm. Generally, IR spectrophotometry is used in qualitative analysis, usually used to identify functional groups in a compound, especially organic compounds. The results of the analysis are usually in the form of a

signal chromatogram of the relationship between IR intensity and wavelength.

2.4 Benefits of Spectrophotometry in Industry

1. Measurement of protein content in milk
2. Analyzing the levels of inorganic compounds in wastewater effluent
3. Quality level testing on vegetable oil
4. Analyze the levels of chemical compounds in the pharmaceutical industry

2.5 Lambert-Beer Law

Lambert formulated the relationship between absorbance and the thickness of the medium layer traveled by light in solution.

$log\ Po = k.\ b$ ……(1)

Where $log\frac{Po}{P}$ = absorbance

P = radiation power that comes out of the medium

Po = radiation power entering the medium

b = medium layer thickness

According to Beer, absorbance is affected by concentration so that

$log\ Po = k.\ c$ ……(2)

If k_1' = f (c) and k_2' = f (b) then the substitutions of equations (1) and (2) are:

$$\frac{f(c)}{c} = \frac{f(b)}{b} = k$$

$$f(c) = k.c\ and\ f(b) = k.b$$

Substitute into the initial equation

$$log\frac{Po}{P} = f(c).b$$

$$log\frac{Po}{P} = f(b).c$$

$$log\frac{Po}{P} = k.c.b$$

$$log\frac{Po}{P} = k.b.c$$

If the concentration of solution in

- mol/liter then k should be written as ε, where ε = molar abortivity

$$log\frac{Po}{P} = \varepsilon.\,b.\,c$$

- grams/liter then k should be written as a where a = absorptivity

$$log\frac{Po}{P} = a.b.c$$

$$A = a.b.c$$

If absorbance (A) = $log\frac{Po}{P}$

$$\%T = \frac{Po}{P}.100\%$$

$$A = log\frac{Po}{P} = log\frac{1}{T} = -\log T = 2 - \log \%T$$

2.6 The Least Square Method

The Least Square method was chosen for the spectrophotometer approach according to Beer's Law which is the basis of light absorption.

A = a.b.c, where:

a = absorbtivity

b = cuvet thickness

c = concentration of the absorbing substance

When A is flowed for c against a sample that is b cm thick, it will produce a region where Beer's law applies to a straight line with slope ab.

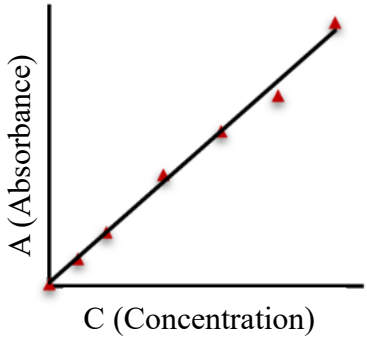

Figure 2.3 Standard curve of absorbance vs concentration relationship

The linear relationship between absorbance and concentration shows that absorbance depends on concentration. The greater the concentration of a substance, the greater the absorbance, and vice versa. Beer's law, A = a.b.c, can

be developed into a linear equation, because absorbance (A) is equal to y, a.b is equal to m, and concentration (c) is equal to the slope (x) in the equation $y = mx + c$.

However, instrumentation obtained graphs that do not fulfill the linear relationship between absorbance and concentration in the determination of absorbance of the solution so that to fulfill Beer's law the curve A vs C is used Least Square method, where:

$$y = mx + c$$

y = absorbance
m = a fixed number (constant), slope
x = solution content
c = a fixed number (constant), y-intercept

$$m = \frac{n\Sigma xy - \Sigma x \Sigma y}{n\Sigma x^2 - (\Sigma x)^2}$$

$$c = \frac{\Sigma x^2 \Sigma y - \Sigma x \Sigma xy}{n\Sigma x^2 - (\Sigma x)^2}$$

CHAPTER III
RESEARCH METHODOLOGY

3.1 Materials and Tools

3.1.1 Materials

1. CuSO4
2. HCl
3. BaCl2.2H2O
4. Aquadest

3.1.2 Tools

1. OPTIMA SP-300 Spectrophotometer
2. Cuvet and place cuvet
3. 50 ml measuring flaskGelas ukur
4. pH paperBeaker glass
5. Pipette

3.1.3 Main Tool Figure

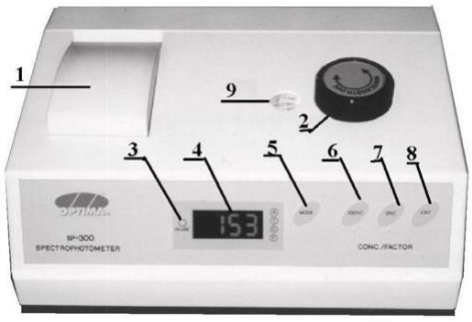

Figure 3.1 OPTIMA SP-300 Spectrophotometer

Image description:

1. Sample place
2. Wavelength Controller
3. ON/OFF power indicator
4. Digital LCD readout
5. Mode change button
6. 100% control button T
7. 0% control button T
8. Print button

9. Wavelength reading window

Figure 3.2 Cuvet

3.2 Experimental Methods

3.2.1 Instrument Calibration

1. The sample holder (1) on the spectrophotometer is emptied.
2. The cuvet (a simple box-shaped but super-quality glass material, quite expensive, so care is needed) is taken and cleaned and then filled with distilled water up to ¾ (called the blank). The outside of the cuvet is cleaned with a cotton swab carefully (do not scratch it).
3. The sample cap on the spectrophotometer (1) is opened, and the cuvet holder is taken out.
4. The cuvet is inserted in the cuvet holder with the clear side facing outward and closed again (the solution height is adjusted according to the markings).
5. Set at a specific wavelength using button (2).
6. The transmittance reading is set to 100% (A=0) for the blank solution using button (6).
7. The cuvet is removed from the sample holder and closed. The transmittance scale reading can be seen on the display (4). At this stage, the transmittance reading must be 100% until the word blank appears. If not, repeat from step 3 until a consistent transmittance reading is obtained.
8. When a consistent 100% transmittance reading is obtained, the cuvet is kept with the blank solution until the lab is completed.
9. The spectrophotometer is ready to be used for optimum wavelength measurements and calibration curves on samples.

3.2.2 Standard Curve Preparation

1. Take X1, X2, X3, X4 ml of CuSO4 mother liquor and put it in a 50 ml volumetric flask.
2. Dilute with distilled water to the limit.
3. Take 10 ml from each measuring flask, then put it into a 50 ml measuring flask.
4. Dilute with distilled water to near the limit mark.
5. Acidify with concentrated HCl until pH = 1. Test the pH using a universal indicator.
6. Add 200 mg BaCl2.2H2O.
7. Dilute with distilled water to the limit.
8. Shake until BaSO4 precipitate forms.
9. The solution was transferred into the cuvet.
10. Measure the transmittance at λ = 480 nm.
11. Make a standard curve of absorbance to concentration relationship.

3.2.3 Sample Solution Measurement

1. Take 10 ml of sample solution with a pipette, put it into a 50 ml measuring flask.
2. Dilute to near the limit mark.
3. Acidify with concentrated HCl until pH = 1. Test pH using universal indicator
4. Add 200 mg of BaCl2.2H2O to the solution.
5. Dilute with aquadest until the limit mark, shake until BaSO4 precipitate forms.
6. The solution was transferred to the cuvet.
7. Measure the transmittance at λ = 480 nm.
8. Calculating the concentration.

3.2.4 Calculation of SO_4^{2-} content

Calculation of SO_4^{2-} levels was carried out on the parent solution and sample solution of each wavelength.

- Calculation of SO_4^{2-} Content in Master Solution

The SO_4^{2-} content in X1 ml of mother liquor, wavelength λ1 nm can be obtained by:

$$C = \frac{X1}{50} x \frac{10}{50} x original\ concentration\ of\ mother\ liquor$$

- Calculation of SO_4^{2-} content in sample solution

  Calculation of SO42- levels in the sample solution can be obtained using the Least Square equation.

$$y = mx + c$$

$$x = \frac{(y - c)}{m} xfp$$

y = absorbance

m = a fixed number (constant), *slope*

x = solution content

c = a fixed number (constant), *y-intercept*

$$m = \frac{n\Sigma xy - \Sigma x \Sigma y}{n\Sigma x^2 - (\Sigma x)^2}$$

$$c = \frac{\Sigma x^2 \Sigma y - \Sigma x \Sigma xy}{n\Sigma x^2 - (\Sigma x)^2}$$

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