DETERMINATION OF THE CONCENTRATION OF LYCOPENE IN TOMATO BY USING ABSORPTION SPECTROSCOPY

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Declaration

This thesis is my original work has not been presented by another person for a degree in any of other university and that all sources or materials used for this thesis have been properly and suitably acknowledged.
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This thesis has been submitted for examination with my approval as University advisor.
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Dedication

This thesis is dedicated

To

My Mother

Contents

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Abstract

In this thesis we report the measurement of optical properties via the molar absorption coefficient and refractive index of Lycopene. The pigment of Lycopene was extracted from raw tomato and/or tomato paste using 50:50 Acetone−Petroleum ether solutions and washed with Sodium chloride and Patassium carbonate. Lycopene was separated from this mixture through Column chromatography and the use of Acetone and Hexane. Five solutions of Lycopene in Hexane were prepared with different concentration for Uv−Vis spectroscopic analysis and a very good correlation coefficient 0.998 was found, thereby the average Molar absorption coefficient was calculated as 168556 L/mol× cm. For 150 grams of raw tomato; the amount of Lycopene was measured as 7.2 mg and 7.5 mg for sample of Genesis Farms/Debre Zeit and Ambo Timer Agro Industry/Ambo, respectively. Whereas, the average content of Lycopene was measured as 8.62 mg (for Merti/Ethiopia) and 8.76 mg (for Meysu/Turkey) for each of 15 grams tomato paste. The average calculated amount of all−trans−Lycopene was more than 85 percent. The refractive index of Lycopene was measured as 1.536 with standard error of the mean and variance of significance ($P < 0.05$).

Key words*: Lycopene, Column chromatography, Uv− Vis spectroscopy, Absorbance, Refractive index.*

Acronyms

Abbreviations	Definitions
Uv-Vis	Ultraviolet-Visible
XRCC1	X-Ray Repair Cross-Complementing Protein 1
DNA	Deoxyribonucleic Acid
SNNPRS	Southern, Nations, Nationalities and Peoples Regional State
ARDM	Agriculture and Rural Development Ministry
EMA	Ethiopian Meteorology Authority
CSA	Central Statistics Agency
HPLC	High Performance Liquid Chromatography
PPM	Parts Per Million
WDXRF	Wavelength Dispersive X-ray Fluorescence
TLC	Thin Layer Chromatography

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Chapter 1

Introduction

1.1 Historical Background and Literature Review

In recent years, the role of diet in preventing cancer has been a popular and important area of research. Tomato is an excellent source of vitamins A and C, aside from that it has content nutrient such as ascorbic acid and especially the Lycopene [**1**,**2**]. Lycopene is very useful to our body. Because of long−chain conjugated double−bond presence, Lycopene has been reported to possess the highest antioxidant activity of all the carotenoids and to be responsible for protecting cells against oxidative damage and thereby decreasing the risk of chronic disease [**3**].

Observational studies in many countries have shown that the risk for some types of cancer is lower in people who have higher levels of lycopene in their blood. Studies suggest that diets rich in tomatoes may account for this reduction in risk. There is strong evidence for lycopene's protective effect against cancer of the lung, stomach, and prostate. It may also help to protect against cancer of the cervix, breast, mouth, pancreas, esophagus, colon and rectum [**4**].

Tomatoes are the most concentrated food source of Lycopene [**5**], although apricots, guava, watermelon, papaya, and pink grapefruit are also significant sources [**6**,**7**]. Eating Lycopene−rich vegetables and fruits together with a small amount of oil or fat (for example, salad oil or cheese on pizza) increase the amount of Lycopene absorbed by the intestines. Lycopene is also available in soft−gel capsule and liquid supplements [**8**,**9**].

Lycopene (Its name is derived from the tomatoes species classification, Solanum Lycopersicum Mill) is an antioxidant compound that gives tomatoes and certain other fruits and vegetables their color as well as protecting cells against damage from the free radicals formed when body cells burn oxygen for energy [**10-12**]. It is one of the major carotenoids in the diet of human beings. Carotenoids are organic pigments that are naturally occurring in the chloroplasts and chromoplasts of plants and some other photosynthetic organisms like algae, some types of fungus and some bacteria. Carotenoids are responsible to give

yellow, red, and orange vegetables and fruits their colors [**13**].

There are over 600 known carotenoids; they are split into two classes, xanthophylls (which contain oxygen) and carotenes (which are purely hydrocarbons, and contain no oxygen) [**14**]. Carotenoids in general absorb blue light. They serve two key roles in plants and algae: they absorb light energy for use in photosynthesis, and they protect chlorophyll from photodamage. In humans, four carotenoids (α-carotene, β-carotene, γ-carotene, and β-cryptoxanthin) have vitamin A activity (meaning they can be converted to retinal), and these and other carotenoids can also act as antioxidants [**15**].

Lycopene is found in different kinds of food. It is found in particularly high amounts in tomatoes and tomato products. The food sources of Lycopene and its concentration is illustrated in table (1.1).

Table 1.1: Amount of Lycopene in different food sources.

Product	Serving Size	Lycopene ($\frac{mg}{serving}$)
Tomato juice	250 mL (1 cup)	25.0
Tomato ketchup	15 mL (1 tbsp)	2.7
Spaghetti sauce	125 mL ($\frac{1}{2}$ cup)	28.1
Tomato paste	30 mL (2 tbsp)	13.8
Tomato soup(condensed)	250 mL prepared	9.7
Tomato sauce	60 mL ($\frac{1}{4}$ cup)	8.9
Chili sauce	30 mL (2 tbsp)	6.7
Cocktail sauce	30 mL (2 tbsp)	5.9
Watermelon	368 gm (1 slice)	14.7
Pink grapefruit	123 gm ($\frac{1}{2}$)	4.9
Raw tomato	123 gm (1 medium)	3.7

Source: Heinz Institute of Nutritional Sciences

Lycopene, whose structural formula is reported in Figure (1.1), specified by the molecular formula $C_{40}H_{56}$ with 89.45% carbon and 10.51% hydrogen. It is highly unsaturated hydrocarbon containing 11 conjugated and 2 unconjugated double bonds [**16**].

Lycopene may be expected to undergo two major changes during processing and storage: Isomerization from all−trans to mono−cis or poly−cis forms and oxidation. In nature, Lycopene exists in all−trans form and seven of these bonds can isomerize from the trans−form to the mono or poly−cis form under the inuence of heat, light, oxygen, or certain chemical reactions [**17**].

Lycopene extract from tomato contains carotenoids (5−15% v/v) as well as non-carotenoid components. The carotenoid fraction of the tomato extract consists mainly of Lycopenes, of which $\approx$ 86% is all−trans−Lycopene, $\approx$ 6% is 5−cis−Lycopene, $\approx$ 2%

is 9−cis−Lycopene and ≈ 2% is 13−cis−Lycopene, and ≈ 4% are other carotenoids. The major non−carotenoid components of tomato extract include fatty acids and acylglycerols (69%−74%), phospholipids (8.9%−14%), and waxes (5%−8.4%) [**18**].

All−trans−Lycopene is an unsaturated acyclic hydrocarbon [**19**,**20**] with chemical formula $C_{40}H_{56}$ and molecular weight of 536.85 gm/mol. The structural formula of all−trans−Lycopene is shown in figure (1.1).

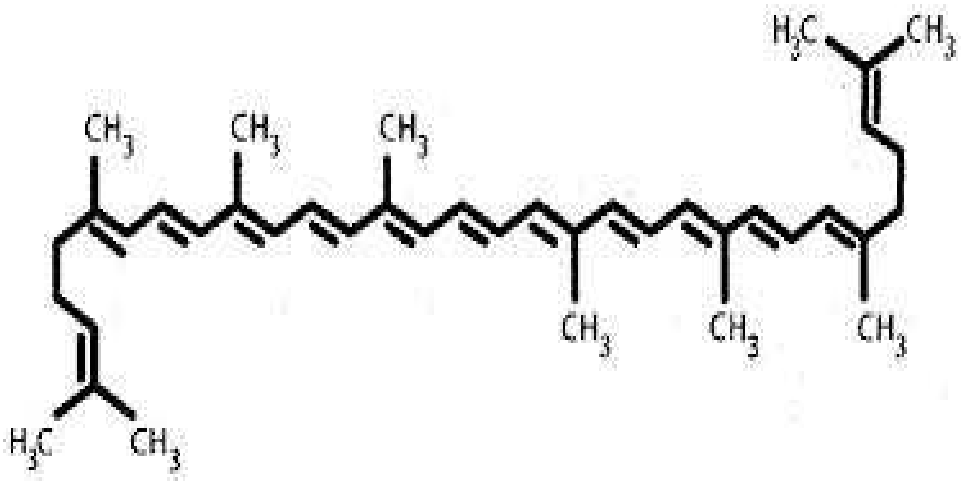

Figure 1.1: Structural formula of all-trans-Lycopene

Some studies have found that a diet high in Lycopene from tomato−based foods was linked with a lower risk of prostate cancer [**21**-**25**]. Other studies, however, found no link between tomato products or other Lycopene−rich foods and prostate cancer [**26**-**28**]. A recent study suggested that variation in a particular gene (known as XRCC1) that helps repair damaged DNA influences whether Lycopene intake will affect a man's prostate cancer risk [**29**]. Conversely, eating fruits and vegetables high in antioxidants is still considered safe during cancer treatment.

Most of the human studies that have been published so far were case control studies or other observational studies, which are more prone to error than clinical trials are. More information from clinical trials (including results of several studies already under way) will be needed to be sure whether Lycopene−rich foods can help prevent or treat cancer.

1.2 Justification

Creating awareness in any kind of technology requires understanding model supported intensive promotional works. Obviously, people all over the world have been suffering from dreadful chronic diseases. Cancer is one of the most killer chronic diseases in human

history. Once somebody victim with cancer, it is difficult and/or impossible to back his/her previous healthy life again.

Based on today's evidence, the foods we eat are likely to play a greater role in preventing cancer than in treating it. The major problem of people living all over the world (especially in Ethiopia) to cause cancer is lack of awareness. When the people consciousness strengthen on the pros and cons of foods that they feed; the probability of being victimized by cancer will decrease.

However, Ethiopia is endowed with a range of vegetables, fruits and crops production including tomato; tomato processing is in its infancy stage in Ethiopia. Currently, there are only two private and one public tomato processing factories in the country [**30**]. As shown in the following table, the daily processing capacities of the privately owned factories range from 1250 to 8000 cans. The public plant processes about 435 tons per year. One of the privately owned plants is located in North Gondar Zone of Amhara region and the other one is located in Sidama Zone of SNNPRS. The public plant is located in the central part of the country.

Table 1.2: Tomato paste processing capacities of existing plants in Ethiopia.

No.	Name	Ownership	Major Products	Plant Production Capacity
1	Melege Wendo Food Processing Factory	Private	Tomato paste (850gm/can) Tomato paste (410gm/can)	5000 per day 8000 per day
2	Gondar Food Processing Factory	Private	Tomato paste (850gm/can)	1250 per day
3	Merti Processing Factory	Public	Tomato paste	43,500 qt/year

1.3 Scope of the study

The scope of this study include following topics:

- Extraction of Lycopene from raw tomato and tomato paste.
- Spectroscopic analysis to find Absorbance of Lycopene by varying the concentration.
- Calculate the refractive index of Lycopene.
- Find the Molar absorption coefficient of Lycopene.
- Evaluate the content of Lycopene in diffrent tomato samples and assess percentage content of all−trans−Lycopene.

1.4 Objective of the study

The major objective of this study is to measure the concentration of Lycopene in raw tomato that could be cultivated in different regions of Ethiopia through developing methodological approach for the rapid, accurate, and sensitive determination of Ultraviolet− Visible Spectroscopy. It has taken into consideration two brands of tomato paste from local and foreign countries (Merti from Ethiopia and Meysu from Turkey) for the purpose of comparison and contrast the effect of Lycopene in processed tomato/tomato paste.

Tomatoes that grow in different places of Ethiopia contain different amount of Lycopene. The variation might comes due to several environmental factors. The common determinant factors are: nature of soil, weather, landscape, topography and annual rainfall found in specified cultivation area; type of fertility provided to the farm; overall planted procedure including spacing, rotation and land preparation.

This project has its own special contribution for the existing burden of investors in line with allocating the productive place of cultivation to grow Lycopene−rich tomato, as well as, advertising the use of tomato (Lycopene) for customers.

Beside to identify which place of Ethiopia is best to grow Lycopene−rich tomato; this research will initiate and encourage host and/or abroad investment via enhanced capacity and skill of Agro−industry products.

In first chapter of this paper the brief historical background and literature review of Lycopene will be presented. In line with the problem of statement, the scope and objective of the study will also be provided.

The concept of Electromagnetic wave in free space and material medium; derivation of Beer−Lambert's law and its application; and the assessment of Ultraviolet−Visible spectroscopy through absorption spectrometry are the core topics of chapter two.

Moreover, the methodological approach and the materials used for the experimental set up of the study will be presented in chapter three. The most important topics of the research are involved in chapter four. In this chapter the detail analysis of experimental results and further discussions on the chemical and physical properties of Lycopene will be provided. Finally, the conclusion of the study and its future trend will present at the end of the chapter.

Chapter 2

Theory

The brief description of theoretical background will be presented in this chapter. In addition to the derivation of electromagnetic radiation through vacuum and material medium, the application of Beer−Lambert's law for testing the concentration of Lycopene in tomato will be evaluated. At the end of this chapter the property and function of ultraviolet−visible spectroscopy will be provided.

2.1 Electromagnetic Wave

Electromagnetic waves are waves which can travel through vacuum and material medium. Electromagnetic waves are created by the vibration of an electric charge. This vibration creates a wave which has both an electric and a magnetic component. An electromagnetic wave transports its energy through a vacuum at a speed of $3.00 \times 10^8 m/s$ (a speed value commonly represented by the symbol **c**). However, the propagation of an electromagnetic wave through a material medium occurs at anet speed which is less than $3.00 \times 10^8 m/s$.

The mechanism of energy transport through a medium involves the absorption and reemission of wave energy by atoms of the material. When an electromagnetic wave impinges upon the atoms of a material, the energy of that wave is absorbed. The absorption of energy causes the electrons within the atoms to undergo vibrations. After a short period of vibrational motion, the vibrating electrons create a new electromagnetic wave with the same frequency as the first electromagnetic wave. While these vibrations occur for only a very short time, they delay the motion of the wave through the medium. Once the energy of the electromagnetic wave is reemitted by an atom, it travels through a small region of space between atoms. When it reaches the next atom, the electromagnetic wave is absorbed, transformed into electron vibrations and then reemitted as an electromagnetic wave. While the electromagnetic wave will travel at a speed of **c** $(3 \times 10^8 m/s)$ through the vacuum of interatomic space, the absorption and reemission process causes the net speed of the electromagnetic wave to be less than **c**.

2.1.1 Electromagnetic Wave in Free Space

This is an appropriate point at which to demonstrate that Maxwell's equations possess propagating wave−like solutions. Let us start from Maxwell's equations in free space (i.e., with no charges and no currents):

$$\nabla \cdot \mathbf{E} = 0 \tag{2.1}$$

$$\nabla \times \mathbf{E} = -\frac{\partial \mathbf{B}}{\partial t} \tag{2.2}$$

$$\nabla \cdot \mathbf{B} = 0 \tag{2.3}$$

$$\nabla \times \mathbf{B} = \mu_0 \epsilon_0 \frac{\partial \mathbf{E}}{\partial t} \tag{2.4}$$

From the above modern 'Heaviside' form of Maxwell's equations; let us search for plane−wave solutions of the form:

$$\mathbf{E}(\mathbf{r}, t) = \mathbf{E}_0 \cos(\mathbf{k} \cdot \mathbf{r} - \omega t) \tag{2.5}$$

$$\mathbf{B}(\mathbf{r}, t) = \mathbf{B}_0 \cos(\mathbf{k} \cdot \mathbf{r} - \omega t - \varphi) \tag{2.6}$$

Here, $\mathbf{E}_0$ and $\mathbf{B}_0$ are constant vectors, $\mathbf{k}$ is called the wave−vector, and ω is the angular frequency. The frequency f in hertz is related to the angular frequency via $\omega = 2\pi f$. The frequency is conventionally defined to be positive. The quantity φ is a phase difference between the electric and magnetic fields. Actually, it is more convenient to write:

$$\mathbf{E} = \mathbf{E}_0 \exp(i(\mathbf{k} \cdot \mathbf{r} - \omega t)) \tag{2.7}$$

$$\mathbf{B} = \mathbf{B}_0 \exp(i(\mathbf{k} \cdot \mathbf{r} - \omega t)) \tag{2.8}$$

Where, by convention, the physical solution is the real part of the above equations. The phase difference φ is absorbed into the constant vector $\mathbf{B}_0$ by allowing it to become complex. Thus, $\mathbf{B}_0 \rightarrow \mathbf{B}_0 \exp(i\varphi)$. A wave maximum of the electric field satisfies:

$$\mathbf{k} \cdot \mathbf{r} = \omega t + n2\pi + \varphi \tag{2.9}$$

Where, n is an integer and φ is phase angle. The solution to this equation is a set of equally spaced parallel planes (one plane for each possible value of n, whose normals lie in the direction of the wave−vector $\mathbf{k}$, and which propagate in this direction with phase−velocity:

$$\nu = \frac{\varphi}{k} \tag{2.10}$$

The spacing between adjacent planes (i.e., the wave−length) is given by:

$$\nu = \frac{\lambda}{k} \tag{2.11}$$

Consider a general plane−wave vector field:

$$\mathbf{A} = \mathbf{A}_0 \exp(i(\mathbf{k}\cdot\mathbf{r} - \omega t)) \tag{2.12}$$

The divergence of $\mathbf{A}$ could easy evaluate as:

$$\nabla\cdot\mathbf{A} = \frac{\partial A_x}{\partial x} + \frac{\partial A_y}{\partial y} + \frac{\partial A_z}{\partial z} = (\hat{i}A_{0x}k_x + \hat{j}A_{0y}k_y + \hat{k}A_{0z}k_z)\exp(i(\mathbf{k}\cdot\mathbf{r} - \omega t)) = i\mathbf{k}\cdot\mathbf{A} \tag{2.13}$$

Now let us calculate the curl of:

$$(\nabla\times\mathbf{A})_x = \frac{\partial A_z}{\partial y} - \frac{\partial A_y}{\partial z} = \hat{i}A_z k_y + \hat{i}A_y k_z = (i\mathbf{k}\times\mathbf{A})_x \tag{2.14}$$

This is easily generalized to:

$$\nabla\times\mathbf{A} = i\mathbf{k}\times\mathbf{A} \tag{2.15}$$

We can see that vector field operations on a plane−wave simplify to replacing the ∇ operator with $i\mathbf{k}$.The first Maxwell equation (2.1) reduces to:

$$i\mathbf{k}\cdot\mathbf{E} = 0 \tag{2.16}$$

Using the assumed electric and magnetic fields (2.7) and (2.8), and Eq.(2.13). Thus, the electric field is perpendicular to the direction of propagation of the wave. Likewise, the third Maxwell's equation (2.3) gives:

$$i\mathbf{k}\cdot\mathbf{B} = 0 \tag{2.17}$$

The magnetic field is also perpendicular to the direction of propagation. Clearly, the wave − like solution of Maxwell's equation is a type of transverse wave. The second Maxwell's equation (2.2) gives:

$$i\mathbf{k}\times\mathbf{E} = i\omega\mathbf{B} \tag{2.18}$$

Where, use has been made of Eq.(2.15). Dotting this equation with $\mathbf{E}$ yields:

$$\mathbf{E}\cdot\mathbf{B} = \frac{\mathbf{E}\cdot(\mathbf{k}\times\mathbf{E})}{\omega} = 0 \tag{2.19}$$

Thus, the electric and magnetic fields are mutually perpendicular. Dotting equation (2.18) with $\mathbf{B}$ yields:

$$\mathbf{B}\cdot(\mathbf{k}\times\mathbf{E}) = \omega\mathbf{B}^{\mathbf{2}} \geq \mathbf{0} \tag{2.20}$$

Thus, the vectors **E**, **B** and **k** are mutually perpendicular, and form a right − handed set. The final Maxwell equation (2.4) gives:

$$i\mathbf{k} \times \mathbf{B} = -i\epsilon_0\mu_0\omega\mathbf{E} \tag{2.21}$$

Combining this with Eq.(2.18) yields:

$$\mathbf{k} \times (\mathbf{k} \times \mathbf{E}) = (\mathbf{k} \cdot \mathbf{E})\mathbf{k} - \mathbf{k}^2\mathbf{E} = -\epsilon_\mathbf{0}\mu_\mathbf{0}\omega\mathbf{E} \tag{2.22}$$

or

$$\mathbf{k}^2 = \epsilon_0\mu_0\omega^2 \tag{2.23}$$

Where use has been made of Eq.(2.16). However, we know from Eq.(2.10) that the phase−velocitycis related to the magnitude of the wave−vector and the angular wave frequency via $c = \frac{\omega}{k}$. Thus, we obtain:

$$c = \frac{1}{\sqrt{\epsilon_0\mu_0}} \tag{2.24}$$

So, we have found transverse wave solutions of the free−space Maxwell's equations, propagating at some phase−velocity, which is given by a combination of ϵ_0 and μ_0 . The constants ϵ_0 and μ_0 are easily measurable. The former is related to the force acting between stationary electric charges, and the latter to the force acting between steady electric currents. Both of these constants were fairly well−known in Maxwell's time. Maxwell, incidentally, was the first person to look for wave−like solutions of his equations, and, thus, to derive Eq.(2.24). The modern values of ϵ_0 and μ_0 are:

$$\epsilon_0 = 8.8542 \times 10^{-12} C^2 N^{-1} m^{-2} \tag{2.25}$$

$$\mu_0 = 4\pi \times 10^{-7} N A^{-2} \tag{2.26}$$

Let us use these values to find the phase−velocity of electromagnetic waves; we obtain:

$$c = \frac{1}{\sqrt{\epsilon_0\mu_0}} = 2.998 \times 10^8 m s^{-1} \tag{2.27}$$

2.1.2 Electromagnetic Wave in Medium

The strength of electromagnetic field will be reduced in transit through a material medium due to absorption. This phenomenon can be explained by the assumption of Lorentz model. He considers a material medium consists of electron vibrating with the same frequency ω_0 (natural frequency of oscillators). When the electric field propagating in Z−direction is given by:

$$E(z,t) = E_0 exp(-i(\omega t - kz)) \tag{2.28}$$

is incident on the matter, some of the electron oscillators will try to align in the direction of electric field (Z−direction). The wave equation which relates electric field and polarization is given by:

$$\nabla^2 E - \frac{1}{c^2}\frac{\partial^2 E}{\partial t^2} = \frac{1}{\epsilon_0 c^2}\frac{\partial^2 P}{\partial t^2} \tag{2.29}$$

The forces acting on the dipole are:

1. Newtons Force: $m\frac{d^2X(t)}{dt^2}$
2. Restoring Force: $k_s X(t)$
3. Frictional force: $bV(t) = b\frac{dx(t)}{dt}$
4. Electrical Force: $eE(z,t) = eE_0 exp(-i(\omega t - kz))$

Where, m = mass of the single electron, k_s = spring(stiffness) constant and b =friction constant The Lorentz− Newton equation of motion of the system is given by:

$$m\frac{d^2X(t)}{dt^2} + b\frac{dx(t)}{dt} + k_s X(t) = eE_0 exp(-i(\omega t - kz)) \tag{2.30}$$

We can rewrite the above equation as:

$$\frac{d^2X(t)}{dt^2} + 2\beta\frac{dx(t)}{dt} + \omega_0^2 X(t) = \frac{e}{m}E_0 exp(-i(\omega t - kz)) \tag{2.31}$$

Where for lateral convenience we have defined: $\beta = \frac{b}{2m}$ and $\omega_0^2 = \frac{k_s}{m}$
We can solve above equation by considering the electric displacement:

$$X(t) = d\exp(-i(\omega t - kz)) \tag{2.32}$$

and after inserting this in to eqn(2.31) we obtain:

$$(-\omega^2 - 2i\beta\omega + \omega_0^2)d = \frac{e}{m}E_0 \tag{2.33}$$

Therefore the assumed solution eqn(2.32) satisfies equation (2.31) if:

$$d = \frac{\frac{e}{m}E_0}{\omega_0^2 - \omega^2 - 2i\beta\omega} \tag{2.34}$$

And the physical relevant solution is:

$$X(t) = R(\frac{\frac{e}{m}E_0}{\omega_0^2 - \omega^2 - 2i\beta\omega})\exp(-i(\omega t - kz)) \tag{2.35}$$

The dipole moment $P = eX(t)$, or $P = \sum_j eX_j$ in the case of many electrons:

$$P = R(\frac{\frac{e^2}{m}E_0}{\omega_0^2 - \omega^2 - 2i\beta\omega}) \exp(-i(\omega t - kz)) \tag{2.36}$$

or

$$P = R\frac{e^2}{m}E_0 \exp(-i(\omega t - kz)) \sum_{j=1}^{z} \frac{1}{\omega_j^2 - \omega^2 - 2i\beta\omega_j} \tag{2.37}$$

The complex polarizability α is defined by the relation between complex dipole moment and complex electric field:

$$P = \alpha(\omega)E_0 \exp(-i(\omega t - kz)) \tag{2.38}$$

By comparing eqn(2.36) and eqn(2.38) we can easily identify the complex polarizability of a Lorentz atom to be:

$$\alpha(\omega) = \frac{\frac{e^2}{m}}{\omega_0^2 - \omega^2 - 2i\beta\omega} \tag{2.39}$$

Given the complex plarizability above, the complex polarization density is:

$$P = Np = N\alpha(\omega)E_0 \exp(-i(\omega t - kz)) \tag{2.40}$$

Using this polarization density in the wave equation (2.28), together with the complex form of the assumed electric field (2.29), we obtain:

$$(-k^2 + \frac{\omega^2}{c^2})E(z,t) = -\frac{\omega^2}{c^2}N\alpha(\omega)E(z,t) \tag{2.41}$$

Therefore k must satisfy the dispersion relation:

$$k^2 = \frac{\omega^2}{c^2}(1 + \frac{N\alpha(\omega)}{\epsilon_0}) \tag{2.42}$$

We can express eqn(2.42) in terms of the refractive index $n(\omega)$ as:

$$k^2 = \frac{\omega^2}{c^2}n^2(\omega) \tag{2.43}$$

In this case, because $\alpha(\omega)$ is complex the refractive index is also a complex:

$$n^2(\omega) = 1 + \frac{Ne^2}{m\epsilon_0}[\frac{\omega_0^2 - \omega^2 + 2i\beta\omega}{(\omega_0^2 - \omega^2)^2 + 4\beta^2\omega^2}] \tag{2.44}$$

$$n(\omega) = \sqrt{1 + \frac{Ne^2}{m\epsilon_0}[\frac{\omega_0^2 - \omega^2 + 2i\beta\omega}{(\omega_0^2 - \omega^2)^2 + 4\beta^2\omega^2}]} \tag{2.45}$$

By using binomial expansion equation (2.45) rewritten as:

$$n(\omega) = 1 + \frac{Ne^2}{2m\epsilon_0}[\frac{\omega_0^2 - \omega^2 + 2i\beta\omega}{(\omega_0^2 - \omega^2)^2 + 4\beta^2\omega^2}] \tag{2.46}$$

Equation (2.46) can split as Real and Imaginary parts as:

$$n(\omega) = n_R(\omega) + in_I(\omega) \tag{2.47}$$

$$n_R(\omega) = 1 + \frac{Ne^2}{2m\epsilon_0}[\frac{\omega_0^2 - \omega^2}{(\omega_0^2 - \omega^2)^2 + 4\beta^2\omega^2}] \tag{2.48}$$

$$n_I(\omega) = \frac{Ne^2}{m\epsilon_0}[\frac{\beta\omega}{(\omega_0^2 - \omega^2)^2 + 4\beta^2\omega^2}] \tag{2.49}$$

The most important consequence of these results is that the electric field in the medium behaves complex characteristics. Because $n(\omega)$ is now complex:

$$E(z,t) = E_0 \exp(-i(\omega t - kz)) = E_0 \exp(-i\omega[t - n(\omega)z/c)]) \tag{2.50}$$

Substituting (2.47) into (2.50), we obtain:

$$E(z,t) = E_0 \exp(\frac{-n_I(\omega)\omega z}{c}) \exp(-i\omega[\frac{[t - n_R(\omega)z}{c}]) \tag{2.51}$$

Note that $E(z,t)$ is no longer pure oscillator. Due to $\mathbf{n_I}(\omega)$, the field decays with increasing distance of propagation. Since the intensity is proportional to the square of amplitude of the electric field, the intensity shows exponential decay with z.

$$I_\omega(z) = I_\omega(0)(\exp(-[\frac{n_I(\omega)\omega z}{c}])^2 = I_0 \exp(-\mathbf{a}(\omega)z) \tag{2.52}$$

Whwere, we call $\mathbf{a}(\omega)$ the absorption coefficient or extinction coefficient:

$$\mathbf{a}(\omega) = \frac{2\mathbf{n_I}(\omega)\omega}{c} \tag{2.53}$$

The physical interpretation of equation (2.53) is the direct relationship via proportionality of the absorption coefficient with the imaginary part of refractive index.

2.2 Beer−Lambert's Law

The Beer−Lambert's law, also called the Beer−Lambert−Bouguer's law or simply Beer's law, is the linear relationship between absorbance and concentration of an absorber of electromagnetic radiation. The general Beer−Lambert's law is written as:

$$A = a_\lambda bc \tag{2.54}$$

Where A is the measured absorbance, a_λ is a wavelength−dependent absorptivity coefficient, b is the path−length and c is the analyte concentration. When working in concentration units of molarity, the Beer−Lambert's law is written as:

$$A = \epsilon_\lambda bc \tag{2.55}$$

Where, ϵ is the wavelength−dependent molar absorptivity coefficient with units of $M^{-1}cm^{-1}$. The wavelength subscript is often dropped with the understanding that a value of ϵ is for a specific wavelength. If multiple species that absorb light at a given wavelength are present in a sample, the total absorbance at that wavelength is the sum due to all absorbers:

$$A = (\epsilon_1 c_1 b) + (\epsilon_2 c_2 b) + ... = \epsilon_i c_i b \tag{2.56}$$

Where, the subscripts refer to the molar absorptivity and concentration of the different absorbing species that are present. Experimental measurements are usually made of transmittance (T), which is defined as:

$$T = \frac{P}{P_0} \tag{2.57}$$

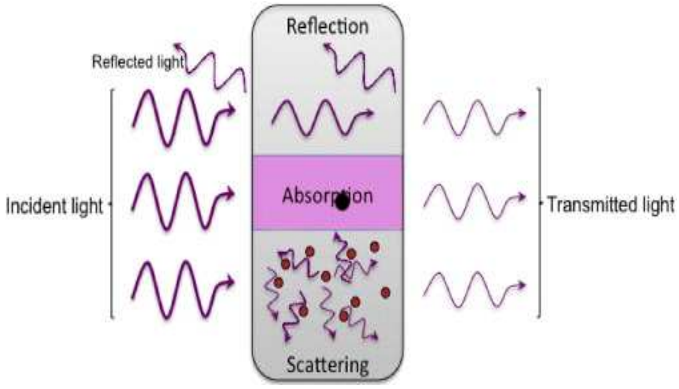

Figure 2.1: The phenomenon of light interacting with matter.

Where, P is the power of light after it passes through the sample and P_0 is the initial light power. The relation between A and T is:

$$A = -\log(T) = -\log(\frac{P}{P_0}) \tag{2.58}$$

The figure above (2.1) shows the case of absorption of light through material medium and includes other processes that decrease the transmittance such as surface reflectance and scattering.

In analytical applications we often want to measure the concentration of an analyte independent of the effects of reflection, solvent absorption, or other interferences. The figure below (2.2) shows the two transmittance measurements that are necessary to determine the concentration of an analyte in solution.

The top diagram is for solvent only and the bottom is for an absorbing sample in the same solvent. In this example, P_s is the source light power that is incident on a sample; P is the measured light power after passing through the analyte solvent and sample holder. The measured transmittance in this case is attributed to only the analyte.

Depending on the type of instrument, the reference measurement (top diagram) might be made simultaneously with the sample measurement (bottom diagram) or a reference measurement might be saved on computer to generate the full spectrum.

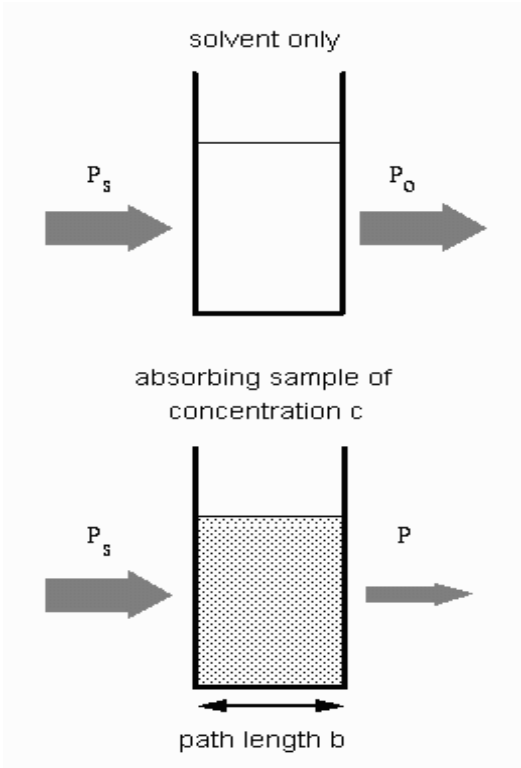

Figure 2.2: Concentration of solvent and sample in sample holder(cuvette).

2.2.1 Derivation of Beer−Lamberts Law

The Beer−Lambert's law can be derived from an approximation for the absorption coefficient for a molecule by approximating the molecule by an opaque disk whose cross−sectional area, σ, represents the effective area seen by a photon of frequency ω. If the frequency

of the light is far from resonance, the area is approximately zero, and if ω is close to resonance the area is a maximum. Taking an infinitesimal slab dZ of sample:

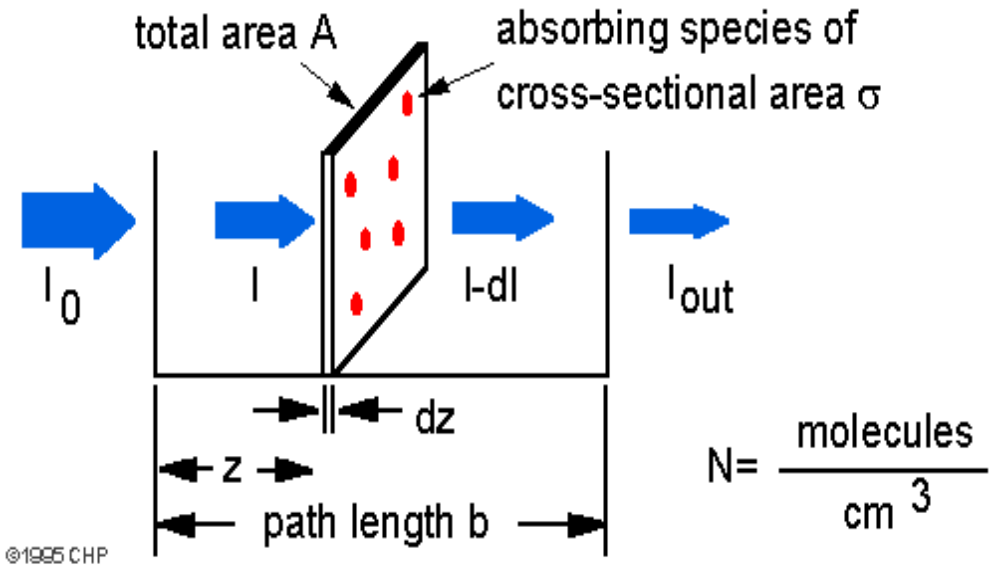

Figure 2.3: Schematics of light absorbing by material medium.

I_0 is the intensity entering the sample at $Z = 0$, Iz is the intensity entering the infinitesimal slab at Z, dI is the intensity absorbed in the slab, and I is the intensity of light leaving the sample. Then, the total opaque area on the slab due to the absorbers is $\sigma NAdZ$. Then, the fraction of photons absorbed will be $\sigma NAdZ/A$. so,

$$\frac{dI}{I_z} = -\sigma N dz \tag{2.59}$$

Integrating this equation from Z=0 to Z=b gives:

$$\ln(\frac{I}{I_0}) = -\sigma N b \tag{2.60}$$

$$I = I_0 exp(-\sigma N b) \tag{2.61}$$

2.2.2 Application of Beer−Lamberts Law

The beer−Lambert's law is a law of spectrophotometery that states the relationship between how a sample absorbs light and the quantity of a known substance within the sample. This is highly useful in many fields of medical testing, where it is applied to determine, among other things, the concentration of a chemical or substance in blood by analyzing how the blood sample absorbs light.

Analytical determination of substances by absorption spectrophotometry is based on the Beer−Lambert's law. The law is used widely in infra−red spectroscopy and near−infrared spectroscopy for analysis of polymer degradation and oxidation (also in biological tissue). The carbonyl group attenuation at about 6 micrometres can be detected quite easily, and degree of oxidation of thepolymer calculated.

Furthermore, Beer−Lambert's law has enormous application in quality control; by measuring and analyzing that whether the given product contains the desired amount of specific compound or not. For instance, the concentration of caffeine in coffee seed or powder, the content of fat and protein in milk, the amount of vitamins and minerals in vegetable and so on.

2.3 Spectroscopy

Spectroscopy deals with the interaction between matter and radiated energy. Historically, spectroscopy originated through the study of visible light dispersion according to its wavelength, by a prism. Later the concept was expanded greatly to comprise any interaction with radiative energy as a function of its wavelength or frequency. Spectroscopic data is often represented by a spectrum, a plot of the response of interest as a function of wavelength or frequency.

Spectroscopic studies were central to the development of quantum mechanics included with Max Planck's explanation of black body radiation; Albert Einstein's explanation of the photoelectric effect and Niels Bohr's explanation of atomic structure and spectra. Spectroscopy is used in physical and analytical chemistry because atoms and molecules have unique spectra. As a result, these spectra can be used to detect, identify and quantify information about the atoms and molecules. Spectroscopy is also used in astronomy and remote sensing on earth. Most research telescopes have spectrographs. The measured spectra are used to determine the chemical composition and physical properties of astronomical objects (such as their temperature and velocity).

One of the central concepts in spectroscopy is a resonance and its corresponding resonant frequency. Resonances were first characterized in mechanical systems such as pendulums. Mechanical systems that vibrate or oscillate will experience large amplitude oscillations when they are driven at their resonant frequency. A plot of amplitude Vs. excitation frequency will have a peak centered at the resonance frequency. This plot is one type of spectrum; with the peak often referred to as a spectral line, and most spectral lines have a similar appearance.

There are different kinds of spectroscopy: Atomic Absorption Spectroscopy (Energy absorbed by the sample is used to assess its characteristics), Fluorescence Spectroscopy (the absorbed energy causes light to be released from the sample), Fourier Transform Spectroscopy (the sample is irradiated by all relevant wavelengths simultaneously for a short period of time), Raman Spectroscopy (scattering of light by molecules), X−ray Spectroscopy

(excitation of inner electrons of atoms), Multiplex or Frequency−Modulated Spectroscopy (optical wavelength that recorded is encoded with an audio frequency), Mass Spectrometry (analyzing the dispersion of ions when they interact with the sample), Gamma−ray Spectroscopy (activation analysis by gamma radiation) and Astronomical Spectroscopy (Energy from celestial objects is used to analyze their chemical composition, density, pressure, temperature, magnetic fields, velocity, and other characteristics): etc... are some examples of spectroscopy.

2.3.1 Absorption Spectroscopy

Absorption spectroscopy uses the range of electromagnetic spectra into which a substance can be absorb. Absorption is the phenomenon that occurs when a transition from a lower level to a higher level takes place with transfer of energy from the radiation field to the atom or molecule.

When atoms or molecules absorb light, the incoming energy excites a structure (in energy quanta) to a higher energy level. The type of excitation depends on the light wavelength. Electrons are promoted to higher orbits by ultraviolet or visible light. Vibrations are excited by infrared light and microwaves excite the rotations.

An absorption spectrum is a way to represent the absorption of light as a function of wavelength. The spectrum of an atom or molecule depends on its energy−level structure, and absorption spectra are useful for identifying compounds.

Absorption spectroscopy is useful in chemical analysis because of its specificity and its quantitative nature. The specificity of absorption spectra allows compounds to be distinguished from one another in a mixture, making absorption spectroscopy useful in wide variety of applications. For instance, Infrared−gas analyzer can be used to identify the presence of pollutants in the air, distinguishing the pollutant from nitrogen, oxygen, water and other expected constituents.

The specificity also allows unknown samples to be identified by comparing a measured spectrum with a library of reference spectra.

An absorption spectrum can be quantitatively related to the amount of material present using the Beer−Lambert's law. Determining the absolute concentration of a compound requires knowledge of the compound's absorption coefficient. The absorption coefficient for some compounds is available from reference sources, and it can also be determined by measuring the spectrum of a calibration standard with a known concentration of the target.

There are a wide range of experimental approaches to measuring absorption spectra. The most common arrangement is to direct a generated beam of radiation at a sample and detect the intensity of the radiation that passes through it. The transmitted energy can be used to calculate the absorption. The source, sample arrangement and detection technique vary significantly depending on the frequency range and the purpose of the experiment.

2.3.2 Ultraviolet−Visible Spectroscopy

UV−Vis spectroscopy is routinely used in the quantitative determination of solutions of transition metal ions highly conjugated organic compounds, and biological macromolecules. Solutions of transition metal ions can be colored (i.e., absorb visible light) because the electrons within the metal atoms can be excited from one electronic state to another. Organic compounds, especially those with a high degree of conjugation, also absorb light in the UV or Visible regions of the electromagnetic spectrum. The solvents for these determinations are often water for water soluble compounds, ororganic substances for organic−soluble compounds. Organic solvents may have significant UV absorption; not all solvents are suitable for use in UV spectroscopy.

Chapter 3

Materials and Methods

This section contains two major topics of study: the study design and the experimental techniques. The research was designed by taking into account the study area, sample size and data collection. Whereas, in the experimental techniques, beside with sample preparation, the instrumentation of UV-Vis spectroscopy and the experimental set up of refractive index will be presented.

3.1 Study Design

The specifications for tomato samples were developed from considerations proposed by the author of this thesis based on the primary data collection method and the availability in market. Personal interview were held to collect the information through questionnaire.

3.1.1 Study Area

Sun−ripened tomatoes were collected from two different regions of Ethiopia. The places were selected based on the fact that they possess different topography and climate. Both districts have altered altitude, longitude, annual rainfall and daily average temperature. Table (3.1) shows the gap of climate and weather condition for both study areas.

Table 3.1: Meteorological data of study area.

Town	Latitude	Longitude	Altitude(m)	Average annual rainfall(mm)	Average Daily Temp(°C)
Ambo	$8°59'$	$37°51'$	2101	968.7	18.5
Debre Zeit	$8°44'$	$38°58'$	1850	800.1	20.15

Source: Ethiopian Meteorology Agency.

Genesis Farms and Ambo Timer Agro Industry are the companies that the raw tomato sample collected from Debre Zeit and Ambo respectively. In line with raw tomato sample collection, compulsory information was gathered through questionnaire. The questionnaire is attached at the end of this paper in Appendix. A great care was taken to confirm that the questionnaire was filled by responsible, well educated, and expertise in Agricultural and related fields. Thus, the author of this thesis could assure that the persons that gave the required information were graduated in pathology and had sufficient experience with treatment of tomato as well.

3.1.2 Sample Size

A total of 4 tomatoes (2 pastes and 2 raw samples) were prepared from selected processing products and sun−ripened tomatoes. The two different branded tomato paste samples are; Merti from Awash Agro Industry (Ethiopia) and Meysu from (Turkey); while the raw samples of tomato were collected from two different agro−industries located in Ethiopian district based on diversified weather condition, altitude, topography and annual rainfall.

3.1.3 Data Collection

The required input data were obtained from the recognized stakeholders of reliable and responsible institutions. ARDM, EMA and CSA are some of the organizations that furnish the desired authentication information for the research. The author of this thesis has found himself in appropriate position to collect the raw tomato samples. In other words, the sun−ripened tomato samples were collected from the original source of the farm. The tomato paste samples were purchased from the market In view of the fact that once they are packed and deliver to market they would not have disparity whether they are gathered from the company they produced or from any market place as long as they kept in safe store.

3.2 Experimental Techniques

The experiment was conducted in three phases; the extraction phase, the column chromatography phase and the refractive index phase. It was taken a great care to calibrate the experimental set up. Moreover, the experiment was performed under darkness with a little bit light to get rid off sun light exposure.

3.2.1 Apparatus and Chemicals

The experiment was carried out with the help of the following devices: beakers, spatula, column tube, flasks, digital electronic beam balance to measure the mass of the sample,

evaporator to suck the solvent and get the sample dried, quartz cuvette to hold the sample for spectroscopic analysis, filter−tip pipet, conical vial, computer linked spectrophotometer (Perkin−Elmer UV/VIS/NIR Lambda 19), plane mirror, convex lens and meter stick.

Reagents used for the experiment were: Alumina (grade I), Silica gel and Sodium sulphate. Furthermore, the solution of: 10% Acetone/Hexane mixture, 50% Acetone/Petroleum ether mixture, saturated Sodium chloride solution and aqueous Potassium carbonate were prepared.

3.2.2 Sample Preparation

Lycopene from tomato paste products was extracted as follows:

1. One gram of tomato sample homogenized into a 15 mL beaker.
2. Extract the solid material by shaking the capped tube with 4 mL of a 50% (by volume) mixture of Acetone and low−boiling Petroleum ether until the solid residue looks dry and fluffy. Then use a flat−bladed microspatula to run and crush it against the sides of the tube.
3. Repeat the shaking and crushing steps several times.
4. Separate the extract with a filter−tip pipet, and transfer it to a second 15 mL beaker. Then repeat the extraction of the solid residue with another 4 mL portion of 50% Acetone/Petroleum ether, and combine the extracts in the second centrifuge tube.
5. Wash the combined extracts with 5 mL of saturated Sodium chloride solution, followed by 5 mL of 10% aqueous Potassium carbonate and another 5 mL portion of saturated Sodium chloride solution.
6. Dry the Lycopene−containig organic layer with anhydrous Magnesium sulphate, collect it in a 5 mL conical vial, and concentrate it to a volume of 0.1−0.2 mL by evaporating most of the solvent under vacuum without heating.

For the case of raw tomato, the following method was taken into consideration before applying the above procedure. The tomatoes were washed and cut longitudinally into several pieces. Once skins have been removed, the tomatoes kept dry with vacuum evaporator to eliminate water and other unwanted solvents from the sample then concentrated at low temperature.

3.2.3 Column Chromatography

Neutral Brockmann grade I Alumina used as an adsorbent to pack chromatography column. The organic solutions, 10 mL of Hexane (the first eluant), 10 mL of 10:90 (%

volume) Acetone/Hexane (the second eluant) were gathered. A small Erlenmeyer flask, (for collecting the Lycopene fraction), and a beaker were prepared before start to run the column.

1. The beaker placed under the column and added the first eluant, Hexane, to the column until the liquid wets all of the Alumina.

2. The extracted sample added to the top of column (used a little of Hexane to rinse the extract vial and added it to the column as well. As soon as the extract entered the Alumina layer, the column filled almost all the way with Hexane.

3. To keep the solvent level in the column relatively constant, Hexane were added as necessary.

4. When the first yellow band started to drain out of the column, the second eluant (10:90% volume acetone/hexane) were added to the top of the column and kept the eluant level constant as before.

5. When the Lycopene layer (Orange−Red) began to leave the column, the layer collected into the Erlenmeyer flask and carried on until the band is almost completely off the column.

TLC was used to differentiate the band gap of the compound that dropped from the column's nozzle.

3.2.4 Instrumentation

Instruments for measuring the absorption of ultraviolet or visible radiation are made up of the following components are: *Sources (UV and visible)*, *Wavelength selector (Monochromator)*, *Sample container (Cuvette)*, *Detector* and *Signal Processor and Readout*

1. Source:- It is important that the power of the radiation source does not change abruptly over its wavelength range. The electrical excitation of deuterium or hydrogen at low pressure produces a continuous UV spectrum. The mechanism for this involves formation of an excited molecular species, which breaks up to give two atomic species and an ultraviolet photon.

Both Deuterium and Hydrogen lamps emit radiation in the range $160 - 375$ nm. Quartz windows must be used in these lamps, and quartz cuvettes must be used, because glass absorbs radiation of wavelengths less than 350 nm.

The tungsten filament lamp is commonly employed as a source of visible light. This type of lamp is used in the wavelength range of $350 - 2500$ nm. The energy emitted by a tungsten filament lamp is proportional to the fourth power of the operating voltage. This means that for the energy output to be stable, the voltage to the lamp must be very stable

indeed. Electronic voltage regulators or constant−voltage transformers are used to ensure this stability.

2. Monochromator:- The other important component of spectroscopy is Wavelength selector (Monochromator). All monochromators contain the following component parts:

- An entrance slit
- A collimating lens
- A dispersing device (usually a prism or a grating)
- A focusing lens
- An exit slit

Polychromatic radiation (radiation of more than one wavelength) enters the monochromator through the entrance slit. The beam is collimated, and then strikes the dispersing element at an angle. The beam is split into its component wavelengths by the grating or prism. By moving the dispersing element or the exit slit, radiation of only a particular wavelength leaves the monochromator through the exit slit.

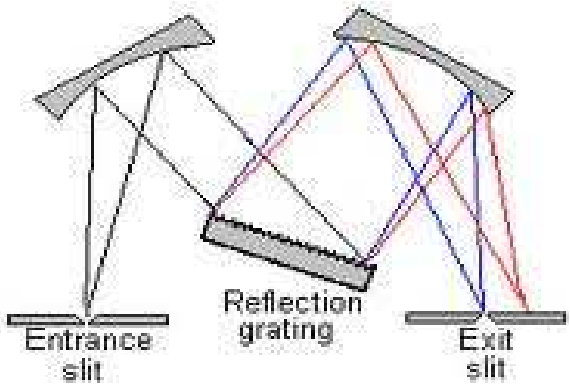

Figure 3.1: Czerney−Turner Grating Monochromator.

3. Cuvette:- The containers for the sample and reference solution must be transparent to the radiation which will pass through them. Quartz or fused silica cuvettes are required for spectroscopy in the UV region. These cells are also transparent in the visible region. Silicate glasses can be used for the manufacture of cuvettes for use between 350 and 2000 nm.

4. Detector:- The photomultiplier tube is a commonly used detector in UV−Vis spectroscopy. It consists of a photo emissive cathode (a cathode which emits electrons when struck by photons of radiation), several dynodes (which emit several electrons for each electron striking them) and ananode.

A photon of radiation entering the tube strikes the cathode, causing the emission of several electrons. These electrons are accelerated towards the first dynode (which is 90 V more positive than the cathode). The electrons strike the first dynode, causing the emission of several electrons for each incident electron. These electrons are then accelerated towards the second dynode, to produce more electrons which are accelerated towards dynode three and so on. Eventually, the electrons are collected at the anode. By this time, each original photon has produced 10^6 upto 10^7 electrons. The resulting current is amplified and measured.

Photomultipliers are very sensitive to UV and Visible radiation. They have fast response times. Intense light damages photomultipliers; they are limited to measuring low power radiation.

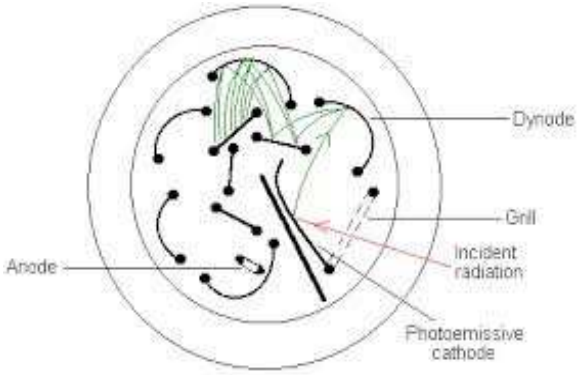

Figure 3.2: Cross-section of a Photomultiplier Tube

Linear photodiode array is an example of a multi channel photon detector. These detectors are capable of measuring all elements of a beam of dispersed radiation simultaneously.

A linear photodiode array comprises many small silicon photodiodes formed on a single silicon chip. There can be between 64 and 4096 sensor elements on a chip, the most common being 1024 photodiodes. For each diode, there is also a storage capacitor and a switch. The individual diode−capacitor circuits can be sequentially scanned.

In use, the photodiode array is positioned at the focal plane of the monochromator (after the dispersing element) such that the spectrum falls on the diode array. They are useful for recording UV−Vis absorption spectra of samples that are rapidly passing through a sample flow cell, such as in an HPLC detector.

Charge−Coupled Devices (CCDs)are similar to diode array detectors, but instead of diodes, they consist of an array of photocapacitors.

5. Signal Processor and Readout:- The output signal from the detector must be monitored, stored and analyzed to make a spectroscopic measurement. As such, the signal-processing and readout system is extremely important to the overall performance

of the system. The particular type of signal processing depends on the form of the output signal, the noise sources expected and the signal level itself. The signal-processing step can perform many conversions such as current-to-voltage, analog-to-digital conversion, amplification, or some mathematical operation designed to improve the measurement of signal-to-noise ratio.

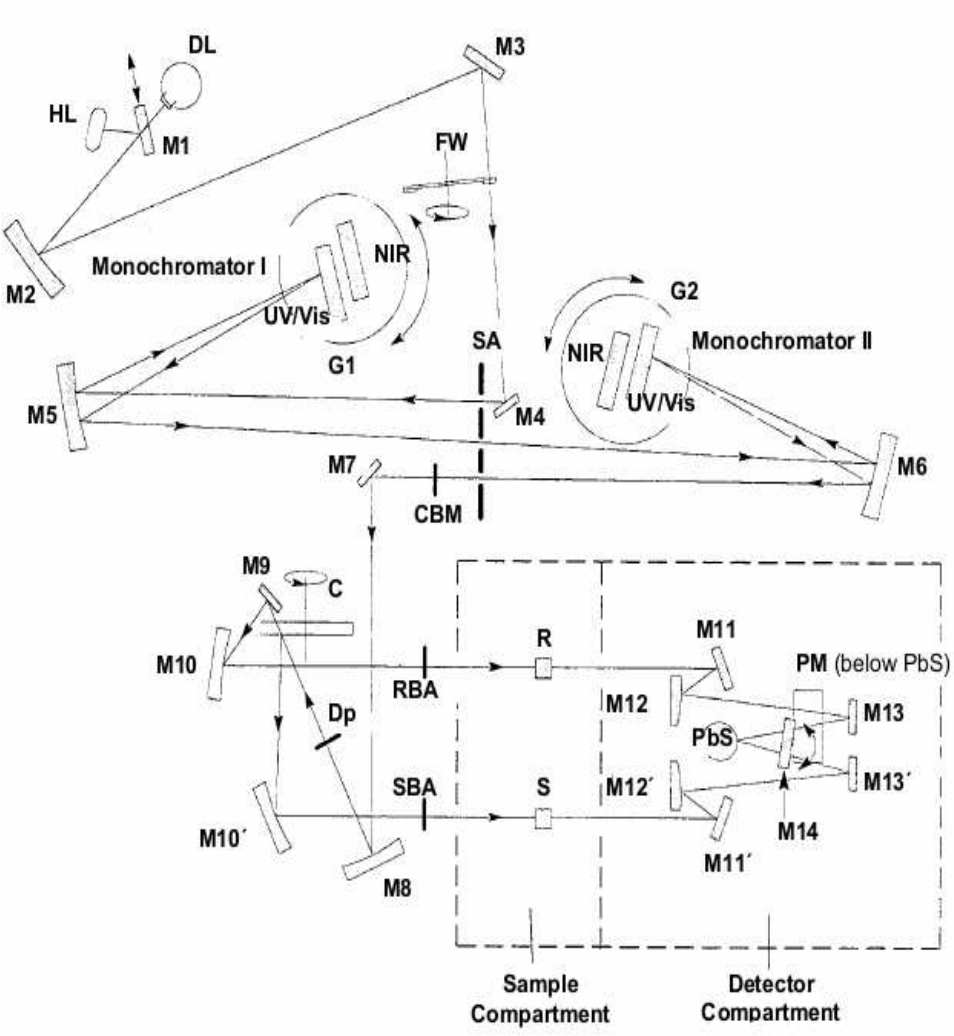

Figure 3.3: Schematics Optical diagrame of Lambda 19 UV/VIS/NIR Spectroscopy

The outputs of most detectors are used in the analog mode. The exception to this is the photomultiplier, which may be used in the photon-counting mode. Nearly all measurements are made by using an analog-to-digital converter to convert the output of a detector to the digital domain for future processing, analysis and display. Even in digital measurements some conditioning, such as filtering or amplifying the analog signal, is

usually necessary to make it suitable for recording by the converter.

Figure (3.3) shows that the schematics of Uv-Vis spectroscopy; how the spectroscopy record datum and gives out the reading result. The so called 'PERKIN−ELMER LAMBDA 19' branded UV-Visible Spectroscopy was utilized for the experiment [**31**].

3.2.5 Experimental procedure of Refractive Index of Lycopene

Experimental set up to investigate the refractive index of Lycopene was carried out by using the following procedures.

1. First take a plane mirror and place it on the horizontal base of iron stand. Then place the convex lens on the plane mirror.
2. Then an object pin is placed above the convex lens on a stand. The position of the object pin is fixed in such a way that a distinct image of the object is visible at the same position as that of the object pin without any parallex.
3. Measure the distance, according to the optical center of the lens. (To do this; first measure the distance from the top of the mirror; and then from the top of the lens; then take the average of those two measurements). This is the focal length of the convex lens (f_1).
4. Now add some drop of Lycopene between the lens and the mirror.
5. Bring the tip of the object pin to the vertical optical center of the lens, so that the tip of the object pin appears to touch the tip of its image without any parallex.
6. Measure the distance again by using step 2. This is the focal length (F) of the combination of the convex lens and the Plano−concave lens formed by Lycopene.

So, Focal length of the lens combination is = F, Focal length of the convex lens is = f_1, Focal length which is formed by Lycopene is f_2. Then,

$$\frac{1}{F} = \frac{1}{f_1} + \frac{1}{f_2} \tag{3.1}$$

Now, we should measure the curvature height of the convex lens using a spherometer, suppose it is **h**. And the distance between the spherometer legs is **a**. So, the radius of the curvature of the lens is:

$$R_1 = \frac{a^2}{6h} \tag{3.2}$$

Suppose μ is the refractive index of Lycopene. Then, $\mu = 1 + \frac{R_1}{f_2}$, since we know R_1 and f_2 , so we can calculate the refractive index of Lycopene. However, the calculation of Radius of curvature seems (3.2); for equi−spherical convex lens its value is simply double of the focal length. i.e $R_1 = 2f_1$.

Chapter 4

Result and Discussion

Experimental results and further discussions will be presented in this chapter. In line with calculation of molar absorption coefficient, the refractive index of Lycopene will be provided. The percentage content of all−trans−Lycopene and isomerization of Lycopene will be mentioned in this chapter. Moreover, this chapter points out the calibration curve and quantitative analysis of the experimental results.

4.1 Molar absorption coefficient

The primary target of studying the characteristics of any substance or compound through absorption spectroscopy is to find the molar absorption coefficient of that substance or compound. Each and every single atom, molecule, substance, or compound has its own specific value of molar absorption coefficient.

According to Beer−Lambert's law, the molar absorption coefficient of the specific compound can be evaluated using the following formula:

$$A = \epsilon cb \tag{4.1}$$

Where, ϵ refers to molar absorption coefficient of the given compound. Letter b represents the distance of the incident wave propagating through the absorbing medium. In other words, it is the interior path length of the quartz cuvette which uses as a sample holder for UV−Vis spectroscopic simulation. The path length of the sample holder (cuvette) is 1 centi meter. The maximum absorbance peak of the compound which can be read from the spectroscopy is denoted by A and c stands for the concentration of the compound to be studied.

From the relation (4.1) it is obvious that absorbance is directly proportional to concentration of the sample. From the spectral analysis of figure (4.1) it can be observed that Lycopene absorbs in the spectral range between 340nm and 564nm. Three maxima peaks are occurred at $\lambda = 448.8nm$, $\lambda = 473.6nm$ and $\lambda = 504nm$.

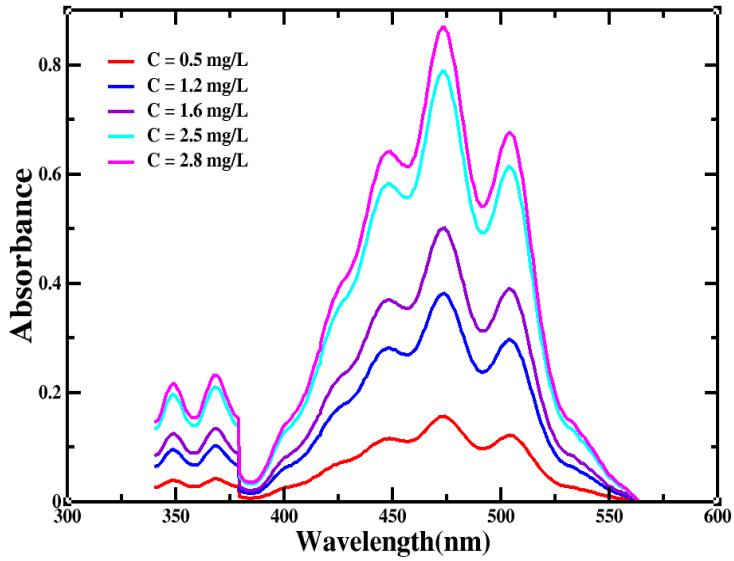

Figure 4.1: Absorbance curve for different Concentration of Lycopene in Hexane

The Molar absorption coefficient of extracted Lycopene samples in hexane solution are summarized in table (4.1). Once the arbitrary units of absorbance read from the spectrophotometry; the molar absorption coefficients can be calculated by using Beer−Lambert's law. Therefore, the molar absorption coefficient of Lycopene at $\lambda = 473.6nm$ was found 168746±1972; and the average coefficient of molar absorption was $168556 L/mol \times cm$. This result is consistent with that of the pure Lycopene sample which is $168571 \pm 2684 L/mol \times cm$.

Table 4.1: Molar Absorption coefficient and peak Wavelength value.

No	Concentration		$\lambda_1(nm)$	$\lambda_2(nm)$	$\lambda_3(nm)$	A at λ_2	$\epsilon(\frac{L}{mol \times cm})$
	m.g/L	mol/L					
1	0.5	0.93×10^{-6}	448.8	473.6	504.0	0.1562	167541
2	1.2	2.24×10^{-6}	448.8	473.6	504.0	0.3816	170718
3	1.6	2.98×10^{-6}	448.8	473.6	504.0	0.5017	168324
4	2.5	4.66×10^{-6}	448.8	473.6	504.0	0.7894	169422
5	2.8	5.22×10^{-6}	448.8	473.6	504.0	0.8698	166774

4.2 Absorbance of pure Lycopene

This research is not fortified with experimental approach of pure Lycopene sample, unfortunately! In other words, the experiment was not done on pure Lycopene sample. Due to its problematic circumstance of obtaining pure Lycopene sample easily. Meanwhile, we Ethiopian are living in the third world; researchers face countless challenges to find various apparatus, chemicals, samples and overall inputs at the proper time and situation as per the need.

Noticeably, the pure sample of most compounds is not accessible in our country, instead of being imported from overseas. In order to get pure sample from abroad, it takes long time (in months and sometimes a year) and requires more finance. Therefore the experimental performance does not involve and figure out the absorbance effect of pure Lycopene.

Figure (4.2) shows the absorption spectrum of the pure extracted Lycopene solution [**32**]. The absorption very closely coincides with the three peaks characteristics of trans−Lycopene at $\lambda = 446nm$, $472nm$ and $505nm$.

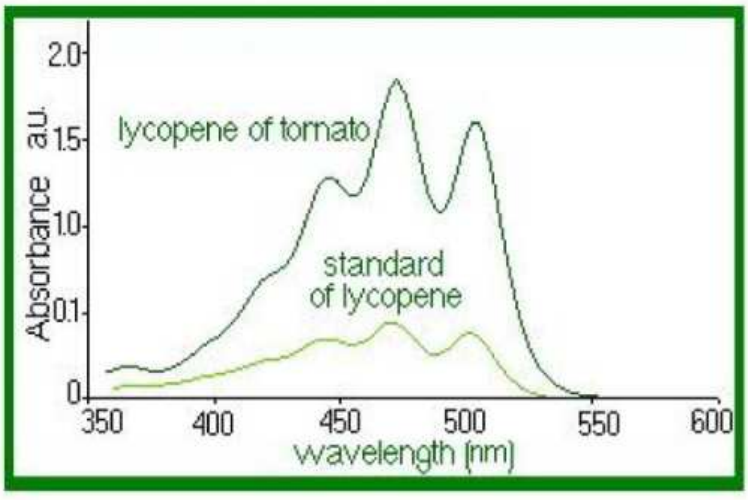

Figure 4.2: The Standard curve of Pure Lycopene.

On the other hand, the experimental result of pure Lycopene was engaged to manipulate with this research. We used Naviglio's curve [**33**] as a standard characteristics of pure Lycopene. This curve is shown in fig (4.3).

The experimental result of figure (4.3) was found for concentration of 1 Mili gram of Lycopene in 1 Liter of solvent (the solvent could be either Dichloromethane, Acetone, Hexane or Chloroform). Figure (4.3) clearly shows that the peak maxima value of absorbance with its corresponding value of wavelength.

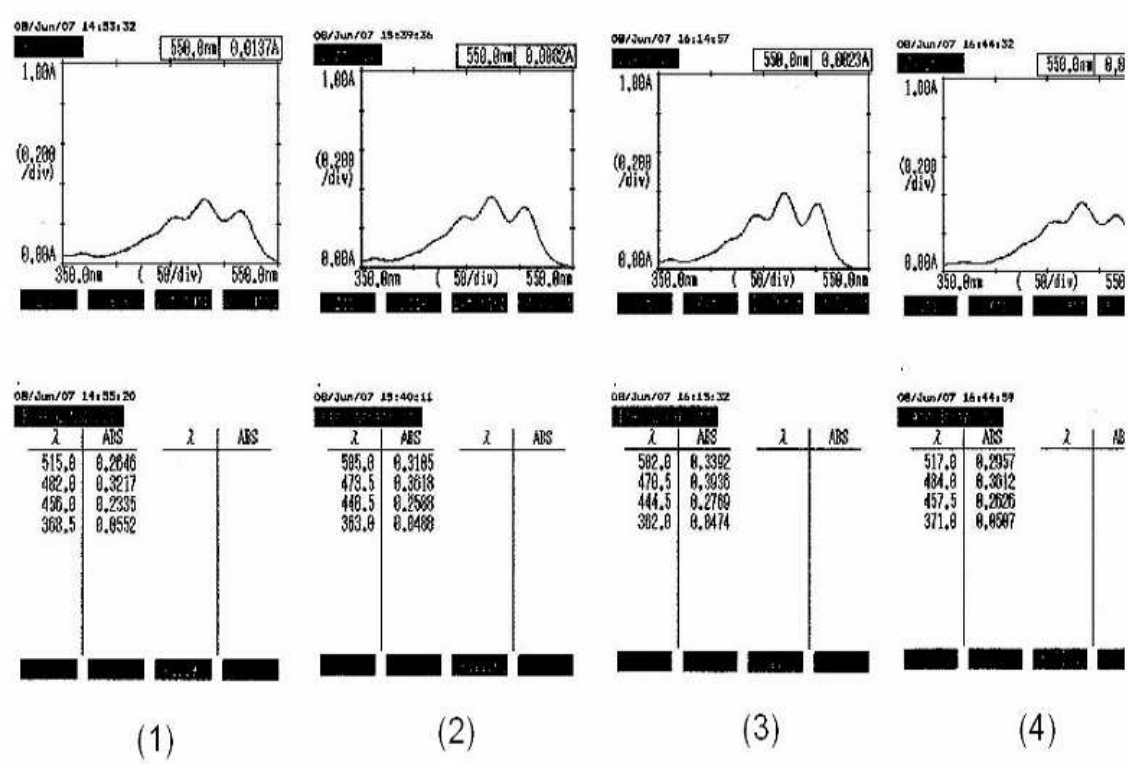

Figure 4.3: Spectrum of Lycopene in different solvent.

For the solution of Lycopene in Dichloromethane (1) the maximum absorbance is 0.3217 at $\lambda = 482.0nm$; the solution of Lycopene in Acetone (2) the maximum absorbance is 0.3818 at $\lambda = 473.5nm$; the solution of Lycopene in Hexane (3) the maximum absorbance is 0.3936 at $\lambda = 470.5nm$ and the solution of Lycopene in Chloroform (4) the maximum absorbance is 0.3612 at $\lambda = 484.0nm$. A very good correlation curve about 0.996 for standard sample and about 0.998 for recovered sample was observed.

Table 4.2: Molar extinction values of 'Recovered' Lycopene in different solvents.

Solvent	$\lambda_1(nm)$	$\lambda_2(nm)$	$\lambda_3(nm)$	$\epsilon(\frac{L}{mol \times cm})$
Hexane	444.5 ± 0.5	470.5 ± 0.5	502.5 ± 0.5	166774 ± 1610
Acetone	448.5 ± 0.5	473.5 ± 0.5	505.5 ± 0.5	153240 ± 1610
Dichloromethane	456.5 ± 0.5	482.5 ± 0.5	515.5 ± 0.5	136308 ± 1610
chloroform	457.5 ± 0.5	484.5 ± 0.5	517.5 ± 0.5	152989 ± 1610

Table (4.2) indicates the molar absorption coefficient of Lycopene in different solvents.

The molar absorption coefficient of standard sample of pure Lycopene in Hexane solution is calculated in the range from $165887 L/mol \times cm$ to $171255 L/mol \times cm$; the molar absorption coefficient of Lycopene in Acetone is from $147795 L/mol \times cm$ to $153163 L/mol \times cm$; the molar absorption coefficient of Lycopene in Dichloromethane is from $130455 L/mol \times cm$ to $135823 L/mol \times cm$ and the molar absorption coefficient of Lycopene in Chloroform is from $148171 L/mol \times cm$ to $153539 L/mol \times cm$.

Table 4.3: Molar extinction values of 'Pure ' Lycopene in different solvents.

Solvent	$\lambda_1(nm)$	$\lambda_2(nm)$	$\lambda_3(nm)$	$\epsilon(\frac{L}{mol \times cm})$
Hexane	445.5 ± 0.5	471.0 ± 0.5	502.0 ± 0.5	168571 ± 2684
Acetone	449.5 ± 0.5	474.0 ± 0.5	505.0 ± 0.5	150479 ± 2684
Dichloromethane	455.0 ± 0.5	482.0 ± 0.5	516.0 ± 0.5	133139 ± 2684
chloroform	456.5 ± 0.5	485.0 ± 0.5	518.0 ± 0.5	150855 ± 2684

On the other hand, the molar absorption coefficient of recovered sample of Lycopene in hexane solution is calculated in the range from $165164 L/mol \times cm$ to $168384 L/mol \times cm$; the molar absorption coefficient of Lycopene in acetone is from $151630 L/mol \times cm$ to $154850 L/mol \times cm$; the molar absorption coefficient of Lycopene in dichloromethane is from $134698 L/mol \times cm$ to $137918 L/mol \times cm$ and the molar absorption coefficient of Lycopene in chloroform is from $151379 L/mol \times cm$ to $154599 L/mol \times cm$.

4.3 Calibration Curve

An amount of Lycopene extracted from column chromatography was dried under a hood (evaporator without heating). Once, Lycopene get dried and the unnecessary solvents removed from it, a stock solution of the extracted Lycopene was prepared by dissolving 10 mg of Lycopene in 100 ml of Hexane. From this standard solution by diluting five solutions containing from 1 to 5 ppm were prepared. The standard solution preparation of Lycopene in hexane is in the range from 0 to 3 mg/L. By using this standard method of solution preparation of Lycopene in hexane; five solutions of ppm were prepared with different concentration as: 0.5 mg/L, 1.2 mg/L, 1.6 mg/L, 2.5 mg/L and 2.8 mg/L.

The experimental result of table (4.4) indicates the proportionality of absorbance and concentration. The statistical significance was determined by analysis of variance $0.6x10^{-5}$ and standard error of the mean is less than 0.002. Figure (4.4): the correlation curve of Concentration versus Absorbance.

Figure (4.4) presents the strong linear relationship of concentration and absorbance with a pretty good proportionality. One could easily understand from the above curve;

Table 4.4: The Correlation coefficient of Absorbance Versus Concentration.

C(mg/L)	λ_{max}	A at λ_{max} (arbitrary unit)	$\frac{A}{C}$(L/mg)
0.5	473.6	0.1562	0.312
1.2	473.6	0.3816	0.318
1.6	473.6	0.5017	0.314
2.5	473.6	0.7894	0.316
2.8	473.6	0.8698	0.311

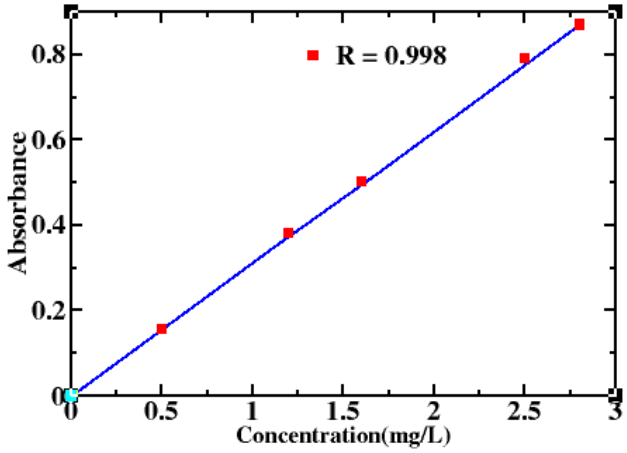

Figure 4.4: Calibration curve (Correlation coefficient) of Lycopene.

when the concentration of Lycopene increases the peak absorbance value will also increases linearly. There was found a very good correlation curve as 0.998.

4.4 Quantity of Lycopene in Tomatoes

Successive and continuous experiment was performed to quantify the content of Lycopene in each tomato sample. Based on the techniques of farming and the mechanism of production; different content of Lycopene were achieved.

Table (4.5) presents the content of Lycopene in raw tomato and tomato paste. According to the tabular datum we can simply generalized that tomato pastes have a better content of Lycopene than that of raw tomatoes. This occurs when the production process eliminate water and unnecessary solvents from the raw material.

Table 4.5: Content of Lycopene in raw-tomato and tomato-paste.

Raw Tomato			Tomato paste		
Sample	Serve	Mg/gm	Sample	Serve	Mg/gm
Genesis	150 gram	7.2	Merti	15 gram	8.62
Ambo Timer	150 gram	7.5	Meysu	15 gram	8.76

4.5 Refractive Index of Lycopene

The refractive index of Lycopene was evaluated by setting up simple experiment. Once the experimental set up was adjusted, the following data was measured.

Table 4.6: Experimental result of Refractive Index of Lycopene.

Trial	$x_0(cm)$	$x_1(cm)$	$f_1(cm)$	$R_1(cm)$	$y_0(cm)$	$y_1(cm)$	$F(cm)$	μ
1	25.8	27	26.4	53.8	36.4	37.6	37	1.5795
2	25	26.2	25.6	51.2	33.9	35.1	34.5	1.5159
3	24.8	26	25.4	50.8	34.4	35.6	35	1.5486
4	26.3	27.5	26.9	53.8	35.3	36.5	35.9	1.5014

Where;

$x_0 \rightarrow$ length from the top of the lens to the point of the object and image coincide.

$x_1 \rightarrow$ length from the top of the mirror to the point of the object and image coincide

$f_1 \rightarrow$ focal length of the convex lens

$R_1 \rightarrow$ radius of curvature for the convex lens

$y_0 \rightarrow$ length from the top of the lens to the point of the object and image coincide before the sample droped.

$y_1 \rightarrow$ length from the top of the mirror to the point of the object and image coincide after the sample droped.

$F \rightarrow$ combinational focal length.

$\mu \rightarrow$ refractive index of Lycopene

Therefore;

$$f_1 = \frac{(x_0 + x_1)}{2} \tag{4.2}$$

$$F = \frac{(y_0 + y_1)}{2} \tag{4.3}$$

$$R_1 = 2f_1 \tag{4.4}$$

The average refractive index of Lycopene calculated as:

$$\mu_{avg} = \frac{\sum_i^n \mu_i}{n} \tag{4.5}$$

Therefore, the average refractive index of Lycopene is 1.536 and it is deviated from the actual value by 0.005. The experimental result of refractive index of Lycopene is in the range of strong presumption against neutral hypothesis. The statistical significance was determined by analysis of variance and standard error in the mean ($P \leq 0.05$).

4.6 Percentage content of all−trans−Lycopene

Lycopene mostly found in all-trans-form. All trans−Lycopene covers more than 85% of the Lycopene portion. An UV-Visible spectrum was revealed three distinct absorption peaks (see fig 4.2); To determine the percentage content of all trans−Lycopene in the sample the following empirical formula was used:

$$All - trans - Lycopene(\%) = \frac{(A)_{\lambda_a}}{(A)_{\lambda_b}} \times 100 \tag{4.6}$$

Where;
$(A)_{\lambda_a} \rightarrow$ arbitrary absorbance value at the highest peak (λ_a) and
$(A)_{\lambda_b} \rightarrow$ arbitrary absorbance value at the higher peak (λ_b)

Table 4.7: Amount of all-trans-Lycopene.

C (mg/L)	$(A)_{\lambda_a}$	$(A)_{\lambda_b}$	all trans−Lycopene(%)
0.5	0.1562	0.1394	89.3
1.2	0.3816	0.2968	77.8
1.6	0.5017	0.4337	86.5
2.5	0.7894	0.6469	82.0
2.8	0.8698	0.8056	92.6

From the experimental result the average content of all trans−Lycopene in the sample was found as 85.7%. The rest 14.3% of the sample is filled with β-carotene and cis−Lycopene.

4.7 Isomerization of Lycopene

When the sample of Lycopene exposed to bright sunlight, some portion of all-trans-Lycopene changes into cis−Lycopene form within half an hour. Therefore, the level of all trans−Lycopene in the sample will decrease, while the level of cis−Lycopene increases. However, it is possible to exposed the sample to direct sunlight to change the all−trans Lycopene into cis−Lycopene form, this method is not advisable, as a result of the sample might be damaged by the rays radiated from the sun light.

The alternative and scientific method to change all trans−Lycopene into cis−Lycopene is; prepare 0.025% solution of iodine in hexane mix it with a single droplet on the sample. Since iodine has a reactive behavior, the sample will immediately change inti cis−Lycopene form within a couple of minutes.

Processing raw tomatoes using heat (in the making of tomato juice, tomato paste or ketchup, for example) actually changes the Lycopene in the raw product into a form that is easier for the body to use. In other words Lycopene is better absorbed by the body when it is consumed in cooked tomato products, rather than fresh tomatoes. In one study, heat processing released up to 2.5 times the Lycopene from tomatoes, making it more available and absorbable in the body.

Chapter 5

Conclusion and Recommendation

This study was undertaken to determine Lycopene content extracted from raw tomato samples from Ambo Timer Agro Industry and Genesis Farms; and from canned tomato pastes Merti and Meysu. On the basis of investigation made by Uv-Vis Spectroscopy it can be concluded that under present experimental conditions:

- Uv-Vis Spectroscopy showed absorption peaks at 448.8 nm, 473.6 nm and 504 nm.
- Absorbance was linear function of concentration which indicates the validity of Beer-Lambers law.
- Lycopene extracted from raw tomata had better amount than that of found in other studies [**34**].
- The refractive index was found excellent precision to the actual data.

In Ethiopian context very little documentation is available on lycopene and its importance in preventing diseases. The view of present work is a step forward to at least analyze the lycopene content in different samples to assess the effect of environmental conditions like temperature, humidity, rainfall patterns etc.

Raw tomato samples were collected from Ambo Timer Agro Industry (Ambo town) had a better content of lycopene than that of Genesis Farms (Debre Zeit town). Whereas, the imported tomato paste (Meysu from Turkey) is found to have a greater content of lycopene than its equivalent paste (Merti from Ethiopia). Therefore, financiers who want to invest their asset on the production of tomato (raw, paste, juice, etc) are advised to choose Ambo rather than Debre Zeit in order to cultivate and/or produce Lycopene-rich tomato; however it requires further study to discover which place of Ethiopia is convenient for Lycopene-rich tomatos production. It is proposed that soil analysis and WDXRF spectroscopy should be done to find the elemental composition of Tomato samples from different parts of Ethiopia.

As stated in chapter 4, the author of this thesis could not get the chance to carry out the experiment on pure/standard sample of lycopene. Due to this reason it was not possible to compare and contrast the chemical properties of lycopene in pure and recovred sample. However, we tried to judge our results against the experimental result performed by researchers who have already had the opportunity to execute experiment on pure sample.

Regardless of its limitation and narrow scope of the study; this research work is a pioneer (To our best of knowledge it is believed that no research has been conducted on the topic of Lycopene at national level). Therefore, this thesis will break new ground for researchers in Ethiopia to study further characteristics phenomenon of lycopene.

Nevertheless, the terminology of Isomerization of Lycopene was emphasized in last subtopic of chapter 4; it calls for advance exploration and research to provide brief and detail analysis. For instance, besides the findings of the amount of lycopene either in raw tomato or tomato pastes, the percentage content of all isomers of lycopene (β-carotene, all-trans and cis-series) could be measured individually from the lycopene itself.

Furthermore, the accommodation of lycopene is not bounded into tomatoes and tomato-processed products. Watermelon, Carrot, Papaya, Guava and the rest of reddish, yellowish and pinkish colored fruits and vegetables could contain lycopene. Therefore, researchers are recommended to investigate the content and other characteristics of lycopene in different vegetables and fruits mentioned above.

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Appendix

Addis Ababa University School of Graduate Studies Physics Department Laser Spectroscopy Specialization

Sample Questionnaire for DCLT Project

Determining the Concentration of Lycopene in Tomato [DCLT] (for the partial fulfillment of thesis at AAU)

- To identify which place of Ethiopia is best to grows Lycopene-rich tomato.
- To implement progressed technological practices by then, reduce workloads for employees and allow more time for other activities, and be more profitable.
- To initiate and encourage host and/or abroad investment for enhanced capacity and skill of agro-industry products, and provide qualified products for customers.

$\Longrightarrow$ Information given by your organization will be utilized for the purpose of the DCLT project of the partial fulfillment of thesis.

$\Longrightarrow$ Please be honest and truth full when you fill this form in a clear and neat ways. This will help us to run as well as move forward for the development of the DCLT and other Tomato based project.

$\Longrightarrow$ The following Person will be available to offer further information if you need clarification.

$\Longrightarrow$ Fill the following 18 Sample Questionnaire and then send it back after you have completed it to the responsible person that has assigned for the DCLT survey assessment.

1. Name of the School: AAU SGS Physics Department
2. Full Address of the school: Addis Ababa, Ethiopia
   - Tell (Office): 0111223931
   - Mobile No: 0911680924
   - Fax No: 0462250996
   - P.O. Box: 184
   - E- Mail Address: tselat2005@yahoo.com
   - Web Address : www.aau.edu.et
3. Name of dedicated person to fill this form _____
   - E-mail Address: _____
   - Phone No: _____
   - Job Position and Title: _____
4. Do you have plant science expert or a stake holder professional whom assess, manage and control the farm?

   YES NO
5. If your answer is YES on question # 4, would you please give us the contact address of the professional (Expert) in space provided below?

It will provide us further information on tomato based movement; plan and other tomato based information of the company which will be helpful for the assessment.

_____
_____.

a. Name: _____
b. Position or Job Title: _____
c. Phone No: _____
d. Office No: _____
e. E-Mail Address: _____

6. If your answer is NO on question #4, what measure is taken at the moment when problem arise? Please provide us your Reason for it.

   _____
   _____.

7. Does the company use irrigation?
   YES NO

8. If your answer is YES on ques. # 7 what is the source of water for irrigation?
   RIVER LAKE GROUND WATER

9. If your answer is YES on ques. # 7, would you please specify the name of the source? _____

10. Does your company provide processed tomato products?
    YES NO

11. Does the company certified with seed production?
    YES NO

12. If your answer is NO on question #11, please specify from where the company gets the seeds?

_____

If YES, by what means does it produce?

_____

13. How many showrooms does the company have?

_____

14. What type of pesticide used to protect tomato from field disease and insect damage?

_____

15. What kind of fertilizers considered growing tomato?

_____

16. How many times in a year does tomato supply to the market?

_____

17. At what distance does the seeds planted (spacing between two consecutive seeds)?

_____

18. Is there any other tomato based System technology is established for the company usage?

    If there is, List the types of system that have been established or Implemented as well as that are under construction / Establishment / or In progress?

_____

_____

_____.