Indonesian Journal of Educational Research and Technology

Journal homepage: http://ejournal.upi.edu/index.php/IJERT/

# How to Read and Interpret UV-VIS Spectrophotometric Results in Determining the Structure of Chemical Compounds

*Restiani Alia Pratiwi*[1], *Asep Bayu Dani Nandiyanto*[1*]

[1]Departemen Kimia, Universitas Pendidikan Indonesia, Indonesia

Correspondence: E-mail: nandiyanto@upi.edu

## ABSTRACTS

UV-VIS spectrophotometry is one of the methods used to perform qualitative and quantitative analysis of organic and inorganic compounds. However, until now there has been no further research that studies and describes the detailed analysis of UV-VIS spectral data in terms of determining the structure of chemical compounds. Therefore, this paper contains guidelines that are used as information on how to read and interpret data from the UV-VIS spectrum in terms of determining the structure of chemical compounds. Steps on how to analyze the UV-VIS spectrum are presented. This paper is expected to provide useful information for researchers and novice students who are studying UV-VIS spectrophotometry.



## ARTICLE INFO

***Article History:***
*Received 28 Apr 2021*
*Revised 8 May 2021*
*Accepted 10 Jun 2021*
*Available online 13 Jun 2021*

***Keyword:***
*Chromophores,*
*UV-VIS Spectrophotometry,*
*UV-VIS Spectrum*

## 1. INTRODUCTION

Spectroscopy is the study of methods for producing spectra. The interpretation of the resulting spectrum can be used to analyze elements and chemical compounds, examine molecular structures, and determine the composition of a material (Danusantoso, 1995). UV-VIS spectrophotometry is one of the analytical methods that is widely used in chemical research for qualitative and quantitative analysis of organic and inorganic compounds. This method is widely applied and is generally used for the determination of compounds in very small quantities (Skoog & West, 1971). One example of the application of the UV-VIS spectrophotometric method is the analysis of chlorophyll content at several positions of palm leaf (Arenga pinnata) shoots with a UV-Vis spectrophotometer (Kamagi *et al*., 2017).

Leong *et al*., 2018; Dachriyanus, 2003 has explained about how to read and interpret data from UV-VIS spectrophotometry. However, most of the research does not explain the stages and the knowledge base for conducting structural analysis of chemical compounds. In fact, a basic and step-by-step understanding of UV-VIS spectrum analysis for researchers and novice students is necessary.

This paper discusses how to read and interpret UV-VIS spectrophotometric data in terms of determining the structure of a chemical compound. Reference analysis from various sources is used to make it easier for readers to understand and interpret the data. The steps in interpreting the data are also shown in this paper descriptively.

Generally, UV-VIS spectra can be used with confirmation by comparing the spectra of the suspected compound (Christian *et al*., 2014). For determining the overall structure, data from other instruments, such as FTIR, NMR and other supporting data are usually used (Pavia *et al*., 2008). This paper can be used as a guide for researchers and novice students in understanding and interpreting UV-VIS spectrum data.

## 2. CURRENT KNOWLEDGE FOR UNDERSTANDING UV-VIS SPECTRA

UV-VIS spectrophotometry is a method for measuring the wavelength and intensity of ultraviolet and visible light absorbed by a sample. The basic principle of the UV-VIS spectrophotometry method is based on the measurement of wavelength and intensity of ultraviolet and visible light absorbed by the sample as a function of wavelength. Samples were given UV radiation (ultraviolet) at a wavelength of 180-380 nm or visible (visible light) at a wavelength of 380-780 nm. The absorption of radiation causes the promotion of electrons from the ground state to the excited state in functional groups called chromophore. This absorption data will be generated by UV-VIS spectrophotometry in the form of transmittance or absorbance that can be read by the spectrophotometer as UV-VIS spectrum (Skoog *et al*., 2016). Electron excitation that occurs in UV-VIS spectrophotometry is recorded in the form of a spectrum expressed as wavelength and absorbance, according to the type of electrons present in the analyzed molecule. The easier the electrons to excite, the greater the wavelength that is absorbed, the more electrons are excited, the higher the absorbance.

UV-VIS spectrophotometry can be used to determine samples in the form of solutions, gases, or vapors. The sample must be converted into a clear solution. The requirements of the solvent used in the sample in the form of a solution are that the dissolution must be carried out completely, the solvent used does not contain conjugated double bonds and is colorless, there is no interaction with the molecules of the compound being analyzed, and has high purity. The solvents that absorb UV light at specific wavelengths are shown in **Table 1**.

DOI: http://dx.doi.org/10. 17509/xxxxt.vxix
p- ISSN 2775-8419 e- ISSN 2775-8427

**Table 1.** UV absorption at maximum wavelengths of some solvents.

| Solvent | λmax | Solvent | λmax |
|---|---|---|---|
| Acetronitrile | 190 | n-hexane | 201 |
| Chloroform | 240 | Methanol | 205 |
| Cyclohexane | 195 | Isooctane | 195 |
| 1-4 dioxane | 215 | Water | 190 |
| Ethanol 95% | 205 | Acetone | 330 |
| Benzene | 285 | Pyridine | 305 |

Generally, researchers use water, ethanol, methanol and n-hexane as solvents in their experiments because these solvents are transparent in the UV region (Suhartati, 2017).

The application of this method is to determine the type of chromophore, conjugated double bond and ausochrome of an organic compound, explain information from the structure based on the maximum wavelength of a compound, and analyze organic compounds quantitatively using Lambert-Beer law which is expressed by Equation (1).

$$A = \varepsilon \times b \times C \qquad (1)$$

where A is the absorbance, is the coefficient of molar exclusion ($M^{-1}$ $cm^{-1}$), b is the thickness of the cuvette (cm), and C is the concentration (M).

The UV-VIS spectrum is in the form of a broad band, the wide band is caused by the absorbed energy, in addition to causing electronic transitions, there are also electron rotational transitions and bonding electron vibrations in molecules. This transition difference can occur from any ground state to a random transition state so that a wide band is obtained. The UV-VIS spectrum was recorded as a plot of absorbance against wavelength. Then the data re-plot was performed. The value of the molar excision is important in determining the structure, because it is related to the determination of allowed electron transitions or forbidden electron transitions. The molar excision value (ε) can be calculated based on the UV-Vis spectrum using the Lambert-Beer equation. The chromophore of the analyzed compound can be estimated from the value (ε). The Lambert-Beer equation can also be used to calculate the concentration of a compound in a solvent.

Most of the applications of UV-VIS spectrophotometry methods on organic compounds are based on n → π* or π → π* because UV-VIS spectrophotometry requires the presence of a chromophore group in the molecule. This transition occurs in the spectral region (about 200 to 700 nm) which is commonly used in experiments. Commercial UV-VIS spectrophotometers usually operate with certain spectral regions, this is because the absorption band of the UV-VIS spectrum is too wide and lacks detail. However, certain functional groups, such as carbonyl, nitro and associated systems, do show characteristic peaks, and often useful information can be obtained about the presence or absence of such groups in the molecule (Day & Underwood, 1986). For example, the absorption spectrum for ethane shows a transition σ → σ * at 135 nm and for water, an n → σ* transition at 167 nm with an exclusion coefficient of 7,000. Benzene has three transitions π → π* of the aromatic ring; two K-bands

DOI: http://dx.doi.org/10.17509/xxxx.vxix
p- ISSN 2775-8419 e- ISSN 2775-8427

at 184 and 204 nm and one B-band at 254 nm with excision coefficients of 60,000, 8,000 and 215, respectively.

There are only 2 types of excited levels of electrons in organic molecules, namely pi star (π*) and star sigma (σ*), so if an organic molecule has sigma, pi, and nonbonding electrons, for example in acetone molecules, then the type The electronic transitions that can be seen in **Figure 1** include σ → σ *, σ → π *, π → π *, π → σ *, n → σ *, n → π *. In order for this electronic transition to occur, energy is needed in accordance with the types of bonding and nonbonding electrons present in organic molecules.

The UV-VIS spectrum is plotted as % T with wavelength (λ), or A with (λ). But in general, researchers use absorbance (A) rather than % T as the ordinate. **Figure 2** shows the B-band on benzene measured in ethanol solvent, resulting in a fine structure band, the shape of the spectrum is sharp. The band at a wavelength of 254 nm from benzene is caused because benzene loses symmetry due to the vibration of the bonds in the benzene molecule, in that state the electron transition of the vibrational energy level from the ground state to the ground state is excited, the vibrations are not observed by the instrument, so that the shape of the spectrum is produced by a fine structure (not wide). The more benzene rings in one molecule, the greater the maximum wavelength, due to increased conjugation and greater resonance-stabilization.

The role of molecular groups such as C = C, C = O, N = N, N = O in shifting light absorption towards the visible region is called the chromophore. Chromophore is a system that can cause absorption of light. The light absorption regions at certain transitions are estimated by **Table 2**.

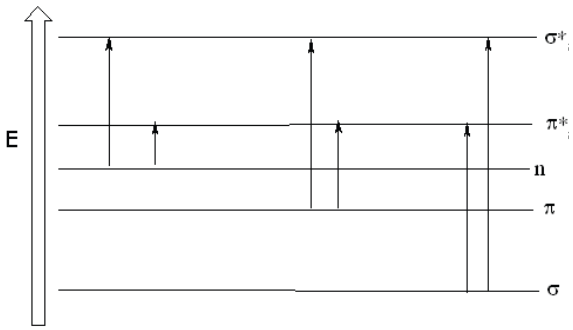



**Figure 1.** Types of electronic transitions in organic molecules (Suhartati, 2017).

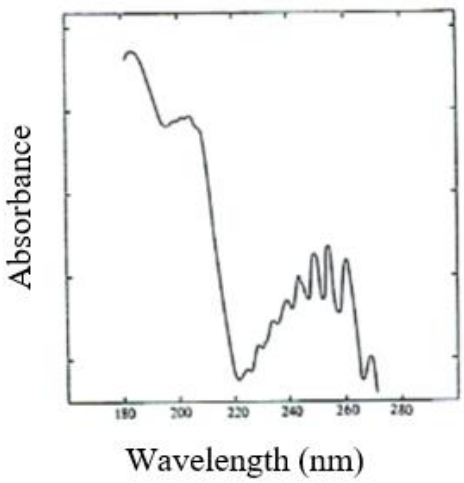



**Figure 2.** UV spectrum at λ max 254 nm of benzene in ethanol (Suhartati, 2017).

DOI: http://dx.doi.org/10. 17509/xxxxt.vxix
p- ISSN 2775-8419 e- ISSN 2775-8427

**Table 2.** Estimated light absorption area.

| Transition | Approximate Absorption Area |
|---|---|
| σ → σ * | 150 nm |
| n → σ * | <200 nm |
| π → π* | <200 nm |
| n → π* | 300 nm |
| The UV vacuum region is below 200 nm | |
| The UV region of quartz is between 200 nm – 400 nm | |

**Table 3.** Estimated wavelengths of colors in the visible light region (Kristianingrum, 2013).

| Wavelength (nm) | Absorbed color | Reflected Color (Complementary) |
|---|---|---|
| 340 – 450 | Violet | Yellow-green |
| 450 – 495 | Blue | Yellow |
| 495 – 570 | Green | Violet |
| 570 - 590 | Yellow | Blue |
| 590 - 620 | Orange | Green - Blue |
| 620 - 750 | Red | Blue - Green |

The different electron environments in the molecule will affect the degree of interaction between the orbitals. If the interaction between the orbitals increases, the bond length formed will decrease and the energy difference between the bonding and anti-bonding molecular orbitals will increase. The position of the maximum absorption of a band is expressed as $\lambda_{max}$..

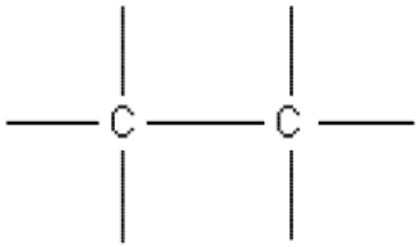
Molecules containing one chromophore group can undergo a change in wavelength. In the measurement of wavelength, the colors in the visible light affect the magnitude of the wavelength. If polychromatic light (white light) which contains the entire spectrum of wavelengths passes through a certain medium, it will absorb other wavelengths, so that the medium will appear colored. Because only the wavelength that is transmitted reaches the eye, it is this wavelength that determines the color of the medium. This color is called a complementary color to the absorbed color. **Table 3** shows the approximate wavelengths of the colors in the visible light region.

There is a close relationship between the color of a substance and its electronic structure. Molecules or ions will exhibit absorption in the visible or ultraviolet region when radiation causes electronic transitions within their structure. Thus, the absorption of light by the sample

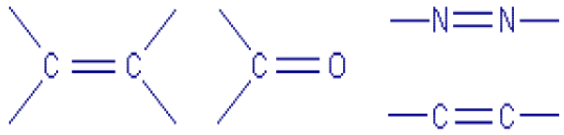
DOI: http://dx.doi.org/10.17509/xxxx.vxix
p- ISSN 2775-8419 e- ISSN 2775-8427

in the ultraviolet or visible region is accompanied by a change in the electronic state of the molecules in the sample. Energy supplied by light will push electrons from ground state orbitals to higher energy, excited state orbitals, or antibonding orbitals. Potentially, three types of ground state orbitals may be involved:

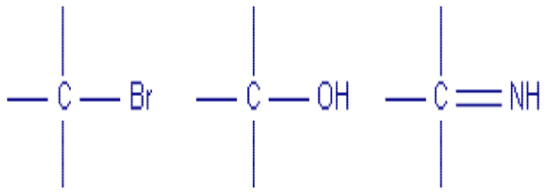
- σ (bonds) molecules as in



- π (bonding) molecular orbitals as in



- n atomic orbitals (non-bonding) as in



n addition, two types of antibonding orbitals may be involved in this transition: σ * orbitals (sigma stars) and π * orbitals (pi stars). There are no n* antibonding orbitals because the n electrons do not form bonds. A transition in which a bonding electron is excited to an antibonding orbital is called a σ → σ * transition. In the same way π → π * represents the transition of one electron from a lone pair (non-bonding electron pair) to an π antibonding orbital. Thus the following electronic transitions can occur with the absorption of ultraviolet and visible light σ → σ *, n → σ *, n → π *, and π → π *.

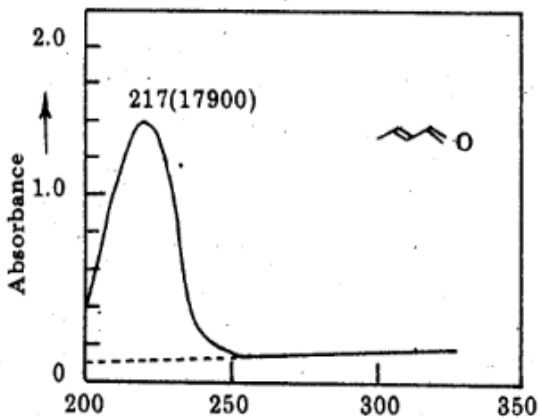



**Figure 3.** UV spectrum of unsaturated aldehydes or ketones -α, β (Kristianingrum, 2013).

DOI: http://dx.doi.org/10. 17509/xxxxt.vxix
p- ISSN 2775-8419 e- ISSN 2775-8427

The UV-VIS spectrum provides some information on the structure of a compound, but it is more useful when combined with infrared and NMR data obtained. Information from the UV-VIS spectrum will be obtained if there is at least a general description of the already known structure, for example in the identification of carbonyl groups, double bonds, aromatic systems, and other important chromophores.

There are six main steps in reading and interpreting the UV-VIS spectrum. Determination of the structure of a compound from UV-VIS spectral data can be done in the following way:

a. Step 1: The overall spectrum pattern is observed. Usually for each spectrum each compound has a distinctive band pattern, and can be easily recognized. Several peaks will appear in the UV-VIS spectrum. **Figure 3** shows an absorption band at 217 nm with ε = 17.900 indicating an unsaturated aldehyde or ketone-α, β.
b. Step 2: The number and intensity of each absorption band that appears is observed. The number of absorption bands indicates the number of chromophore contained in the sample compound. It is possible that there is more than one absorption band for one type of chromophore. The second step: The number and intensity of each absorption band that appears is observed. The number of absorption bands indicates the number of chromophore contained in the sample compound. It is possible that there is more than one absorption band for one type of chromophore.
c. Step 3: The absorbance magnitude and wavelength for the emerging absorption band are identified. The value of ε is important in determining the structure associated with the allowed electron transitions. This value also affects the chromophore would be expected of the compound to be analyzed. The amount of ε and the wavelength range is typical for any chromophore.
d. Step 4: Possible chromophore can be identified based on the data amount, the identity, the magnitude of ε, and wavelength for each absorption band that appears. Each identified chromophore has its own characteristic UV-VIS spectrum band. Based on the existing literature (Kristianingrum, 2013; Pavia *et al*., 2008; Skoog *et al*., 2016), there are several characteristic bands produced in the UV-Vis spectrum for various chromophore and compounds, including:
    - The tape was a single low intensity towards moderate (ε = 100 to 10,000) at wavelengths less than 220 nm generally shows the transition n → σ *. The possibilities are amines, alcohols, ethers, and thiols, provided the unbonded electrons are not included in the conjugated system. Exceptions to this point is that the transition n → π * group cyano (-C≡N :) appears in the region, but with a weak transition (ε <100), and a cyano group are easily identified in the infrared.
    - A single band with lower intensity (ε = 10 to 100) in the region of 250 to 360 nm, without a large absorption at shorter wavelengths (200 to 250 nm) usually show a transition n → π *. Because absorption does not occur in the long wave or contain conjugated chromophore which generally contain atoms of O, N, or S. Examples such as C = O, C = N, N = N, -$NO_2$, -COOR, -COOH, or -$CONH_2$.
    - Two bands of moderate intensity (ε = 1,000 to 10,000) having λmax above 200 nm, which is generally two bands indicated the aromatic system. Substitution on the aromatic ring increases the molar absorptivity above 10,000, especially if the substituent extends the conjugated system.

DOI: http://dx.doi.org/10.17509/xxxx.vxix
p- ISSN 2775-8419 e- ISSN 2775-8427

- Ribbons with high intensity (ε = 10,000 to 20,000) that appears above 210 nm generally showed the compound α, β-unsaturated ketones diene, or polyene. The greater the length of the conjugate system, the greater the observed wavelength. For the diene, λmax can be calculated using Rule Woodward - Fieser.
- Compounds sedehana ketones, acids, esters, amides, and other compounds that contain the system π and lone pair shows two absorption: the transition n → π * at wavelengths longer (> 300 nm, low intensity) and the transition π → π * at wavelengths lower (<250 nm, high intensity. with their conjugations, λmax ribbon π → π * shifted to wavelengths greater and predictable with the Rules of Woodward. Pengan conjugation, the value of ε always be above 10,000 and because of very strong possibility of this compound will obscure or cover the transition n → π * weaker.
- Highly colored compounds may have conjugated long ring systems or polycyclic aromatic chromophores. Benzenoid compounds can be colored if they have sufficient conjugate substituents. For nonaromatic systems it always requires a minimum of four to five conjugated chromophore to produce absorption in the visible region. However, some simple nitro compounds, azo, nitroso, α-diketo, polibromo, and poliiodo may also removing the color, as in many compounds with kuinoid structure.
- Some inorganic species can also absorb UV-Vis radiation and thus the chromophore or its compounds can be determined directly. Typical absorptions on common chromophores are shown in **Table 4**. Some inorganic anions emit UV absorption bands resulting from the excitation of nonbonding electrons. For example, the nitrate ion (313 nm), carbonates (217 nm), nitrite (360 and 280), Azido (230 nm), and tritiocarbonate (500 nm).

e. Step 5 : Shifting / shifting in the absorption band is observed. Substituents bound to the chromophore structure can change the position and intensity of the absorption band of the chromophore. Ausochrome is a substituent that can increase the absorption intensity and wavelength of a chromophore. Common ausochromes are methyl, hydroxyl, alloxy, halogen, and amino groups. There are four types of shifting that can affect the absorption of a chromophore, namely bathochromic (the maximum transfer of absorption to a longer wavelength or lower energy from blue to red), hypsochromic (the maximum transfer of absorption from red to ultraviolet occurs at shorter wavelengths or energy). higher), hyperchromic (wavelength shift that occurs due to an increase in absorption intensity), hypochromic (wavelength shift that occurs due to a decrease in absorption intensity). Changes in the shift in wavelength or absorption intensity can be illustrated in **Figure 4**.

f. Step 6: The spectrum obtained is adjusted to the possible compounds present.

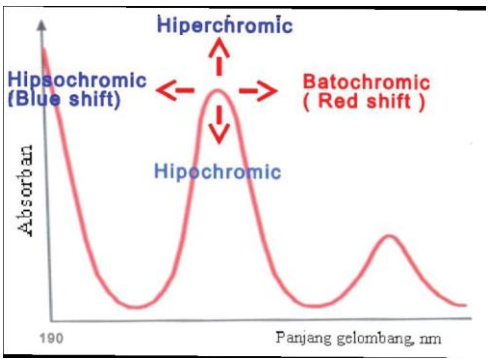



**Figure 4.** Illustration of the term shift in the UV-Vis spectrum (Suhartati, 2017).

DOI: http://dx.doi.org/10. 17509/xxxxt.vxix
p- ISSN 2775-8419 e- ISSN 2775-8427

## 3. METHODS

To understand and interpret UV-VIS spectral data, this paper describes step by step in determining the structure of simple chemical compounds. The simple compounds used were ethene, acetone, 1,3 butadiene, isoprene and polyene. Several other spectrum patterns were also used for the chemical compounds caffeine, benzoic acid, paracetamol, and NADH.

In short, Sari *et al* (2013) conducted a study using energy drink samples to analyze the content of caffeine and benzoic acid. The absorption spectrum and calibration curve were determined by making standard solutions of caffeine and benzoic acid with various concentrations, one of the standards was then made to determine the maximum wavelength. Norazemi *et al* (2017) conducted a study on a drug delivery system to analyze the content of paracetamol using gellan gum and paracetamol (drug model) dissolved in deionized water separately. Rovel *et al* (1998) conducted a study to evaluate the level of NADH degradation. The absorption spectra of NADH were obtained from buffer solutions of 0.1 M PIPES (at pH 6.8 and 7.8) and 0.1 M phosphate (at pH 6.8 and 7.8).

**Table 4.** Typical Absorption on Some Common Chromophores (Kristianingrum, 2013).

| Chromophores / Compounds | λmax (nm) | εmax | Transition |
|---|---|---|---|
| Alkene | 177 | 13000 | $\pi \rightarrow \pi^*$ |
| Alkyne | 178-225 | 10000-160 | $\pi \rightarrow \pi^*$ |
| Carbonyl | 186-280 | 1000 | $n \rightarrow \sigma^*$ |
| Carboxyl | 204 | 41 | $n \rightarrow \pi^*$ |
| Amido | 214 | 60 | $n \rightarrow \pi^*$ |
| Azo | 339 | 5 | $n \rightarrow \pi^*$ |
| Nitro | 280 | 22 | $n \rightarrow \pi^*$ |
| Nitroso | 300-665 | 100-20 | $n \rightarrow \pi^*$ |
| Nitrate | 270 | 12 | $n \rightarrow \pi^*$ |
| Olefin | 184 | 12 | Delocalization n* |
| Triolefin | 250 | 10000 | Delocalization n* |
| Diolefin | 217 | - | Delocalization n* |
| Ketones | 282 | 21000 | $n \rightarrow \pi^*$ |
| $H_2O$ | 167 | 24 | $n \rightarrow \sigma^*$ |
| Methanol | 184 | 1480 | $n \rightarrow \sigma^*$ |
| Methyl Chloride | 173 | 15 | $n \rightarrow \sigma^*$ |
| Dimethyleter | 184 | 200 | $n \rightarrow \sigma^*$ |
| Methylamine | 215 | 2500 | $n \rightarrow \sigma^*$ |
| Benzene | 204 | 900 | $\pi \rightarrow \pi^*$ |
| Toluene | 207 | 7000 | $\pi \rightarrow \pi^*$ |
| Phenol | 211 | 6200 | $\pi \rightarrow \pi^*$ |

DOI: http://dx.doi.org/10.17509/xxxx.vxix
p- ISSN 2775-8419 e- ISSN 2775-8427

| | | | |
|---|---|---|---|
| Aniline | 230 | 8600 | π → π* |
| Naphthalene | 286 | 9300 | π → π* |
| Styrene | 244 | 12000 | π → π* |

## 4. RESULTS AND DISCUSSIONS

Determination of the structure of chemical compounds can be done by analyzing the resulting UV-VIS spectrum pattern. The analysis can be carried out based on the steps described in Section 2.

### 4.1. Ethene UV-VIS spectrum

The UV-VIS spectrum of ethene can be seen in **Figure 5**. Based on the step-by-step analysis of how to read and interpret the UV-VIS spectrum, it can be concluded as follows:

1) Step 1: Identify the number of peaks appearing in the UV-VIS spectrum. **Figure 5** shows several peaks indicating the presence of an excited electron. The easier the electrons are excited, the greater the wavelength that is absorbed, the more electrons are excited, the higher the absorbance.
2) Step 2: Identify the absorption band with high intensity. **Figure 5** shows 1 absorption band with high intensity. The absorption band that appears indicates the presence of a chromophore group in the spectrum. **Figure 5** shows the presence of 1 chromophore that appears.
3) Step 3: Identify the absorbance and wavelength of each peak that appears. The magnitude of the absorbance and the wavelength are usually typical for each chromophore. **Figure 5** shows a wavelength of about 160-165 nm.
4) Step 4: Identify possible chromophore based on the absorption band that appears. **Figure 5** shows the presence of 1 chromophore that appears. Theoretically, the maximum absorption for ethene has a wavelength of 165 nm.
5) Step 5: Identify shifts in the absorption band. **Figure 5** has a shift that causes an increase in wavelength due to the conjugated double bond.
6) Step 6: Identify the compound. From the UV-VIS spectrum in **Figure 5.** Based on the observation of the absorption band and identification of possible chromophore, it can be assumed that the chromophore absorption band that appears in the above spectrum is ethene.

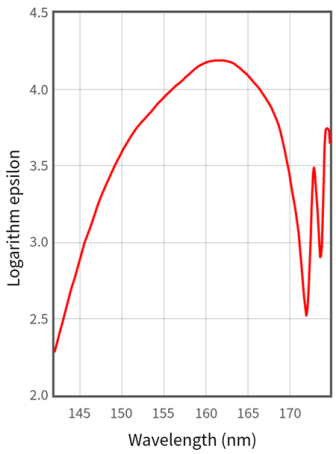



**Figure 5.** UV-VIS spectrum of ethene.

DOI: http://dx.doi.org/10. 17509/xxxxt.vxix
p- ISSN 2775-8419 e- ISSN 2775-8427

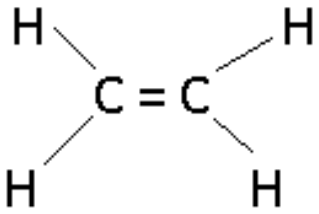



**Figure 6.** Structure of ethene.

The compound ethene has 2 carbon atoms linked by a double covalent bond. Generally, ethene is used in industry as a material for the manufacture of plastics. **Figure 6** shows the structure of ethene.

### 4.2. Acetone UV-VIS spectrum

The UV-VIS spectrum of acetone can be seen in **Figure 7**. Based on the step-by-step analysis of how to read and interpret the UV-VIS spectrum, it can be concluded as follows:

1) Step 1: Identify the number of peaks appearing in the UV-VIS spectrum. **Figure 7** shows a peak indicating the presence of an excited electron. The easier the electrons are excited, the greater the wavelength that is absorbed, the more electrons are excited, the higher the absorbance.
2) Step 2: Identify the absorption band with high intensity. **Figure 7** shows 1 absorption band with high intensity. The absorption band that appears indicates the presence of a chromophore group in the spectrum. **Figure 8** shows the presence of 1 chromophore that appears.
3) Step 3: Identify the absorbance and wavelength of each peak that appears. The magnitude of the absorbance and the wavelength are usually typical for each chromophore. **Figure 7** shows a wavelength of 270.5 nm.
4) Step 4: Identify possible chromophore based on the absorption band that appears. **Figure 7** shows the presence of 1 chromophore that appears.
5) Step 5: Identify shifts in the absorption band. **Figure 7** has a shift. In these compounds, there is a transition n → π *. In a conjugated system the energy separation between the ground state and the excited state is reduced and the system then absorbs at longer wavelengths and with increasing intensity. In addition, due to the reduced energy difference, the transition n → π * for their presence and the heteroatom lone pair, then the ribbon-R also had a red shift with little change in intensity.
6) Step 6: Identify the compound. From the UV-VIS spectrum in **Figure 7**. Based on the observation of the absorption band and identification of possible chromophore, it can be assumed that the chromophore absorption band that appears in the above spectrum is acetone.

Acetone is also known as dimethyl ketone, 2-propanone, dimethylformaldehyde. It is generally used as a polar solvent in organic reactions. Acetone is a colorless, flammable liquid. Acetone is commonly used in industry as a reaction intermediate for the production of other components, a direct solvent, as a solvent and diluent. Acetone has a carbonyl group that has a carbon-oxygen double bond consisting of one bond and one bond. **Figure 8** shows the structure of acetone.

### 4.3. 1,3 Butadiene UV-VIS spectrum

DOI: http://dx.doi.org/10.17509/xxxx.vxix
p- ISSN 2775-8419 e- ISSN 2775-8427

The UV-VIS spectrum of 1,3 butadiene can be seen in **Figure 9**. Based on the step-by-step analysis of how to read and interpret the UV-VIS spectrum, it can be concluded as follows:

1) Step 1: Identify the number of peaks appearing in the UV-VIS spectrum. **Figure 9** shows a peak indicating the presence of an excited electron. The easier it is for the electrons to be excited, the greater the wavelength that is absorbed, the more electrons are excited, the higher the absorbance.
2) Step 2: Identify the absorption band with high intensity. **Figure 9** shows 1 absorption band with high intensity. The absorption band that appears indicates the presence of a chromophore group in the spectrum. **Figure 9** shows the presence of 1 chromophore that appears.
3) Step 3: Identify the absorbance and wavelength of each peak that appears. The magnitude of the absorbance and the wavelength are usually typical for each chromophore. **Figure 9** shows a wavelength of 217 nm.
4) Step 4: Identify possible chromophore based on the absorption band that appears. **Figure 9** shows the presence of 1 chromophore that appears. Theoretically, the maximum absorption for alkenes has a wavelength of 177 nm.
5) Step 5: Identify shifts in the absorption band. **Figure 9** has a shift that causes an increase in the wavelength due to the conjugated double bond.
6) Step 6: Identify the compound. From the UV-VIS spectrum in **Figure 9**. Based on the observation of the absorption band and identification of possible chromophore, it can be assumed that the chromophore absorption band that appears in the above spectrum is 1,3 butadiene compound.

The compound 1,3-Butadiene is a conjugated diene with the chemical formula $C_4H_6$. This compound is an important industrial compound used as a monomer in the production of synthetic rubber. **Figure 10** shows the chemical structure of the compound 1,3 butadiene.

7)

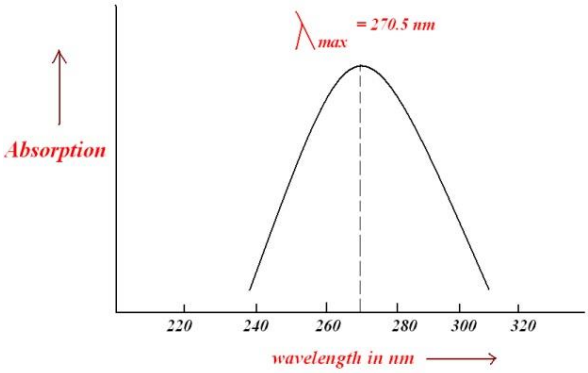



**Figure 7.** UV-VIS spectrum of acetone.

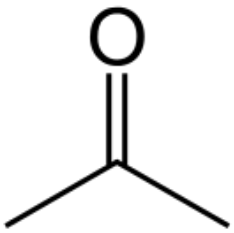


**Figure 8.** Structure of acetone.

DOI: http://dx.doi.org/10. 17509/xxxxt.vxix
p- ISSN 2775-8419 e- ISSN 2775-8427

### 4.4. Isoprene UV-VIS spectrum

The UV-VIS spectrum of isoprene can be seen in **Figure 11**. Based on the step-by-step analysis of how to read and interpret the UV-VIS spectrum, it can be concluded as follows:

1) Step 1: Identify the number of peaks appearing in the UV-VIS spectrum. **Figure 11** shows a peak indicating the presence of an excited electron. The easier the electrons are excited, the greater the wavelength that is absorbed, the more electrons are excited, the higher the absorbance.
2) Step 2: Identify the absorption band with high intensity. **Figure 11** shows 1 absorption band with high intensity. The absorption band that appears indicates the presence of a chromophore group in the spectrum. **Figure 11** shows the presence of 1 chromophore that appears.
3) Step 3: Identify the absorbance and wavelength of each peak that appears. The amount of absorbance and wavelength are usually typical for any chromophore. **Figure 11** shows a wavelength of 222 nm.
4) Step 4: Identify possible chromophore based on the absorption band that appears. **Figure 11** shows the presence of 1 chromophore that appears.
5) Step 5: Identify shifts in the absorption band. **Figure 11** undergoes a shift caused by the ausochrome of the methyl group.
6) Step 6: Identification of compounds. From the UV-VIS spectrum in **Figure 11**. Based on the observation of the absorption band and identification of possible chromophore, it can be assumed that the absorption band of the chromophore that appears in the above spectrum is an isoprene compound.

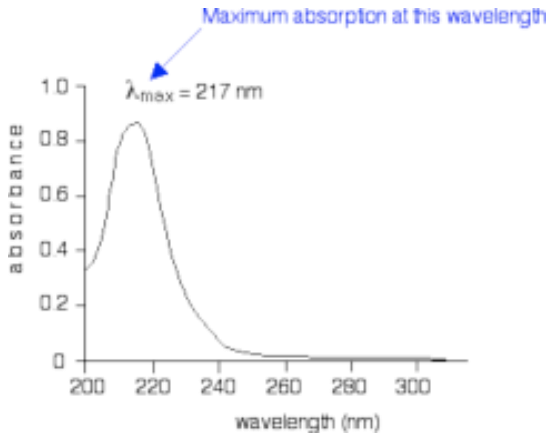



**Figure 9.** UV-VIS spectrum of 1,3 butadiene.

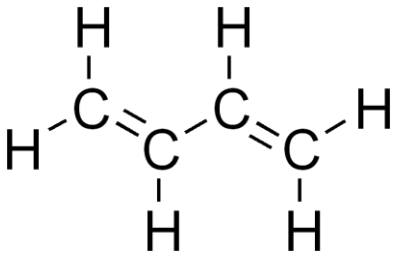


**Figure 10.** Structure of 1,3 butadiene.

DOI: http://dx.doi.org/10.17509/xxxx.vxix
p- ISSN 2775-8419 e- ISSN 2775-8427

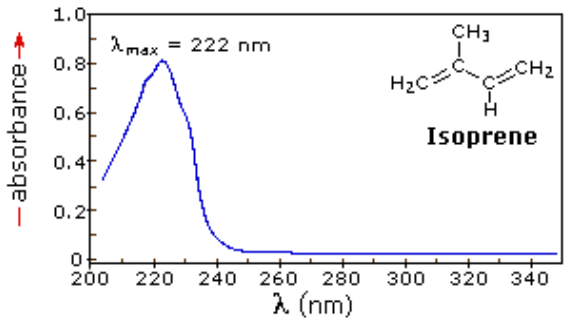



**Figure 11.** Isoprene spectrum.

### 4.5. Polyene UV-VIS spectrum

The UV-VIS spectrum of polyenes can be seen in **Figure 12**. Based on the step-by-step analysis of how to read and interpret the UV-VIS spectrum, it can be concluded as follows:

1) Step 1: Identify the number of peaks appearing in the UV-VIS spectrum. **Figure 12** shows several peaks indicating the presence of an excited electron. The easier the electrons are excited, the greater the wavelength that is absorbed, the more electrons are excited, the higher the absorbance.
2) Step 2: Identify the absorption band with high intensity. **Figure 12** shows 1 absorption band with high intensity and 3 bands with high intensity. The absorption band that appears indicates the presence of a chromophore group in the spectrum.
3) Step 3: Identify the absorbance and wavelength of each peak that appears. The magnitude of the absorbance and the wavelength are usually typical for each chromophore. **Figure 12** shows 1 high-intensity band in the 230 nm region and 3 high-intensity bands in the 250-300 nm region.
4) Step 4: Identify possible chromophore based on the absorption band that appears. **Figure 12** shows a typical spectrum such as that of polyene.
5) Step 5: Identify shifts in the absorption band. **Figure 12** does not shift.
6) Step 6: Identify the compound. From the UV-VIS spectrum in **Figure 12**. Based on the observation of the absorption band and identification of possible chromophore, it can be assumed that the chromophore absorption band that appears in the above spectrum is a polyene compound.

### 4.6. UV-VIS spectrum of caffeine and benzoic acid

The UV-VIS spectrum of caffeine and benzoic acid can be seen in **Figure 13**. Based on the step-by-step analysis of how to read and interpret the UV-VIS spectrum, it can be concluded as follows:

1) Step 1: Identify the number of peaks appearing in the UV-VIS spectrum. **Figure 13** shows 2 peaks indicating the presence of an excited electron. The easier the electrons are excited, the greater the wavelength that is absorbed, the more electrons are excited, the higher the absorbance.
2) Step 2: Identify the absorption band with high intensity. **Figure 13** shows 2 peaks where the absorption band has a high intensity for both peaks.
3) Step 3: Identify absorbance and wavelength for each peak that appears. From the UV-VIS spectrum, two absorption bands with high intensity at 273 nm and 230 nm can be seen. The peak formed in the spectra is the peak where caffeine and benzoic acid provide maximum absorption. The maximum absorption wavelength of caffeine in dilute acid

DOI: http://dx.doi.org/10. 17509/xxxxt.vxix
p- ISSN 2775-8419 e- ISSN 2775-8427

solvents according to the literature is 273 nm (Clarke, 1986). According to Spangenberg, Poole, and Weins (2011), wavelength shifts up to ± 2.5 nm are still acceptable, while differences beyond ± 2.5 nm require re-reading.

4) Step 4: Identify possible chromophore based on the absorption band that appears. **Figure 13** has several possible chromophores, namely carbonyl groups, conjugated double bonds and aromatic compounds.
5) Step 5: Identify shifts in the absorption band. **Figure 13** indicates a shift due to solvent changes due to the conjugated system.
6) Step 6: Identify the compound. From the UV-VIS spectrum above, based on the observation of the absorption band and identification of possible chromophore, it can be assumed that the chromophore absorption band that appears in the above spectrum is a compound of caffeine and benzoic acid.

The maximum wavelength for the two substances must not be close together (Gandjar *et al*., 2008). Prior to measurement, the maximum wavelength was determined. This is because the maximum wavelength provides the highest absorption of the sample so as to minimize errors. According to some literature, the maximum wavelength for caffeine is 272 nm.

The maximum wavelength used in this study was 273 for caffeine and 230 for benzoic acid. This wavelength was obtained from the results of wavelength scanning with standard solutions of caffeine and benzoic acid with various concentrations. The result of UV-VIS spectrum analysis shows that the resulting peak absorbs at a specific wavelength.

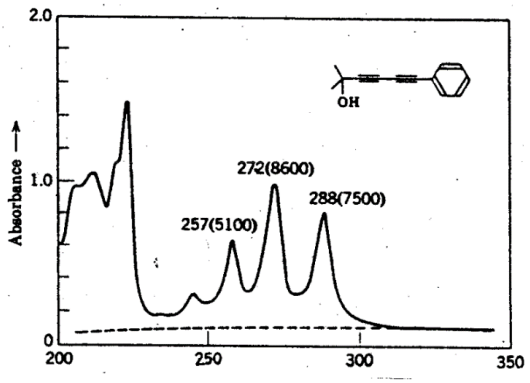



**Figure 12.** UV-VIS Spectrum of Polyene.

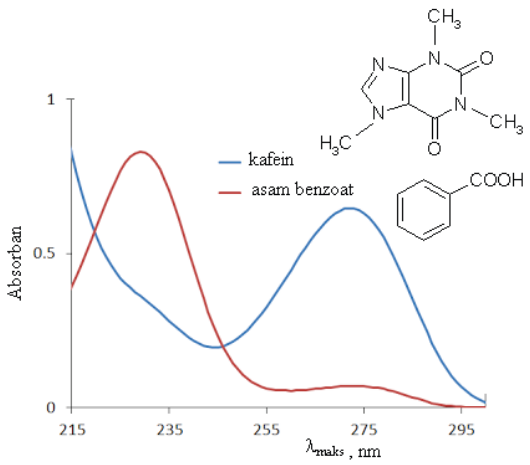



**Figure 13.** UV-VIS spectrum of caffeine and benzoic acid.

DOI: http://dx.doi.org/10.17509/xxxx.vxix
p- ISSN 2775-8419 e- ISSN 2775-8427

The determination of the maximum wavelength is determined because at the maximum wavelength the sensitivity is also maximum. This happens because of a change in absorbance, the maximum concentration around the maximum wavelength has a flat absorbance curve shape and under these conditions the Lambert-Beer law will be fulfilled, in addition if repeated measurements are made, the error caused by re-pairing the wavelength will be very small (Rohman, 2007). A mixture of caffeine and benzoic acid can be analyzed using a UV-Vis spectrophotometer because it contains a chromophore.

Benzoic acid ($C_7H_6O_2$) is a chemical compound that has a solid crystalline powder form, colorless, odorless, slightly soluble in water, but soluble in ethanol and very soluble in benzene and acetone. Benzoic acid is commonly used in food as a preservative. But beyond that, it can also be used as a corrosion inhibitor. **Figure 14** shows the structure of benzoic acid. Benzoic acid has a carboxylic acid functional group and there is a conjugated double bond.

Caffeine ($C_8H_{10}N_4O_2$) is a xanthine alkaloid compound in the form of white crystals or white shiny needles which is odorless and has a bitter taste. Caffeine is found in tea, coffee, chocolate, and some other soft drinks. Caffeine is often added in the manufacture of beverages to stimulate the consumer's central nervous system so that it can reduce drowsiness and increase enthusiasm. Caffeine works as a psychoactive stimulant drug and a mild diuretic (Wilson & Gisvold, 1982). **Figure 15** shows the structure of caffeine. Caffeine is an aromatic heterocyclic organic compound that has a pyrimidine ring and an imidazole ring coupled.

The analysis of caffeine and benzoic acid was carried out simultaneously because the determination of the levels was carried out without having to be separated first.

### 4.7. Paracetamol UV-VIS spectrum

The UV-VIS spectrum of paracetamol can be seen in **Figure 16**. Based on the step-by-step analysis of how to read and interpret the UV-VIS spectrum, it can be concluded as follows:

1) Step 1: Identify the number of peaks appearing in the UV-VIS spectrum. Figure 16 shows one peak which indicates the existence of excited electrons. The easier it is for the electrons to be excited, the greater the wavelength that is absorbed, the more electrons are excited, the higher the absorbance..
2) Step 2: Identify the absorption band with high intensity. **Figure 16** shows 1 absorption band with high intensity. The absorption band that appears indicates the presence of a chromophore group in the spectrum. **Figure 16** shows the presence of 1 chromophore that appears.

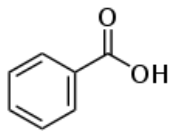



**Figure 14.** Structure of benzoic acid.


**Figure 15.** Caffeine structure.

DOI: http://dx.doi.org/10. 17509/xxxxt.vxix
p- ISSN 2775-8419 e- ISSN 2775-8427

3) Step 3: Identify the absorbance and wavelength of each peak that appears. The magnitude of the absorbance and the wavelength are usually typical for each chromophore. **Figure 16** shows a wavelength of 243 nm with an absorbance value of 0.643. Theoretically, the maximum absorption for paracetamol with methanol and water solvent has a wavelength of 243 nm.
4) Step 4: Identify possible chromophore based on the absorption band that appears. **Figure 16** shows the presence of 1 chromophore that appears,
5) Step 5: Identify shifts in the absorption band. The maximum absorption wavelength shift for paracetamol is 1 nm. This shift still meets the requirements, where if there is a shift in the maximum absorption wavelength with a shift range of ± 2 nm with the same solvent as the reference standard, then the wavelength can be used as the maximum absorption wavelength.
6) Step 6: Identify the compound. From the UV-VIS spectrum above, based on the observation of the absorption band and identification of possible chromophore, it can be assumed that the chromophore absorption band that appears in the above spectrum is paracetamol compound.

The spectrum of paracetamol can be observed in the UV wavelength due to a chromophore that provides orbital π electrons in an easy excited to a higher energy level, namely π * when subjected to UV radiation has an energy corresponding to the energy required for the excitation. Chromophore group and ausokrom on paracetamol can be seen from **Figure 17**.

### 4.8. NADH UV-VIS spectrum

The UV-VIS spectrum of the NADH compound is shown in **Figure 18**. Based on the analysis of the steps to determine the structure of the compound on how to read and interpret the UV-VIS spectrum, the following conclusions were drawn:

1) Step 1: Identify the number of peaks appearing in the UV-VIS spectrum. **Figure 18** shows 2 peaks indicating the presence of an excited electron. The easier the electrons are excited, the greater the wavelength that is absorbed, the more electrons are excited, the higher the absorbance.

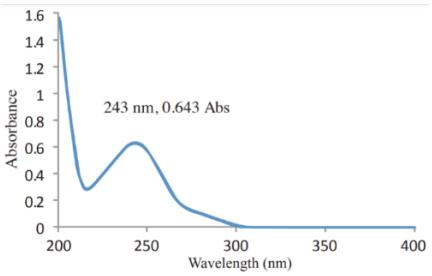



**Figure 16.** UV-VIS spectrum of paracetamol.

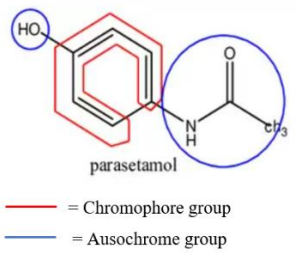



**Figure 17.** Chromophore and ausochrome groups in paracetamol.

DOI: http://dx.doi.org/10.17509/xxxx.vxix
p- ISSN 2775-8419 e- ISSN 2775-8427

2) Step 2: Identify the absorption band with high intensity. **Figure 18** shows 2 absorption bands with high intensity. The absorption band that appears indicates the presence of a chromophore group in the spectrum.
3) Step 3: Identify the absorbance and wavelength of each peak that appears. The magnitude of the absorbance and the wavelength are usually typical for each chromophore. **Figure 18** shows 2 absorption bands with intensities at 260 nm and 340 nm.
4) Step 4: Identify possible chromophore based on the absorption band that appears. From the UV-VIS spectrum above, it is possible that the chromophore that appears in the absorption band is a conjugated C-N bond.
5) Step 5: Identify shifts in the absorption band. The binding of substituents to the basic structure of the chromophore can change the position and intensity of the absorption band of the chromophore. The shift is caused by the presence of $NH_2$ and OH substituents. Each compound will provide maximum absorption at a certain wavelength. If the maximum absorption of the analyte at the time of the study is right or is within the limits of ± 2 nm from the theoretical wavelength, then the analyte is the compound in question. Based on the literature, for maximum wavelength of 260 nm has a value of ε = 18000 L.mol literature$^{-1}$.cm$^{-1}$. These compounds absorb light in the UV region because of the conjugated pi bond system. The absorption band with a maximum wavelength of 340 nm has = 6290 L.mol$^{-1}$ cm$^{-1}$.
6) Step 6: Identify the compound. From the UV-VIS spectrum above, based on the observation of the absorption band and identification of possible chromophore, it can be assumed that the chromophore absorption band that appears in the above spectrum is NADH compound.

In metabolism, these compounds accept and donate electrons in redox reactions, involving the release of two hydrogen atoms from the reactants in the form of a hydride ion and a proton. If double-stranded DNA is gradually heated, it will begin to melt or break as the temperature increases. As the melting process progresses, the absorbance value for the sample increases. Lambert-Beer's law and UV spectroscopy provide an easy way for a variety of redox reactions. In biochemistry, the oxidation of organic molecules often occurs simultaneously with the reduction of nicotinamide adenine dinucleotide ($NAD^+$) to NADH as shown in **Figure 19**.

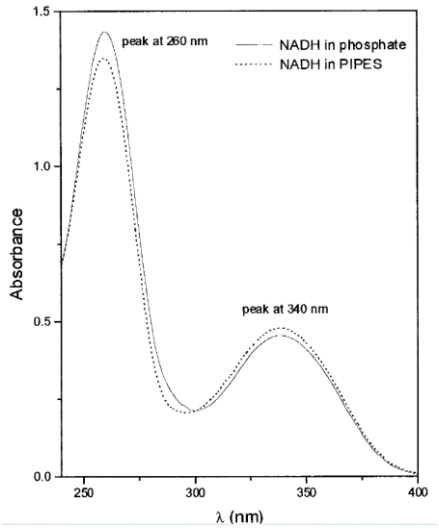



**Figure 18.** UV-VIS spectrum of NADH.

DOI: http://dx.doi.org/10. 17509/xxxxt.vxix
p- ISSN 2775-8419 e- ISSN 2775-8427

Both $NAD^+$ and NADH absorb at a wavelength of 260 nm. However, NADH has a second absorption band with a maximum wavelength of 340 nm. The structure of NADH is shown in **Figure 20**.

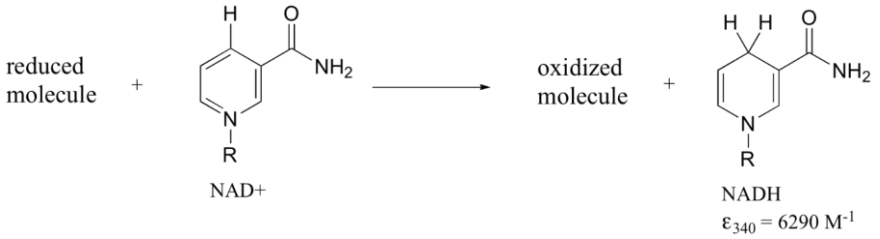



**Figure 19.** The process of redox reaction of compounds $NAD^+$.

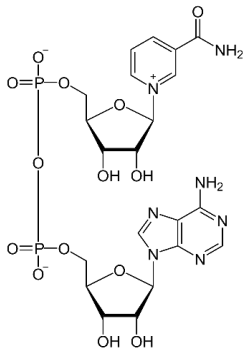


**Figure 20.** Structure of NADH.

## 5. CONCLUSION

This paper shows the steps for reading and interpreting the UV-VIS spectrum. The steps for reading and interpreting the UV-VIS spectrum are described in detail. The UV-VIS spectrum provides some information on the structure of a compound, but it is more useful when combined with infrared and NMR data obtained. With this study, we believe that this paper could be used as a basis to understand and interpret the UV-VIS spectral data.

## 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

## 7. REFERENCES

Christian, G. D., Dasgupta, P. K., & Schug, K. A. (2014). *Analytical Chemistry, Seventh Edition*. New York: Wiley.

Clarke, E. G. C., .(1986). *Isolation and Identification of Drugs in Pharmaceutical Body Fluids and Post Mortem Material*. London: The Pharmaceutical Press

Dachriyanus. (2004). *Analisis Struktur Senyawa Organik Secara Spektroskopi*. Padang: Lembaga Pengembangan Teknologi Informasi dan Komunikasi (LPTIK) Universitas Andalas.

DOI: http://dx.doi.org/10.17509/xxxx.vxix
p- ISSN 2775-8419 e- ISSN 2775-8427

Friedel, R. A., & M. Orchin. (1951). *Ultraviolet Spectra of Aromatic Compounds.* New York: John Wiley and Sons.

Gandjar, Gholib, & Rohman, A. (2008). *Kimia Farmasi Analisis*. Yogyakarta: Pustaka Pelajar

Kamagi, L. P., Pontoh, J., & Momuat, L. I. (2017). Analisis kandungan klorofil pada beberapa posisi anak daun aren (Arenga pinnata) dengan spektrofotometer UV-Vis. *Jurnal MIPA*, *6*(2), 49-54.

Kristianingrum & Susila. (2013). *Handout Spektroskopi Ultra Violet dan Sinar Tampak.* Yogyakarta: Universitas Negeri Yogyakarta.

Suhaimi, H., Rose, L. C., Chan, S. Y., Norazemi, N. F., & Amin, K. A. M. (2017). Coated Gellan Gum Hydrogel As A Drug Carrier For Colon Targeted Drug Delivery. *Journal of Sustainability Science and Management Special Issue Number 2: Fundamental Interdisciplinary Pathways To Future Sustainability*. *2*, 36-41

Pavia, D. L., Gary M. L., George S. K., & James R. V. (2008). *Introduction to Spectroscopy, Fourth Edition.* USA: Brooks/Cole.

Rohman, A. (2007). *Kimia Farmasi Analisis*. Yogyakarta: Pustaka Pelajar

Skoog, D. A., West, D. M., Holler, F. J., & Crouch, S. R. (2013). *Fundamentals of Analytical Chemistry, Ninth Edition.* USA: Brooks/Cole.

Skoog, D. A., West, D. M., Holler, F. J., & Crouch, S. R. (2016). *Principles of Instrumental Analysis, Seventh Edition.* USA: Cengage Learning.

Skoog, D., & West, D. M. (1971). *Principles of Instrumental Analysis*, New York: Holt, Rinehart and Winston, Inc.

Sari & Asri Puspita. (2013). *Spectrophotometric UV-VIS Analysis Of A Mixture Caffeine And Sodium Benzoate Value In Power Drink*. Bogor: Institut Pertanian Bogor.

Spangenberg, B., Poole, C. F., and Weins, C. (2011). *Quantitative Thin Layer Chromatography: A Practical Survey.* New York: Springer Science & Business Media.

Suhartati, T. 2013. *Dasar-Dasar Spektrofotometri UV-Vis dan Spektrofotometri Massa untuk Penentuan Struktur Senyawa Organik*. Lampung: Aura.

Underwood, A. L. & Day, R. A. (1986). *Analisis Kimia Kuantitatif*. Jakarta: Erlangga

DOI: http://dx.doi.org/10. 17509/xxxxt.vxix
p- ISSN 2775-8419 e- ISSN 2775-8427