DETERMINATION OF CAFFEINE CONCENTRATIONS OF ETHIOPIAN EXPORT STANDARD COFFEE SAMPLES AND THE INVESTIGATION OF OPTICAL AND QUANTUM MECHANICAL TRANSITIONAL PROPERTIES OF CAFFEINE MOLECULE BY UV/VIS-ABSORPTION SPECTROSCOPY

BY:

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This is to certify the thesis prepared by **ALENE SEYOUM MITIKU** entitled of ‘‘Determination of caffeine concentrations of Ethiopian export standard coffee samples and the investigation of Optical and Quantum mechanical transition properties of caffeine molecule by UV/Vis- absorption spectroscopy’’ submitted in partial fulfillment of the requirements for the degree of **Master of Science in Physics.** Compiles with the regulations of the university and meets the accepted standards with respect to originality.

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DECLARATION

This thesis is my original work, the results reported in this work were obtained by research carried out by me under the supervision of my Advisors in the College of Natural Sciences, Department of Physics, Addis Ababa University.

This thesis has not been presented for a degree in any other university and that all sources of materials used for the thesis have been duly acknowledged. No part of this work shall be published in scientific journals or reported in the media or presented at a conference without the knowledge and consent of my advisors, who are the principal scientists responsible for any publication. Furthermore if the work is published the institutional address given should be that of the Physics Department, AAU.

Name of author: Alene Seyoum Signature_____ Date: 12 June 12, 2017

This thesis has been submitted for examination with my approval as university advisor.

Prof. A.V.P Gholap Signature _____ Date : 12 June 12, 2017

Date of submission June 12, 2017

DEDICATION

This Work is dedicated to:

My Father: Seyoum Mitiku

And

My mother: Belaynesh Alemayehu

STATEMENT OF THE AUTHOR

Alene Seyoum

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ACRONYMS

Abrivations	Deffinations
UV/Vis	Ultra violate-visible
BLL	Beer Lambert's Law
IAT	Integrated absorption technique
DCM	Dichloromethane
CNS	Central nervous system
TLC	Thin Layer chromatography
SPE-HPLC	Solid phase extraction-High performance liquid chromatography
ATR	Attenuated total refraction
FT-IR	Fourier transform infrared radiation
NIR	Near infrared radiation
RS	Radiation source
MC	Monochromator
PD	Photo detector
BS	Beam splitter
DA	Difference amplifier
RO	Readout

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Abstract

In this thesis, determination of caffeine concentration in aqueous solution of medium roasted export quality coffee bean samples performed by using UV/Vis absorption spectroscopy. Using UV/Vis absorption spectroscopy the molar decadic absorption coefficients and transitional dipole moment of pure caffeine in distilled water and DCM were obtained at 273 and 276 nm. The molar decadic absorption coefficients of caffeine in water and dichloromethane at these wavelengths were 1125.035 and 376.06m^2mol^{-1}, respectively. The calculated values for the transitional dipole moment of caffeine in distilled water and in DCM were 6.70 x $10^{-30}$and 11.63 x 10^{-30}C m for the given concentration in wave number range of 2,800,000 to 4,100,000m^{-1} respectively. After characterizing caffeine in water and DCM, a simple, rapid, cost-effective, and environmentally friendly UV/Vis absorption spectroscopy method was developed that enables to quantify the concentration of caffeine in coffee beans. Caffeine extracted with dichloromethane (DCM) from aqueous solution of coffee in distilled water to get UV/Vis absorption spectrum. The inference matrix was eliminated by fitting Gaussian function to the spectrum band of the experiment. From absorption spectrum optical & quantum transition mechanical properties such as; integrated absorption coefficient, integrated absorption cross-section, oscillator strength, dipole moment, and Einstein B coefficient as well as number density of caffeine molecule were calculated in DCM and distilled water. Optical & quantum transition mechanical property shows us the intrinsic ability of molecules to absorb light and these were proportional to the intensity of transition. The concentration of caffeine in medium roasted Ethiopian export standard coffee samples expressed in percentage ranged from 1.34% to 2.51% by BLL and by IAT. These finding will help Ethiopian coffee storage & liquoring board, Ethiopian standard agency, and Farming & agricultural ministry of Ethiopia for specifying caffeine concentration in each coffee samples.

The highest caffeine concentration in this work was found in Teppi & washed Sidama coffee samples and the lowest caffeine concentration was found in Limu coffee sample. The caffeine concentration for Ethiopian export standard roasted coffee in this work was in a good agreement with other researchers reported on roasted Arabica coffee samples by the same method.

Key words: - Export standard, optical transition, caffeine, concentration, absorbtion spectroscopy, Einstein B coefficient

Chapter 1

1.Introduction

Backgrounds related to coffee growing and compounds are presented. The chemical and physical properties of the compounds, the physiological and psychological effects in biological systems, and their roles in determining the quality of coffee beans are discussed. Moreover, the different physical and chemical methods to analyze these compounds in coffee beans and their interaction with aromatic compounds and metal ions are reviewed.

1.1. Background

Coffee plant is believed to be discovered during the 6th century in Ethiopia around KAFFA, the name coffee is derived from the southwestern massive highlands of Ethiopia, KAFFA region. Arabs introduced coffee to Yemenis in 13th century. Latter it developed as a habit of drink around 15thcentury. This habit spread all over the world and popular coffee houses became favorite meeting places for both Social and Economic purposes starting from mid 17thCentury. Now coffee is primary Agricultural commodity next to Oil as a source of foreign exchange for developing countries and it is the first source of foreign exchange for Ethiopia. In Africa Coffee tree is indigenous and can still be found growing wild in the hills of Ethiopia. Today coffee is cultivated over four continents and more than 80 countries and becomes the base for their economy. The biggest coffee producing countries are Brazil, Colombia, Indonesia, Vietnam and Mexico. The coffee tree is a tropical evergreen shrub (genus coffee) and two beans are generally contained in each fruit, which when ripe resembles a red cheery [1, 2, 3].

The two most commercial important species of Coffee are Robusta and Coffee Arabica. Coffee Arabica is native to the southwestern highlands of Ethiopia. It is the most cultivated coffee species throughout the world. About 90% of the world's coffee production is coffee Arabica and 10% is Robusta. Coffee Arabica grown at higher altitude requires less rain and its beans have lower caffeine content than that of Robusta. It grows mainly in Central and Southern America, East Africa, India. It is the most important foreign currency earner for more than 80 developing countries. It is the only species occurring in Ethiopia and Robusta coffee is widely grown in Western and central Africa, Malaysia, Brazil, and India[4, 5, 6]. It originated in the humid lowland forest of tropical Africa, which stretches from Guinea to Uganda and Angola. It has stronger flavor than Arabica with a full body and a woody after test, which is useful in creating blends and especially useful in instant coffee. It is grown at lower altitude[7].

Ethiopia is the 3rd largest producer of coffee in Africa and the 6th from the world next to Uganda and Ivory Coast[8]. The agricultural based economy of Ethiopian is highly dependent on Arabica .c, as it contributes more than 60 % of the country's foreign exchange earnings. There are four types of production system of coffee in Ethiopia: forest coffee, semi forest coffee, garden coffee and plantation coffee. Forest coffee is found in south and south- western Ethiopia Zones. It

accounts 10 % of Ethiopia's total coffee production. Semi - forest coffee production system is also found in the south and south - western parts of the country. It accounts 35 % of Ethiopia's total coffee productions. Garden coffee is grown in the vicinity of farmers' residences, mainly in the southern and eastern parts of the country. It accounts for about 35 % of Ethiopia's total coffee production. Plantation coffee includes that is grown in plantations owned by the former state and some well managed smallholder coffee farms. The former state plantation accounts about 5 % of total production and well-managed smallholder coffee farms account 15 % of the Ethiopia's total production[1, 9].

These Four types of production system of coffee in Ethiopia are collected and exported to other countries which seek Ethiopian coffee because of its quality production. Ethiopian coffee export association collects coffee from all over the country depending on the coffee standard given by Ethiopian coffee board and liquoring center in the case of category, origin and grade. These standards are used to identify the type of coffee for collection purpose. The type of export standard coffees of Ethiopia in category are classified as washed and unwashed; by origin (Yirgacheffe, Sidama, Limmu, Bebeka, Teppi, Harer, Lekempti and Djimmah) and by grade for each unwashed category; grade 1,2,3,4,5,& under grade(UG) and for each washed category; grade 1, grade 2 & under grade (UG)). Ethiopia has five types of coffee in Washed category viz. Yirgacheffe, Sidama, Limmu, Bebeka, & Teppi and four types in Unwashed coffee category which are (Harer, Sidama, Lekempti, & Djimmah). In this work, we did the determination of caffeine concentration in Ethiopian export standard coffee samples. Coffee is a brewed drink prepared from roasted coffee beans, which are the seeds of berries from the Coffee plant. Coffee is a widely and extensively consumed as recreational beverage. It is a worldwide favorite. Everybody everywhere at all times enjoys a fresh cup of coffee. Coffee is a big deal in almost all regions of Ethiopia. In most places of the country, it is culturally prepared and served one up to three times a day. Coffee is served in special occasions such as birth, marriage, ceremonies, burials, and holidays.

Fig1.1:- Image of the coffee plant, green coffee beans, roasted coffee and cup of coffee

Coffee is chemically composed of over 1000 compounds such as; caffeine, minerals, trigonelline lipids, total chlorogenic, aliphatic acids, oligosaccharin, total polysaccharides proteins and hommic acid. The chemical composition of green coffee depends on the species and variety.

Other factors such as agricultural practices, degree of maturation and storage conditions determines its chemical composition to a lesser extent. Caffeine is the most commonly found alkaloid in coffee beans that belongs to a class of organic compounds called methylxanthines. Caffeine or 1,3,7-trimethylxanthine is a xanthine molecule with methyl groups replacing all of the three hydrogen's bound to nitrogen in xanthine. In pure form, caffeine is a crystalline white powder moderately soluble in water and a wide range of organic solvent such as ethanol, ethyl acetate methanol, benzene, dichloromethane. This powdered form of caffeine is actually the scientific definition of bitter, that is why many beverages containing caffeine also contains copious amounts of sugar or other sweeteners. Its molecular weight is 194.19 g/mol. with chemical formula $C_8H_{10}N_4O_2$, melting point is 236^0C, which sublimes at 178^0C at atmospheric pressure. The advantage of drinking coffee on human depends on concentration of caffeine consumed. Consumption of high concentration of this compound causes various physiological and psychological problems. Caffeine acts as a mild psychoactive stimulant drug, works by stimulating the central nervous system (CNS), heart, muscles, and the centers that control blood pressure, may raise blood pressure However, moderate consumption is advantageous due to its anti-oxidant property. It is also used as a painkiller for preventing and treating headaches, after epidural anesthesia. It acts as a "water pill" that increases urine flow, for treating migraine headaches, for asthma, gallbladder disease, shortness of breath in newborns, and low blood pressure, for weight loss and type 2 diabetes, gastric acid secretion and dieresis Bolton, Caffeine creams are used to the skin to reduce redness and itching in dermatitis.

As it has an advantage, caffeine may actually harm voice quality, aggravates heart disease, carcinogenesis, kidney malfunction and asthma.

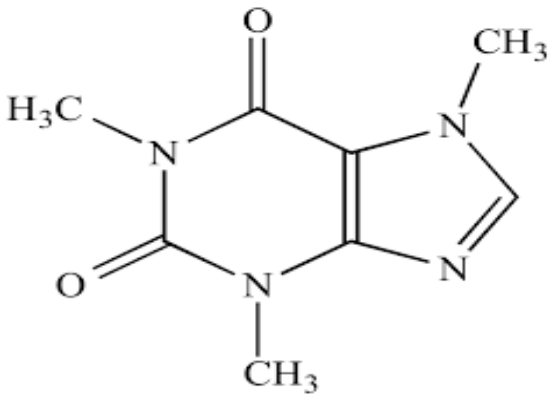

Figure 1.2:- The Chemical Structure of Caffeine

Ethiopian Coffee graded in export standard with the objective of producing the best quality and there by securing the best price possible. However, there is no universal grading system. Each producing country has their own national standard which fulfills the minimum export quality requirement suggested by the market. Most probably the flavor and quality of coffee depends on the coffee beans selected, the roasting process, the brewing method and the fineness of the

ground coffee used. In Ethiopia, coffee grading is conducted through the combination of two methods. They are green coffee analysis and cup tests (liquoring). Green coffee analysis involves visual inspection of physical characteristics of coffee bean. This includes screen analysis which makes size assessment, defect count, appearance or color test and shape that usually refers to the structure of beans. Cup test is based on roasted ground coffee analysis (chemical process) by which aroma, acidity, and other flavor components are tested. From the overall grading methods of coffee, green analysis accounts 40% and cup test accounts 60% in the quality inspection processes. In light of these, the most economical and faster system which saves time and more accurate chemical analysis of caffeine concentration in coffee beans is also used as an additional tool for evaluating coffee quality by reducing observer effects of biases pertaining to the quality standard that enhances the commercial needs[4,9].

1.2.Literature Review

Caffeine is determined in coffee samples mostly with the use of HPLC [10, 11]. Caffeine was also determined using Thin Layer Chromatography (TLC), Solid Phase Extraction and High Performance Liquid Chromatography (SPE-HPLC) [12, 13], Capillary Zone Electrophoresis (CZE), Fourier Transform Infrared (FT-IR) and electrochemical methods have been reported[14, 15, 16]. Although these methods are used widely for caffeine determination, the methods require very costly instrumentation, higher skilled technician, complicated, and time consuming procedures. The use of attenuated total reflection (ATR) accessories in conjunction with Fourier transform infrared (FT-IR) spectrometers provides for the non-destructive measurement of samples and mid-infrared approaches have a huge potential for gaining rapid information about the chemical composition and related properties of coffee[17]. In addition to its ability for effectively quantifying and characterizing caffeine content of coffee in aqueous solution, it is also able to measure multiple chemical constituents simultaneously avoiding extensive sample preparation.

On the other hand, the derivative spectrophotometer is relatively easy[18]; however, it is not reliable for the determination of small concentration of caffeine in samples. With HPLC methods, the use of expensive equipments and demand of more operator awareness for application in small industrial laboratories results to perform few analyses in each day. Other methods such as FT infrared, Raman spectroscopy and NIR reflectance spectrometry are equally versatile for the measurement of caffeine and do not require expensive chemicals however, such instruments are expensive and are not available in most laboratories[19, 20, 21]. The method of UV/Vis-absorbance spectroscopy is available in most laboratories. Moreover the method, is easy fast and cheap for the determination of the caffeine contents in coffee beans [22, 23, 24, 25, 26, 27, 28].

In this thesis one of such best method UV/Vis-absorption spectroscopy used for roasted, grounded and dissolved coffee solutions. UV/Vis-absorption spectroscopy is an applied spectroscopic technique that used to study the physical system when it interacts with electromagnetic radiation (EMR). More over it is a spectroscopic technique that studies about the

Absorption, transmission and detection of light with relation to the optical properties of a material medium. The methods are characterizing pure caffeine in distilled water and dichloromethane, and based on these several techniques were developed to determine caffeine in coffee beans. These are extracting caffeine from coffee using dichloromethane solution. After caffeine is extracted from coffee using dichloromethane, still there exist some interfering substances from chlorogenic-acid related compounds. Finally, these interfering matrices eliminated by fitting the Gaussian function.

1.3. Scope of the study

The scope of this study includes the following

- ✓ Extraction of caffeine from medium roasted coffee samples
- ✓ Analyzing of spectrum for measuring absorbance of pure caffeine by varying the concentration
- ✓ Calculating the molar decadic absorption coefficient
- ✓ Finding the concentration of caffeine in roasted coffee samples
- ✓ Finding optical and quantum mechanical transition property of caffeine

1.4.Objective of the study

- In the agricultural production of export standard coffee quality of Ethiopian coffee; coffee users in all over the country, association of coffee exporters and the countries of Ethiopian coffee importer needs to know how much caffeine is found in the coffee to ensure the quality of products which was not done yet for all export standard level. One of the requirements to be a good coffee is whether it contains a reasonable and moderate quantity of caffeine or not. Therefore, quantitative determination of the amount of caffeine in roasted coffee of Ethiopian export standard coffee is necessary.

The general objective of this study is:

Determination of caffeine concentration in Ethiopian export quality coffee samples. Investigation of optical and quantum mechanical transition properties of caffeine molecule by UV/Vis-absorption spectroscopy.

The specific objective of this study is to:

- Develop simple, rapid, cost-effective, and environmentally friendly UV/Vis-absorption spectroscopy method for the quantitative determination of caffeine concentration in medium roasted coffee samples, which are prepared in export quality
- Investigate the optical & quantum mechanical transition property of caffeine molecules.
- Compare the results of this work with results reported by literatures.

1.5. Statement of the problem

Like many other foods, the composition of coffee that we eat and drink are complex. However, coffee contains a very wide range of macro and micronutrients and as one of the most popular beverages consumed worldwide it has nutritional contribution to our diet. Many compounds in coffee are often thought to have implications upon human health. These include caffeine, micronutrients, chlorogenic acid and other bioactive components. In this study, caffeine has been studied as a compound of interest due to the reason that consumed daily in our real life through different beverages.

1.6. Significance of the study

The aim of this study is to produce a quick and cost effective method for the routine analysis of caffeine in coffee beans. Developing a system based on UV/Vis absorption spectroscopy to assess the coffee quality and quantity parameters would bring economical benefits to the coffee industry by increasing consumer's confidence in the quality products. Since there is no report in literature the amount of caffeine directly determined in aqueous solution of coffee beans by using spectroscopic techniques for example in the UV-Vis absorption spectroscopy is difficult to quantify directly in aqueous solution of coffee beans owing to matrix effect[23].

In this research work, water and DCM have been used as a solvent for caffeine determination in roasted coffee beans using optical methods. DCM and water used for coffee extraction are environmentally friendly, decrease cost, toxicity and time. Due to this reason, it is desirable to develop methods of caffeine determination in aqueous solution, which is similar with the actual caffeine intake through coffee beverages.

The thesis is organized as follows. In **Chapter 1,** Backgrounds related to coffee growing and compounds are presented. The chemical and physical properties of the compounds, the physiological and psychological effects in biological systems, and their roles in determining the quality of coffee beans are discussed. Moreover, the different physical and chemical methods to analyze these compounds in coffee beans and their interaction with aromatic compounds and metal ions are reviewed. **In Chapter2**, The derivation of electromagnetic radiation from Maxwell's equations and the quantum mechanical derivation that is often used for qualitative and quantitative understanding how transitions are induced in molecular system when it interacts with electromagnetic radiation are presented. The Schrodinger's equation is introduced and simple problems to illustrate its relation to quantities that are important in UV-Vis absorption spectroscopy are solved.

In Chapter 3, The materials and methods used in this work are presented. This chapter has two sections. the first section of this chapter deals with the description of various chemicals, samples and instruments used to carry out this research and the second section of this chapter deals with

the methods of measuring the optical & quantum mechanical transition properties of as well as the mathematical and experimental procedures used to analyze these compounds in coffee beans are presented. **In Chapter 4**, The results and discussion of the thesis are presented. The calculated values of molar decadic absorption coefficients, the optical and quantum mechanical transition properties (transitional dipole moment, oscillator strength, integrated absorption cross-section and Einstein B coefficient) as well as number density of caffeine are reported. In addition, the caffeine concentration measured in coffee beans both in Beer-Lambert's law and integrated absorption coefficients techniques are presented using UV/Vis absorption spectroscopic method. Finally, conclusion is given in Chapter 5.

Chapter 2

2. Theory

The derivation of electromagnetic radiation from Maxwell's equations and the quantum mechanical derivation that is often used for qualitative and quantitative understanding how transitions are induced in molecular system when it interacts with electromagnetic radiation are presented. The Schrodinger's equation is introduced and simple problems to illustrate its relation to quantities that are important in UV-Vis absorption spectroscopy are solved.

2.1. Spectroscopy

Spectroscopy is the study of how light interacts with matter. Spectroscopy is an experimental charting of the energy level structure of physical systems for the purpose of transition processes, spontaneous or induced between different energy states are studied, and it is normally a means of analysis for various types of electromagnetic radiation or particle emission[17, 28, 38]. Spectroscopy is a tool for studying the structure, dynamics of molecules & for exploring the micro world of atoms and molecules. The structure of atoms and molecules are studied based on spectroscopic investigations[38].

2.2. Band of Spectrum

Molecules produce band spectrum and are the groups of lines, which are closely spaced to one another. These spectra are characteristics of molecular gases or chemical compounds. For molecules, the UV/Vis absorption usually occurs over a wide range of wavelengths because molecules have many excited modes of vibration and rotation at room temperature. In fact the vibration of molecules cannot be completely frozen out even at absolute zero. Consequently, molecules have their members in many states of vibration and rotational excitation. The energy levels for these states are quite closely spaced, corresponding to energy differences considerably smaller than those electronic levels. The rotational and vibration levels are thus super-imposed on the electronic levels. A molecule may therefore undergo electronic and vibration or rotational excitation simultaneously. Because there are so many possible transitions that one differing from others by only a slight amount & each band spectrum consists of a vast number of lines very spaced to each other closely at the sharp edge but spaced at the end. What observed from these types of combined transitions is the UV/Vis-absorption spectrum of a molecule usually consists of a broad band of absorption[23, 28].

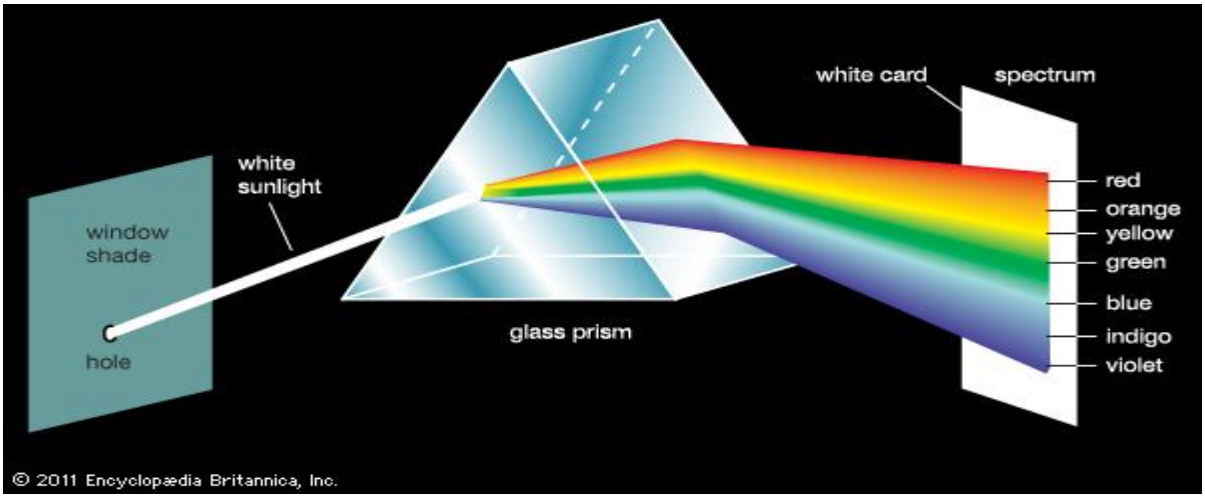

Fig 2.1: - Diagram of spectrum band

2.3. Nature of Molecular Absorption

When continuous radiation passes through a transparent material, a portion of the radiation may be absorbed. If that occurs the residual radiation when it passed through a prism, yields a spectrum with gaps in it, called an absorption spectrum. Because of absorption of energy, atoms or molecules pass from a state of lower energy to a state of higher energy. The absorbed electromagnetic radiation has excited energy state and ground energy states. In the case of ultraviolet and visible Spectroscopy, the transition results absorption of electromagnetic radiation in region of the spectrum are transitions between electronic energy levels. As a molecule absorbs energy, an electron was promoted from an occupied orbital to an unoccupied orbital of higher potential energy. Molecular absorption is more complex than atomic absorption because many more potential transitions exist. When a molecule interacts with photons in UV/Vis region, the absorption of energy results in displacing an outer electron of the molecule and the quantum energy hv equals the energy difference between the two energy levels at resonant condition as a result the atom gains a quantum of energy.

2.4. Transition Dipole Moment

The Transition dipole moment is the electric dipole moment associated with the transition between the two states. It is complex vector quantity include the phase factors associated with the two states. Its direction gives the polarization of the transition, which determines how the system will interact with an electromagnetic wave of a given polarization and while the square of the magnitude gives the strength of the interaction due to the distribution of charge within the system.

The transition dipole moment for the $2 \longrightarrow 1$ is given by the relevant off-diagonal element of the dipole matrix, which can be calculated from an integral taken over the product of the wave functions of the initial and final states of the transition,

$$\mu_{12} = -e \int \psi_1^*(r)(\vec{r})\Psi_2(r)d^3r \quad = \left\langle \Psi_1 \middle| (\mu) \middle| \Psi_n \right\rangle \tag{2.1}$$

where $\Psi_1$and Ψ_2 are the wave functions of states 1 and 2, respectively, μ is dipole moment operator, μ_{21} is molecular transition of dipole moment, e is elementary electron charge and $\vec{r}$ is the position vector.

The rate of transition probability that any molecule will go to excited sate 2 because of absorption under the perturbing effect of the electric field related to transition dipole moment by the following equation

$$\frac{d}{d\tau}\left(C_2(t)C_2^*(t)\right) = \pi\frac{\rho(\nu)}{3\varepsilon_0 h^2}\left|\mu_{12}\right|^2 \tag{2.2}$$

Where C(t) are the coefficient of time dependent probability amplitudes of the two molecular states of 1 and 2, $C^*(t)$ is the conjugate part, $\rho(\nu)$ is the incident energy density of the sample at frequency ν of electromagnetic radiation per volume, h is Plank's constant and ε_0 is permeability constant of vacuum.

In order to compare the theoretical expressions with the experimentally measurable quantities consider the following. If $\frac{d}{d\tau}\left(C_2(t)C_2^*(t)\right)$, is the rate of probability for a single molecule to change as a result of absorption of radiation under perturbing effect of electric field radiation then, $\frac{d}{d\tau}\left(C_2(t)C_2^*(t)\right)$Ndl is the number of molecules excited in a layer dl with energy absorption. Therefore, the loss in intensity becomes[30, 31]

$$-dI = \frac{d}{dt}\left(C_2(t)C_2^*(t)\right)NhV_{12}dl \tag{2.3}$$

Further, the loss in intensity of light when light passes through material whose concentration is the ratio of number density with number of Avogadro, $C = \frac{N}{N_a}$, and molar decadic absorption coefficient, ε given by

$$-dI = c\rho\ln(10)\varepsilon(\nu)\frac{N}{N_a}dl \quad = 2.303c\rho\varepsilon(\nu)\frac{N}{N_a}dl \tag{2.4}$$

From comparison of equations (2.3), and (2.4) we can express rate of probability as follows,

$$\frac{d}{dt}\left(C_2(t)C_2^*(t)\right) = \frac{2.303\varepsilon(\nu)c\rho}{N_a hV} \tag{2.5}$$

If the energy density assumed to be constant throughout the bands, the total rate of probability for the entire absorption band obtained by integrating over the entire frequency range. The total intensity of the band are obtained by measuring ε in the region of absorption usually determined by integrating the area under the graph[31, 32, 33].

So the integrated area due to transition for purely theoretical expression of transition dipole moment related to experimentally measurable quantities of molar decadic absorption coefficient is given by the following equation [32, 33].

$$I_A = \int \frac{\varepsilon(\nu)}{\nu} d\nu = S\frac{|\mu_{12}|^2}{3} \quad (2.6)$$

Where, ($S = 2.9352 \times 10^{60} C^{-2} mol^{-1}$ the cross-section of the incident beam) and N_a is number of Avogadro 6.022 x 10^{23}/mol, h is Plank's constant 6.27 x10^{-34}Js & ħ is 1.055 x 10^{-34}Js, I_A is integrated area & μ_{12} transition dipole moment and ε molar decadic absorption coefficient. eq (2.7) relates experimentally measured molar decadic absorption coefficient with the quantum mechanical expression of the transition dipole moment. The transition dipole moment is a vector that depends on both ground state and excited states of wave function and couples the transition to the electric field of light. This transition dipole moment was expressed from eq (2.6) as

$$\mu_{12} = \sqrt{\frac{3}{S} I_A} \quad (2.7)$$

2.5. The Einstein relation and Einstein B Coefficient

The stimulated emission of radiation in a two-level atomic system, characterized by the wave function Ψ_1 of the lower level and Ψ_2 of the upper level for an electric dipole transition is determine by the dipole matrix element

$\mu_{21} = -\int \Psi_2^* er \Psi_1 dV$, where dV is volume element. (2.8)

We are looking for relations between the Einstein coefficients in the interaction of a two-level atomic system when radiation occurs via absorption, stimulated and spontaneous emission. By describing the three processes of rate equations that correspond to differential equations of first order, we can describe Einstein B coefficient as

$\mathbf{B} = \frac{\mu_{21}^2}{6\varepsilon_0 \hbar^2}$, Where μ_{21} is molecular transition of dipole moment (2.9)

where, B is Einstein coefficient of stimulated emission and equal to Einstein coefficient of absorption

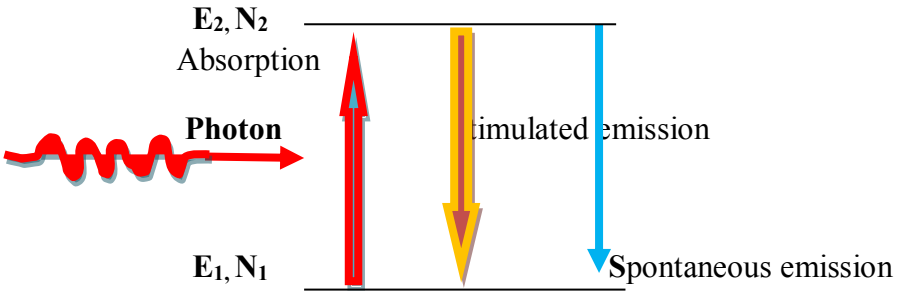

Fig 2.2:- Absorption, stimulated emission, and spontaneous emission where N_1 and E_1 are number of population & energy density of the ground level and N_2 & E_2 are number of population & energy density of the excited level.

2.6. Optical and Quantum mechanical transition property of molecule

Optical & quantum mechanical transition property is an optical absorption and emission that occurs through the interaction of optical radiation with electrons in a material system that defines the energy levels of the electrons. Depending on the properties of a given material, electrons that interact with optical radiation can be either those bound to individual atoms or those residing in the energy-band structures of a material such as a semiconductor. The absorption or emission of a photon by an electron is associated with a resonant transition of the electron between a lower energy level |1> of energy $E_1$and an upper energy level |2> of energy E_2.

In this thesis since the optical & quantum mechanical transition properties of the molecules are important to characterize an electron transition and to interpret the absorption spectra, we see the properties of electrons interacting with optical radiation which residing in the energy-band of caffeine molecules. The optical & quantum mechanical transition properties (oscillator strength, dipole moment, integrated absorption coefficient, integrated absorption cross-section and peak absorption cross-section) calculated in different solvents are the intrinsic ability of molecules to absorb light and they are proportional to the intensity of transition. Experimental determinations of the optical &quantum mechanical transition probabilities are important for direct applications to absorption, emission and dispersion radiation.

2.7. Beer-Lambert's Law (BLL)

The BLL allows us to measure the absorbance of a particular sample and to deduce the concentration of the solution from that measurement. We can measure the concentration of a particular chemical species in a solution as long as we know the species absorbs light of a particular wavelength. In optics, BLL relates the absorption of light to the properties of materials through which the light travels. Firstly, BLL considered the changes in the intensity of radiation passing through the absorbing material of the system. Further, Beer modifies the change in the intensity of radiation also depend on the concentration of the absorbing material. Here law states that there is a logarithmic dependence between the transmission T of light through substance, the absorption coefficient of molecule (α_λ), and the distance of light travel through the material (the path length l). The absorption coefficient can in turn be written as a product of either a molar decadic absorption coefficient (**ε**) of the absorber, path length (l) and the concentration (C) of the absorbing species in material or the absorption cross-section (σ_λ), path length and number density N of the absorbers as eq (2.9) and (2.10).

For liquids and solutions, these relations usually written as

$$T = \frac{I_t}{I_0} = e^{-\alpha_t l} = e^{\varepsilon C l} = 10^{-\alpha_t l} = 10^{\varepsilon C l} \qquad (2.10)$$

Where, I_0 , I_t, α_t, l, ε and C are the intensities of the incident light, intensity of the transmitted light, absorption coefficient, path length of the substance, molar decadic absorption coefficient, and concentration of the substance respectively. The transmission for liquid substances expressed

in terms of absorbance as follows and Absorbance is a direct measure of how much light absorbed by our sample.

$A = \log\left(\frac{I_0}{I_t}\right) = \log\left(\frac{1}{T}\right) = \log\left(e^{\varepsilon Cl}\right)$, since it is easier to use logarithm to the base ten rather than natural logarithm as:

$$A = \log\left(\frac{I_0}{I_t}\right) = \log\left(10^{\varepsilon Cl}\right) \qquad (2.11)$$

$A = \varepsilon Cl$

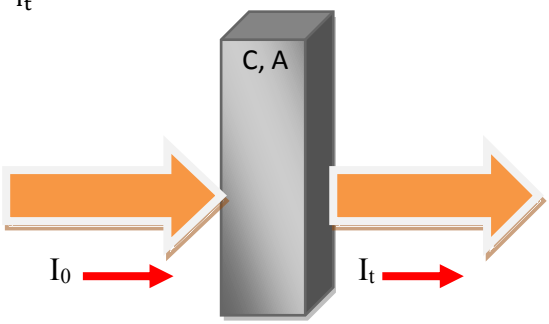

Fig 2.3:- Absorbance, incident and transition of light for a given caffeine concentration in cuvette quartz cell.

An important extension of Beer's law is the "law of additivity" which states that the absorption of the radiation by one species will be unaffected by the presence of other materials, whether they absorb or not.

$A = \sum_i^n \varepsilon_i C_i l$, where i = 1, 2, 3 , ... n (2.12)

From equation (2.10), absorption coefficient can be expressed as

$$\alpha_{(\lambda)} = \frac{1}{l}\log\left(\frac{I_0}{I_t}\right) \qquad (2.13)$$

The absorption cross-section $\sigma_{(\lambda)}$ is related to the absorption coefficient $\alpha_{(\lambda)}$ at a single frequency for N number of molecules per unit volume expressed by the following relation[34,35,36].

$$\alpha_{(\lambda)} = \sigma_{(\lambda)}N \qquad (2.14)$$

However, in a UV/Vis-absorption spectroscopy, the absorption of molecules in a liquid occurs over a certain range of frequencies rather than at a single frequency. Therefore, absorption coefficient measured at any single frequency may not express the true intensity of the molecular transition in BLL. Due to this limitation of BLL, we can use another technique.

2.8. Integrated Absorption Technique (IAT)

IAT is the preferable technique of finding integrated absorption coefficient, which is the sum of absorption coefficients for all frequencies in the band of spectrum. In such cases, the technique is useful for different applications since it is independent of the line function, which may vary by parameters like pressure, temperature, concentration of the solute and solute-solvent interaction.

In addition, the technique is very important in the absence of a high-resolution spectroscopy. Therefore, in liquids and solutions where the above effects observed, the true integrated absorption intensity of a band should be defined by the following equations:

$$\alpha_t = \int \alpha d\nu \qquad (2.14)$$

By using equation (2.10), we can rewrite the integrated absorption coefficient (α_t) as

$$\alpha_t = \frac{1}{l} \int \log\left(\frac{I_0}{I_t}\right) d\nu \quad \text{and} \qquad (2.15)$$

$$\sigma_{(t)} = \frac{1}{Nl} \int \log\left(\frac{I_0}{I_t}\right) d\nu = \frac{1}{N} \int \alpha d\nu \qquad (2.16)$$

Where $\sigma_{(t)}$ is integrated absorption cross-section, **N** is number density of the molecule and **l** is path length

On the other hand, oscillator strength was considered as the other useful parameter providing the intensity of transition. It expresses the relative strength of electron transition. Oscillator strength is one of the most fundamental quantities in analytical absorption spectroscopy. In practice, it determines the sensitivity of a given atomic resonance line and needs to be accurately known if one needs to relate the magnitude of the absorption signal to its concentration. Oscillator strength can be determined directly through absolute emission, absorption or dispersion measurement. Oscillator strength is related to the molar decadic absorption coefficient by the following equation

$$f = 4.32 \times 10^{-9} \text{mol cm L}^{-1} \int \varepsilon(\nu) d\nu = 4.32 \times 10^{-10} \text{mol m}^{-1} \int \varepsilon(\nu) d\nu \qquad (2.17)$$

Measurements of emission, absorption and dispersion intensities of the molecules give their number density and oscillator strength. Absorption and dispersion measurement involves the number density of the lower level of the transition, and emission measurement involves that of the upper level. An equation relating integrated absorption coefficient with number density and the oscillator strength given by

$$\int \alpha(\nu) d\nu = 2.65 \times 10^{-6} Nf \qquad (2.18)$$

Where N is number density in molecules m^{-3}, $\alpha(\nu)$ in $m^{-2}$and ν in m^{-1}, and f is dimensionless oscillator strength of the transition molecule. From equation (2.18), we can express the number density of caffeine molecule as follows;

$$N = f\, 10^6 \frac{1}{2.65} \int \alpha(\nu) d\nu = 3.774 \times 10^5 \int \alpha(\nu) d\nu \qquad (2.19)$$

Chapter 3

3. Materials and methods

The materials and methods used in this work are presented. This chapter has two sections. the first section of this chapter deals with the description of various chemicals, samples and instruments used to carry out this research and the second section of this chapter deals with the methods of measuring the optical & quantum mechanical transition properties of as well as the mathematical and experimental procedures used to analyze these compounds in coffee beans are presented.

3.1. Material

3.1.1. Chemicals and samples

Commercially bought Chemical Dichloromethane (Aldrich, Germany) and distilled water (Dallul pharmaceuticals plc, Ethiopia) were used. For standard solution preparation, caffeine sample that was (Evan, England) bought from the local market.

Arabica Coffee samples were obtained from Ethiopia coffee & Tea development and marketing authority for coffee quality inspection and storage board, Ethiopia Four of these were unwashed (Harer, Sidama, Nekempti, & Djimmah) and other five were washed (Yirgacheffe, Sidama, Limmu, Bebeka, & Teppi). All samples were export quality. These coffee samples collected from the association of coffee exporters and farmers in Ethiopia and stored at the center. The coffee samples used for this research medium roasted level at 215^{o}c for 15 minute. Then the roasted coffee beans were grounded and sieved with micro sieve.

3.1.2. Instrumentation

The laboratory instruments used for the experiment are the following. Carbolite Oven (Bamford,sheffield®, ENGLAND,S302AU) to roast coffee, Beakers, measuring cylinders, pipettes, volumetric flasks, spatula, magnetic stirrer with hot plate, funnel, separatory funnel, glass filter, filter paper, 1cm quartz cuvette and 300µm sieve are some of the apparatus used. All glassware thoroughly cleaned, rinsed with distilled water and dried before use. For measuring the mass of caffeine and coffee samples digital electro balance with maximum measuring of 0.5g were used. For electronic absorption measurement of standard solutions and coffee samples a double monochromator UV-Vis-NIR spectrophotometer, Perkin Elmer Lambda 19 (PerkinElmer, D-7770 Ueberlingen, Germany) with wavelength ranges of 170-3200 nm was used. The instrument operated by a powerful software package termed UVCSS. It provides a wide range of operating mode for the instrument and it also includes comprehensive data handling and file management capabilities. Scanning speed of UV-Vis-NIR spectrophotometer was 266.75 nm per min and slit width 2nm were used during spectral data acquisition. The instrument is a PC-driven

spectrometer. The spectrum was interfaced with computer, which was operated by origin®2015 software.

Fig 3.1:- Photograph of some experimental instruments used in UV/Vis absorption spectroscopy measurement of caffeine.

3.1.3. Components of UV/Vis- spectroscopy

The basic components of double beam spectroscopy are light sources, monochromator, focusing devices, sample cell compartment. The sources are a deuterium lamp, which covers ultraviolet (UV) range and tungsten-halogen lamp for visible (VIS) and near infrared (NIR) ranges[39]. The monochromator isolate radiant energy of desired wavelength by dispersing the beam in to its components and placing the light path in a slit that only passes through a narrow wavelength band. The focusing devices are a combination of lenses, slits, and mirrors inserted in to light path to render the light rays parallel or to isolate narrow portion of the light beam or its spectrum. The detectors are photosensitive materials, mostly a photomultiplier tube for UV and VIS ranges. When light strikes the PMT, it generates electron that passes through a series of dynode that amplify the signal several times.

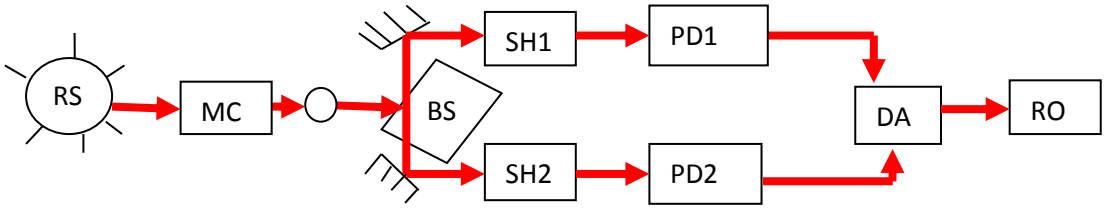

Fig 3.2:- Schematic diagram of double beam UV/Vis absorption spectroscopy.

In the UV/Vis spectrophotometer, there are two types of radiation sources from the entire ultraviolet to near IR region[31]. For the UV region, the radiation source is Deuterium discharge lamp that emits polychromatic UV- radiation, which can filtered into monochromatic UV - radiation. For the visible region, the radiation source should change to a tungsten filament similar to the ones found in a common incandescent light bulb. The monochromator or wavelength selector disperses the light from radiation source into separate wavelength. The wavelength selector consists of an entrance slit, collimating lenses, dispersing device a focusing lens and an

exit slit. A radiation of only a particular wavelength leaves the monochromator through an exit slit. The monochromatic light that emerges from exit slit is pulsed by a chopper and split into sample and reference beams by the beam splitter. A reference beam passes through a sample holder or a quartz cuvette that contains only a solvent. The sample beam passes through a sample holder or a quartz cuvette that contains a sample solution. The radiation beams that pass through the detectors is amplified by different amplifiers, and finally reaches the recorder (RO), where the results are recorded digitally, in a personal computer attached to a spectrophotometer.

3.1.4. UV/Vis cut-off

Every solvent has a UV/Vis absorbance cut-off wavelength. The solvents cut-off is the wavelength below which the solvent itself absorbs the entire incident light. So when choosing a solvent we should be aware of its absorbance cut-off that tells us where the compound under investigation is to be absorbing. With this procedure, we can get the free spectral range of our compound caffeine in the two solvents. In this experiment, the solvents that we used are distilled water and dichloromethane (DCM). The UV cut-off for DCM is 233nm and for water is 190nm as (Burdick & Jackson *https://www.researchgate.net/post/What_is_the_UV_cut-off*). However, in our finding, the UV/Vis cut-off for DCM is 228nm and for distilled water is 196nm.

3.1.5. Preparation of standard solutions

For standard solution preparation, commercially bought caffeine mass of 0.0045g dissolved in distilled water and in DCM. The volume was raised to 50ml. The solutions were stirred for 30min using magnetic stirrer. We prepare the working solution from the above prepared stock solution with a series of one millimeter (1ml, 2ml, 3ml, 4ml, 5ml and 6ml) and dissolved again each working solution until the volume of each working solution became to 25ml with DCM. The concentration for working solution of caffeine in DCM calculated as follows and these were listed in appendix A.

$$\text{Concentration(C)} = \frac{\text{Given mass of caffeine (g)}}{\text{Molar mass of caffeine } \left(\frac{\text{g}}{\text{mole}}\right) \text{ x Volume of solution (L)}} \qquad (3.1)$$

Given mass of caffeine = 0.0045g

Molar mass of caffeine = 194.19g/mol

Volume of solution = 50ml = 0.05liter

C =?

$$C = \frac{0.0045\text{g}}{194.19\frac{\text{g}}{\text{mol}} \text{x} 0.05\text{L}}$$

$C = 4.635 \times 10^{-4}\frac{\text{mol}}{\text{L}}$, since $1\text{L} = 0.001\text{m}^3$ then the concentration is given by

$C = \underline{463.5 \times 10^{-3} \text{ mol m}^{-3}}$ (3.2)

This was the concentration of the stock solution for caffeine standard in DCM and we calculated the working solutions with the solvent DCM which was used to lowering the concentration by applying the following equation;

$CV = C_1V_1$ (3.3)

Where C was the concentration of the stoke solution, V is volume of our concentration that we took as working solution from stoke solution, V_1 is the amount of solution we want to reach which was the sum of V and additional volume of solvent DCM that can used to lowering our concentrations.

Then we can calculate the concentration ($C_1, C_2, C_3, C_4, C_5, C_6$) of working solutions as follows;

$C_1 = CV / V_1$ (3.4)

Here C & V_1 had constant value for each calculations 463.5 x 10^{-3} mol m^{-3} and 25 ml respectively. The only change is in the value of V for each working solutions.

When we have V = 1ml

$C_1 = 463.5 \times 10^{-3} (\text{mol m}^{-3}) \times \frac{1\text{ml}}{25\text{ml}}$

$C_1 = 18.4 \times 10^{-3} \text{ mol m}^{-3}$

$C_1 = \underline{1.84 \times 10^{-2} \text{ mol m}^{-3}}$ (3.5)

By the same procedure we can calculate the concentrations of the working solution at V 2ml, 3ml, 4ml, 5ml & 6ml and these values are presented in appendix A.

Similarly, stock solution for 0.1g mass of caffeine dissolved in one liter of distilled water was stirred for 30 minutes using hotplate magnetic stirrer. Then working solution were prepared with in a series of 4 milliliter which are 1ml, 4ml, 8ml, 12ml, 16ml and 20ml dissolved in distilled water till its volume will be 30ml. The concentration for standards caffeine in distilled water was calculated as follow by using equation (3.1)

$C = \frac{0.1\text{g}}{194.19 \frac{\text{g}}{\text{mol}} \times 1\text{L}}$

$C = 5.15 \times 10^{-4} \text{mol/L}$, since $1\text{L} = 0.001\text{m}^{-3}$ then the concentration is given by

$C = \underline{515 \times 10^{-3} \text{ mol m}^{-3}}$ (3.6)

This was the concentration of the stoke solution for caffeine standard in distilled water and we calculated the concentrations of each working solutions with the solvent which was used to lowering the concentration by applying equation (3.4).

Here also C & V_1 have constant value 515 x 10^{-3}mol m^{-3} and 30 ml respectively. The only change is in the value of V.

When we have V = 1ml

C_1 = 515 x 10^{-3}mol m^{-3} x1ml/30ml

C_1 =1.762 x 10^{-2} mol m^{-3}

C_1 = $\underline{176.2 \times 10^{-4}}$ mol m^{-3} (3.7)

By the same procedure, we calculated the concentration at 4ml, 8ml, 12ml, 16ml & 20ml and these values were represented in appendix B.

After preparing the desired concentration of that standard caffeine, solutions for both solvents were stirred gently with magnetic stirrer for 30 minutes and the absorbance versus wave length of each solution was measured by UV/Vis- absorption spectroscopy at room temperature in the wavelength range of 200nm-500nm. The maximum wavelength (λ_{max}) for maximum absorbance of caffeine standards in DCM and distilled water were 276nm and 273nm respectively.

From the maximum absorbance and the corresponding concentrations of caffeine standards the molar decadic absorption coefficient was obtained in DCM and water as in appendix C.

3.1.6. Coffee sample preparation

Coffee beans from the samples are medium roasting level at 215°c for 15 minutes by Carbolite Oven (Bamford, sheffield ®, ENGLAND, S302AU). The roasted coffee was ground and the powder was sieved through 300µm sieve to get a uniform texture. An accurately weighed amount of sieved coffee 0.050g for each sample dissolved in 25 ml of distilled water. The solution stirred gently for 30 minutes using a stirrer of 80°C magnetic hot plate to remove caffeine easily from the solution. In addition, the solutions filtered by using glass filter and filter paper to get rid of particle from solution. In this case, for extraction of caffeine distilled water and DCM were used as solvents. Because, it had been observed that many interfering matrices were extracted with water than dichloromethane. The efficiency of DCM to extract caffeine from coffee beans is 98 - 99 % and caffeine is 140 mg·ml^{-1} times more soluble in DCM than it is 22 mg·m^{-1} times in water[3, 23, 26, 29, 37]. To extract caffeine from coffee, the coffee solution prepared above under coffee sample preparation should mixes with 25ml DCM. Then first, this mixture of the solution was stirred for 10 minutes for each sample. After this process by using separatory funnel, caffeine extracted with DCM from the solution and coffee with water remains as a residue. This is due to the density difference between the two solvents and caffeine is more soluble in DCM, the extracted caffeine with DCM easily separated from coffee with water solution. The extraction of caffeine repeated for three times with 25 ml dichloromethane at each round. The caffeine extracted by dichloromethane at each round was stored in one volumetric flask. Finally, the absorbance of caffeine was measured by UV/Vis-absorption spectroscopy in the wavelength

range of 200nm – 500nm against the corresponding blank of DCM. The total content of caffeine extracted from coffee samples was stored in the same container. After extraction, there is still interfering substances that eliminated by Gaussian function based on non-linear curve fitting. Coffee samples with their maximum absorbance at peak wavelength of 276nm represented in appendix C.

3.1.7. Data collection

The data for the six standard caffeine concentrations in DCM and distilled water with their measured maximum absorbance at peak wavelength of each solvent and the data for nine roasted coffee samples concentration & their measured maximum absorbance at peak wavelength of 276nm collected and listed at the Appendices.

3.2. Methods

3.2.1. Beer Lambert's Method of measuring caffeine

Beer Lambert's Law (BLL) based on peak absorbance was the most popular method to investigate the concentration of the targeted molecule in a sample. Recently, the caffeine concentration of coffee beans had been reported using UV-Vis absorption spectroscopy with BLL by extracting caffeine from coffee solution with solvent DCM. In this method, there is change in the intensity of radiation passing through the caffeine molecule & the change in intensity of radiation depends on the concentration of the absorbing material. The results obtained using BLL are satisfactory, cost-effective, timely and reproducible at room temperature for particular wavelength.

However, in a UV/Vis- absorption spectroscopy, the absorption of molecules in a liquid occurs over a certain range of frequencies rather than at a single frequency. Therefore, integrated absorption coefficient measured at any single frequency may not express the true intensity of the molecular transition in BLL. In addition, when there is no high-resolution spectroscopy since a finite slit width is used, the radiation is not monochromatic under these conditions; thus, the band determined experimentally comprises no true physical constants of the absorbing molecule but depends on the instrumental conditions employed. Due to these limitations of BLL, we can use another technique.

3.2.2. Integrated Absorption technique (IAT) of Measuring Caffeine

Measuring the intensity of absorption by the IAT provides additional information about the nature of the absorbing molecules and establishes accurate evaluation of UV-Vis absorption band intensity. Recently, an IAT becomes alternative methods instead of BBL to determine the concentration of caffeine in coffee beans optical & quantum mechanical transition property and number density of caffeine in coffee beans. It is independent of the line function, which may vary by parameters like pressure, temperature, concentration of the solute and solute-solvent interaction. In liquids and solutions, IAT used to improve the interference matrix and we can

deconvolute the overlapped spectra of caffeine to determine the area under peak of Gaussian function that fitted to each bands of spectrum for caffeine.

Therefore, in this research, IAT proposed to determine optical & quantum mechanical transition property and number density of caffeine molecules in different standards and roasted coffee beans. In IAT we calculate the area under the spectrum band for optical & quantum mechanical transition property and number density of caffeine depending on their variables. after we obtained the area deconvolution of Gaussian fit of Gaussian function for each spectrum bands to improve interference matrices related to chloregenic acid in roasted coffee of Ethiopian export standard coffee beans[34, 35, 40].

In this study, we used Origin 2015 software for data analysis of the spectra; for integrated the absorption coefficient, integrated absorption cross-section and molar decadic absorption coefficient across the entire absorption band.

3.2.3. Least Square Method

One does not accept the molar decadic absorption coefficient value determined from one known concentration; rather, several concentrations are prepared and the corresponding absorbance value plotted against concentrations. In any real experiment there will be a random errors arising from the limitation of the experiment.

Based on Beer-Lambert's equation absorbance $A = \varepsilon Cl$, it is customarily assumed that concentrations are known (i.e. more accurately than the absorbance value). The deviation of experimental values from the equation written as

$$A_i - \varepsilon C_i l = e_i \tag{3.8}$$

the deviations e_i, are squared and the sum of them for all the experimental points. It is required that this sum of the squares of the deviation be minimum. This achieved by setting the derivative with respect to the adjustable parameter, ε or εl equal to zero.

$$\frac{\partial}{\partial \varepsilon l}\sum_{i=1}^{n} e_i^2 = \frac{\partial}{\partial \varepsilon l}\sum_i (A^2_i - 2\varepsilon l C_i A_i + \varepsilon l C_i^2) = 2\sum_i (\varepsilon l C_i^2 - C_i^2 A_i^2) = 0 \tag{3.9}$$

The least square condition for molar decadic absorption is therefore,

$$\varepsilon = \frac{\sum_i^n A_i^2 C_i^2}{l \sum_i^n C_i^2} \tag{3.10}$$

Where A is absorbance, ε is the molar decadic absorption coefficient, l is a distance in the absorbing medium (the path length) and C is the concentration of the absorbing caffeine molecule. By this criterion the molar decadic absorption coefficient of caffeine (the ability of caffeine molecule to absorb light) in DCM and water was calculated.

3.2.4. Non-linear curve fitting Gaussian function

In this research, there were many interfering bands in caffeine spectrum from the other coffee components extracted by DCM and the peak of these interfering bands observed at the

wavelength below 246nm & above 312nm for roasted coffee beans. The compound responsible for these peaks is related to chlorogenic acid compounds. This interfering band had an effect on the maximum peak of caffeine. Therefore, the interference matrix was eliminated by fitting the following equation of Gaussian fit to the experimental data.

$$y = y_0 + Ae^{-2\left(\frac{x-x_c}{w}\right)^2} \quad (3.11)$$

Where y_0 represents the minimum point, A is amplitude, x_c is the central wavelength and w is full width at half maxima of the given spectrum. It was fitted by Non-linear curve fitting based on the Origen 2015 software. The four quantities of Gaussian function (y_0, A, x_c, & w) served as searching parameters in order to achieve minimum discrepancy between the experimental data and Gaussian function. Thus, the peak absorbance for calculating the concentration of caffeine obtained after subtracting the fitted Gaussian function from the total caffeine spectrum.

Chapter 4

4. Results and Discussion

The results and discussion of the thesis are presented. The calculated values of molar decadic absorption coefficients, the optical and quantum mechanical transition properties (transitional dipole moment, oscillator strength, integrated absorption cross-section and Einstein B coefficient) as well as number density of caffeine are reported. In addition, the caffeine concentration measured in coffee beans both in Beer-Lambert's law and integrated absorption coefficients techniques are presented using UV/Vis absorption spectroscopic method.

4.1. Calibration curve of pure caffeine in dichloromethane and in distilled water

The molar decadic absorption coefficient of caffeine in water and dichloromethane obtained by measuring the intensity of the absorption for a series of caffeine concentration in DCM and distilled water. Caffeine concentration of ($1.84 \times 10^{-2} - 11.04 \times 10^{-2}$) mol·m^{-3} and ($1.762 \times 10^{-2} - 34.3 \times 10^{-2}$) mol·m^{-3} was prepared in DCM and distilled water respectively for calibration curves. From the analysis of calibrations (Figure 4.1 & 4.2) are good in linear relationships ($R = 0.99993$, $R^2 = 0.99783$ & S.D = 0.01611 and $R = 0.99913$, $R^2 = 0.9998$ & S.D = 0.00463) were observed for variance concentrations of caffeine in distilled water and in DCM respectively for absorbance versus concentration of caffeine molecule, which is convenient for the determination of caffeine in coffee beans. From the analysis of calibrations, a linear dataset was obtained. Where, R = linear regreation coefficient and S.D = standard deviation of the measurement. Similarly, with peak height measurements, a linear fit with (R = 0.999) was obtained. Therefore, the methods are valid in terms of sensitivity. Over all method of repeatability was also determined by calculating the coefficient of variance (C.V) and a value ranged from (0.99 - 5.56) % were obtained. These results suggested that the proposed method is valid in terms of precision. The molar decadic absorption coefficient for caffeine molecule in distilled water at a maximum peak wavelength (λ_{max} = 273nm) was calculated from appendix B by using equation (3.10)

$$\varepsilon = \frac{A_1C_1+A_2C_2+A_3C_3+A_4C_4+A_5C_5+A_6C_6}{l(C_1^2+C_2^2+C_3^2+C_4^2+C_5^2+C_6^2)} \quad (4.1)$$

$$\underline{\varepsilon = 376.06}\ m^2\ mol^{-1} \quad (4.2)$$

It is molar decadic absorption coefficient of caffeine in distilled water.

Similarly the molar decadic absorption coefficient measuring the intensity of optical absorption for caffeine in DCM at a maximum wavelength of (λ_{max} = 276nm) can be calculated from appendix A as

$$\varepsilon = \frac{A_1C_1+A_2C_2+A_3C_3+A_4C_4+A_5C_5+A_6C_6}{l(C_1^2+C_2^2+C_3^2+C_4^2+C_5^2+C_6^2)}$$

$$\varepsilon = \underline{1228.035} m^2\ mol^{-1} \quad (4.3)$$

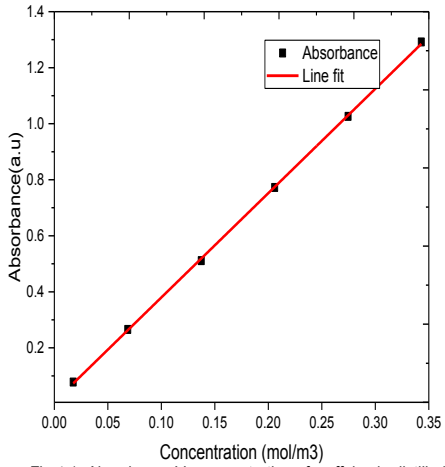

Fig 4.1;-Absorbance Vs concentration of caffeine in distilled water

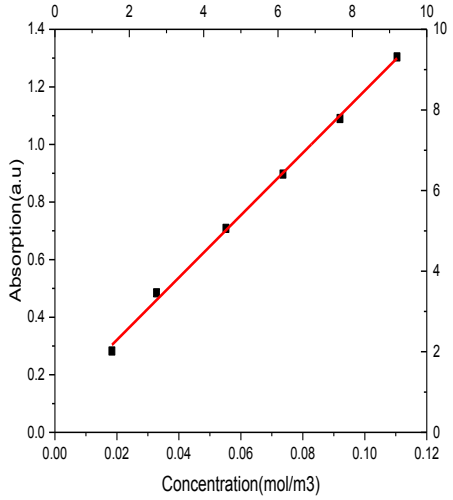

Fig 4.2:- Absorbance Vs Concentration of caffeine in DCM

4.2. Absorption versus concentration relation

To determine the concentration of caffeine in solutions of roasted coffee beans different concentrations of standard caffeine in DCM & distilled water were prepared and the maximum absorbance of the standard solutions was recorded to find molar decadic absorption coefficient at λ_{max}= 276 & 273nm respectively. In this work absorbance versus wavelength graphs of different concentrations of standard caffeine are normalized for both solvents shown in Figure 4.3(a) and 4.3(b).

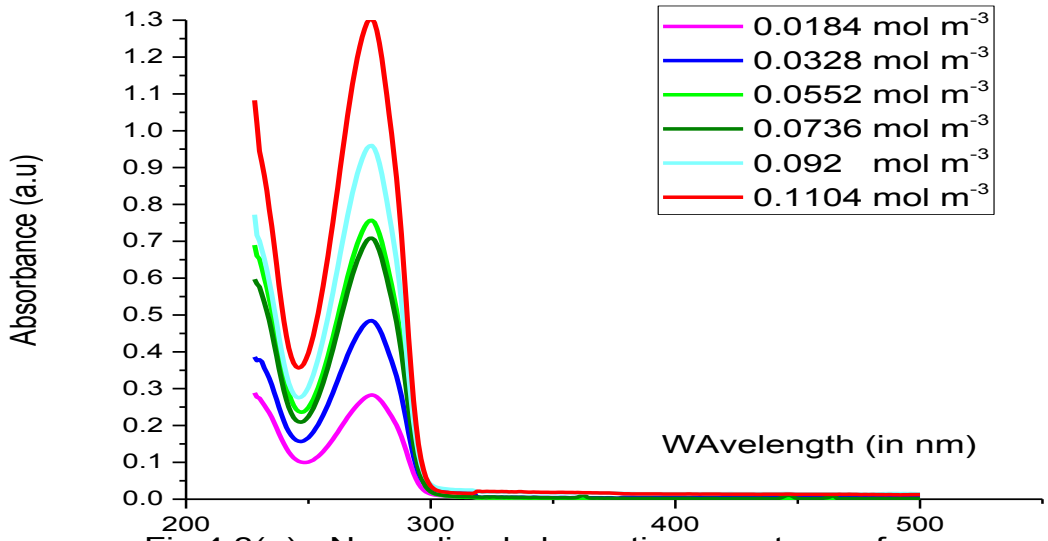

Fig 4.3(a);- Normalized absorption spectrum of pure caffeine in DCM

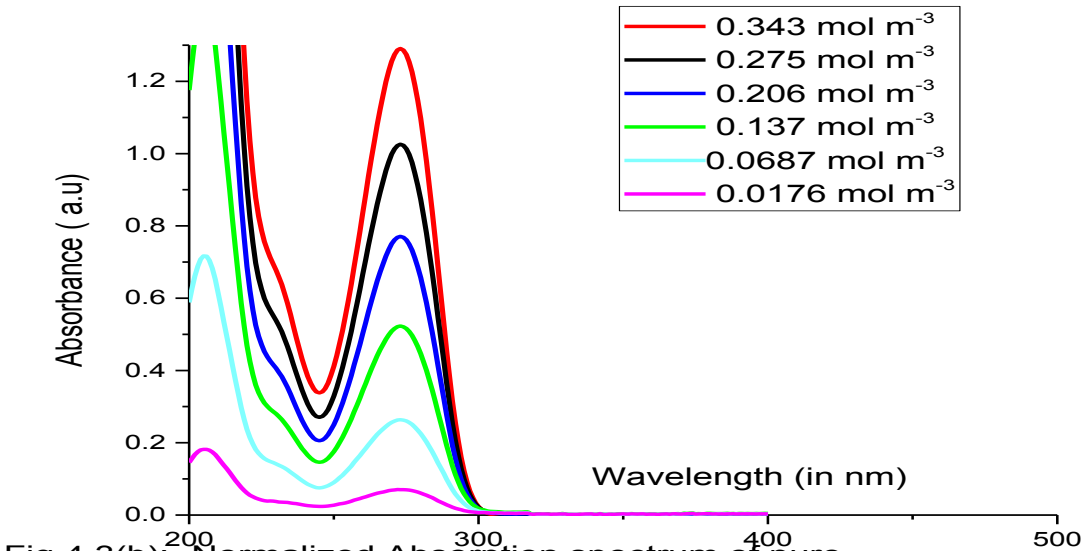

Fig 4.3(b):- Normalized Absorption spectrum of pure caffeine in distild water

4.3. UV-Vis Absorption of Caffeine

Fig 4.3(c) shows absorbance versus wavelength of caffeine in DCM has a maximum absorbance at 276 nm. The intensity of caffeine in DCM drops to zero for wavelengths greater than 312 nm and rise below this wavelength, on the other hand it has a shoulder at 246nm and a new peak absorption is noticed at a wavelength below 246nm. This new spectrum is due to the solvent interference. The maximum peak absorbance of caffeine observed in DCM at wavelength 276nm is 1.30409 for the concentration of C = 1.1 × 10^{-1}mol m^{-3}. The molar decadic absorption coefficient for the intensity of optical absorption at λ_{max}= 276 nm was calculated using Equation (4.3). This molar decadic absorption coefficient of caffeine in DCM is ε_{max} =1228.035 m^2 mol^{-1}.

Similarly, Fig 4.5 shows caffeine has peak at 273nm in distilled water. The intensity of caffeine in distilled water drop to zero for wavelength greater than 302nm and start to rise below this wavelength, more over a new peak absorbance was noticed at a wavelength below 245 nm. This new spectrum was due to the solvent interference. The maximum peak absorbance of caffeine observed in distilled water at wavelength 273nm is 1.2907 for concentration of C = 3.43 x 10^{-1}mol m^{-3}. The molar decadic absorption coefficient for the intensity of optical absorption at λ_{max}=273 nm was calculated by Equation (4.2). This molar decadic absorption coefficient value of caffeine in distilled water is ε_{max} = 376.06 m^2 mol^{-1}. From these discussions, molar decadic absorption coefficient of caffeine in DCM is greater than that of in distilled water.

The wavelength of caffeine in DCM and distilled water found in this work matches with Atomssa and Gholap et al 2011.

4.3.1. Integrated absorption coefficient

The total intensity of the band is obtained by measuring ε(ν) in the region of absorption for caffeine molecules due to transition of electromagnetic radiation in the spectrum band and usually determined by integrating the area under the graph of molar decadic absorption coefficient over wave number versus wave number. It is also an important to express transition dipole moment of caffeine molecule. So the integrated absorption coefficient due to transition can be express as eq (2.6)[30, 31, 33]

The integrated absorption coefficient I_A is calculated from the spectra of caffeine standard dissolved in DCM [Fig 4.4] by using the Origin 2015 software and the result is $I_A = 132.37227$ m^2mole^{-1} . It was obtained by integrating the molar dicadic absorption coefficient over wave number versus wave number in the wave number range of (2,800,000 – 4,100,000) m^{-1} for the concentration $C = 1.1 \times 10^{-1}$mol m^{-3}.

Similarly, the integrated absorption coefficient of caffeine in distilled water [fig 4.6] is $I_A = 43.93985 m^2mole^{-1}$ in wave number range of (2,800,000 – 4,100,000) m^{-1} for the concentration of $C = 3.43 \times 10^{-1}$mol m^{-3}.

4.3.2. Transition dipole moment (μ_{21}) of caffeine

The transition dipole moment is a vector that depends on both ground state (1) and excited state (2) of wave function and couples the transition to the electric field of light. The transition dipole moment of caffeine, which related to the molar decadic absorption coefficient was calculated by using the result of the integrated absorption coefficient of caffeine spectra with eq (2.7).

$$\mu_{21} = \sqrt{\frac{3}{S} I_A}$$

$$\mu_{21} = \sqrt{\frac{3}{2.9352} \times 132.37227 \times 10^{-60} m^2C^2}$$

$\mu_{21} = 11.6316222 \times 10^{-30}$C m (4.4)

The transitional dipole moment of caffeine in DCM $= 11.6316222 \times 10^{-30}$C m

Similarly, transition dipole moment of caffeine dissolved in water was

$\mu_{21} = 6.70 \times 10^{-30}$Cm (4.5)

4.3.3. Einstein B Coefficient

The Einstein B coefficient for standard caffeine in DCM & in distilled water can be expressed using the transition dipole moment of caffeine standards using eq (2.9), $B = \frac{\mu_{21}^2}{6\varepsilon_0 \hbar^2}$ and these are given in tables (4.1- 4.4).

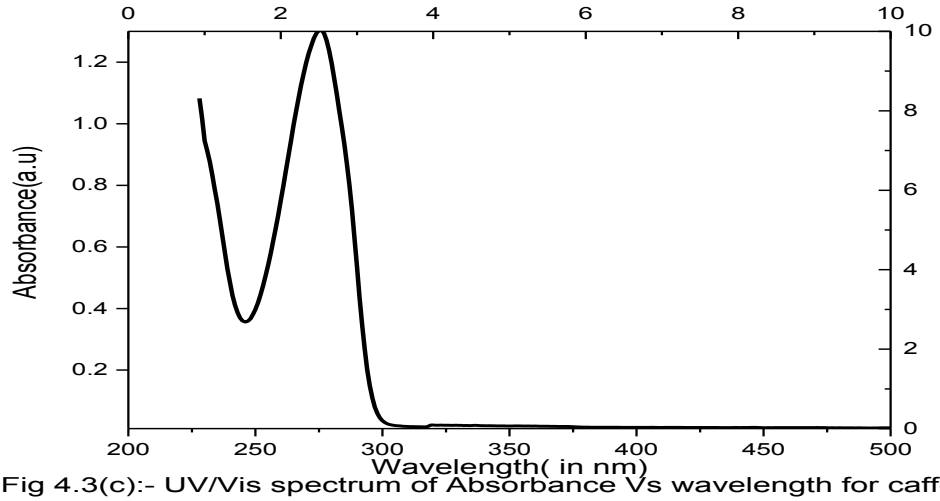

Fig 4.3(c):- UV/Vis spectrum of Absorbance Vs wavelength for caffeine in DCM

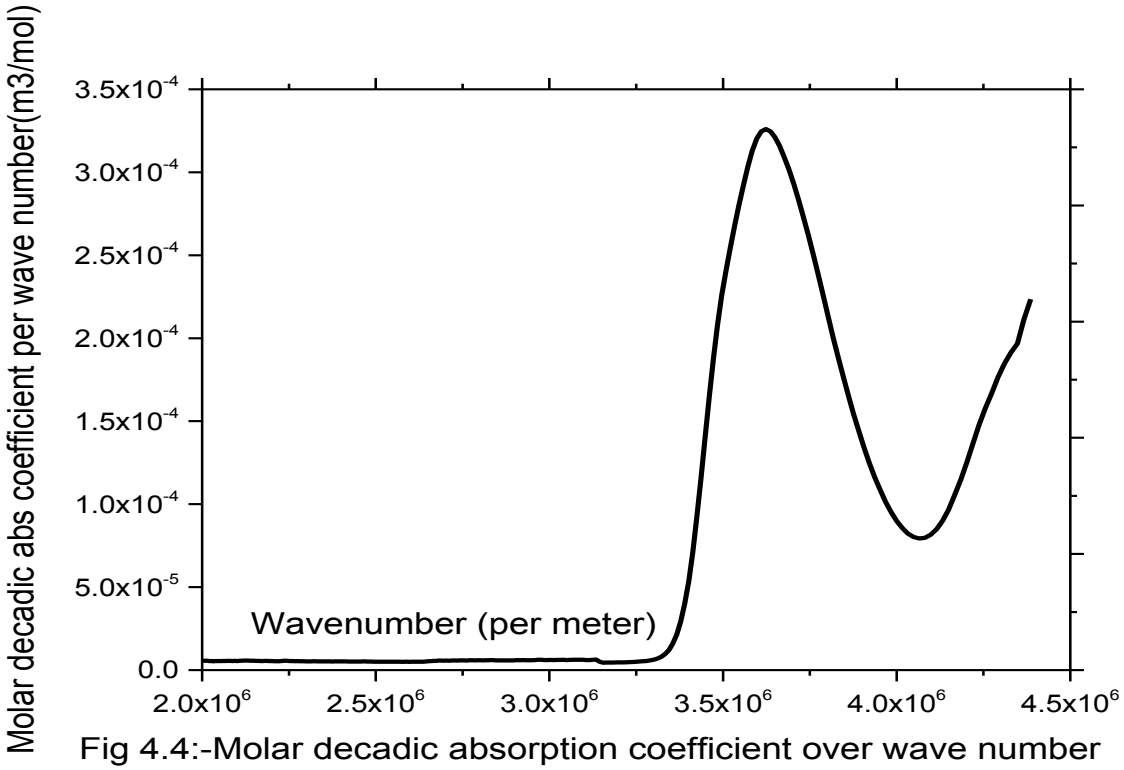

Fig 4.4:-Molar decadic absorption coefficient over wave number Vs wavenumber of caffeine in DCM

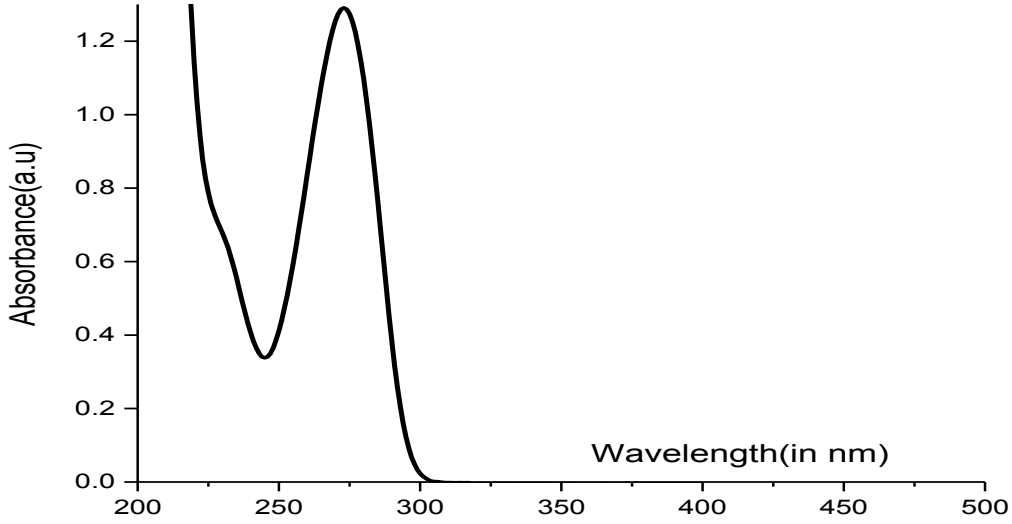

Fig 4.5:- UV/Vis spectrum of Absorbance Vs Wavelength for caffeine in water

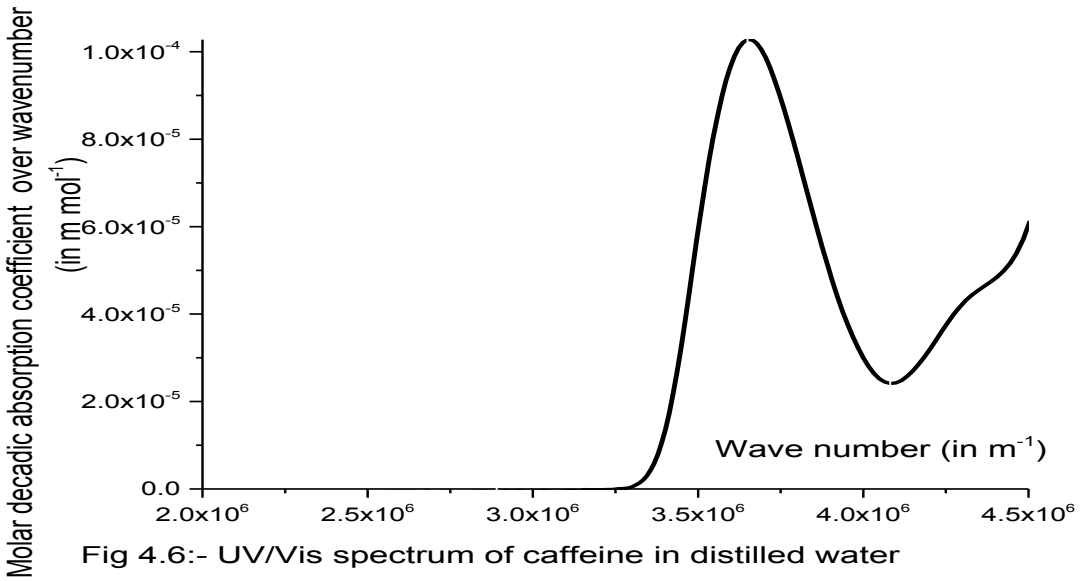

Fig 4.6:- UV/Vis spectrum of caffeine in distilled water

4.4. Optical and Quantum mechanical Transition Properties of caffeine molecule in Beer Lambert's Law (BLL)

When an electromagnetic radiation is incident on caffeine molecules, there are transitions of molecules from lower energy state to the higher energy state due to interaction of molecules with incident radiation. From UV-visible absorption spectra, by using BLL we can study the optical & quantum mechanical transition properties (integrated absorption coefficient, integrated

absorption cross-section, oscillator strength) & number density of caffeine calculated in DCM and distilled water to compare the strength of transition.

4.4.1. Integrated absorption coefficient (a_t)

In this work the integrated absorption coefficient of molecules in a liquid using UV/Vis-absorbance spectroscopy was expressed as absorption coefficient over path length [33, 34].

$$a_t = \frac{1}{l} \int \log\left(\frac{I_0}{I_t}\right) d\tilde{\nu} = \int a_{\tilde{\nu}} d\tilde{\nu} \qquad (4.6)$$

These results were calculated from the spectrum band of absorption coefficient versus wave number of caffeine standard in DCM and distilled water [Fig 4.3 & 4.5] & presented in Table 4.1 & 4.2.

4.4.2. Integrated absorption cross-section (δ_t)

The integrated absorption cross-section δ_t related to the integrated absorption coefficient a_t at a single frequency of number density N of caffeine molecule is given by [33, 34].

$$\delta_t = \frac{a_t}{N} \qquad (4.7)$$

4.4.3. Oscillator Strength (f)

Oscillator strength related to molar decadic absorption coefficient can described as[34, 35]:

$$f = 4.32 \times 10^{-10} \text{mol m}^{-1} \int \varepsilon(\tilde{\nu}) d\tilde{\nu} \qquad (4.8)$$

The oscillator strength of caffeine in DCM using equation (4.13) was obtained by integrating the spectra of molar decadic absorption coefficient versus wave number at concentration C = 1.1 x 10^{-1} mol m^{-3} of Fig 4.7 from 3,200,000 m^{-1} – 4,100,000 m^{-1} is

$$\int \varepsilon(\tilde{\nu}) d\tilde{\nu} = 4.97371 \text{x} 10^8 \text{m mol}^{-1} \qquad (4.9)$$

$f = 4.32 \text{ x } 10^{-10} \text{ mol m}^{-1} \text{ x } (4.97371 \text{ x } 10^8 \text{ m mol}^{-1})$

f = 0.214864272 is the oscillator strength of caffeine in DCM. (4.10)

Similarly, by using the spectra of Fig 4.8 the oscillator strength for caffeine in distilled water at concentration C = 3.43 x10^{-1} mol m^{-3} from wavelength range of 3,200,000m^{-1}– 4,100,000$m^{-1}$was 0.06996672.

4.4.4. Number density of caffeine molecule (N)

The number density of caffeine molecule dissolved in DCM & in distilled water calculated by using the integrated absorption coefficient and the oscillator strength of each bands of spectrum. It is given by $N = \frac{\int a_{\tilde{\nu}} d\tilde{\nu}}{f\ 2.65\ x\ 10^{-6}}$ (4.11)

The number density & oscillator strength of caffeine in DCM and in distilled water given in Table 4.1 & 4.2 respectively.

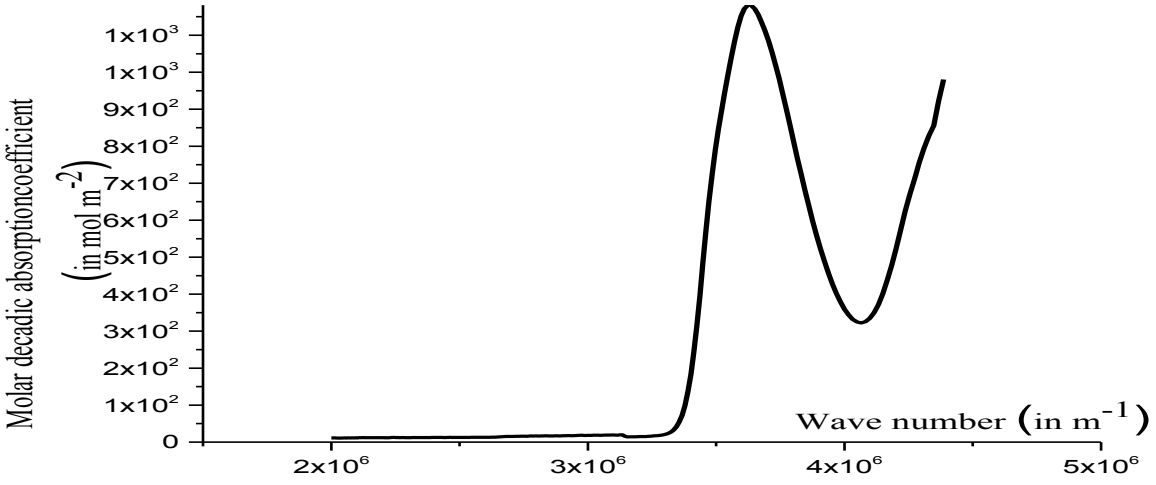

Fig 4.7:- UV/Vis spectra of standaed caffeine solution in DCM

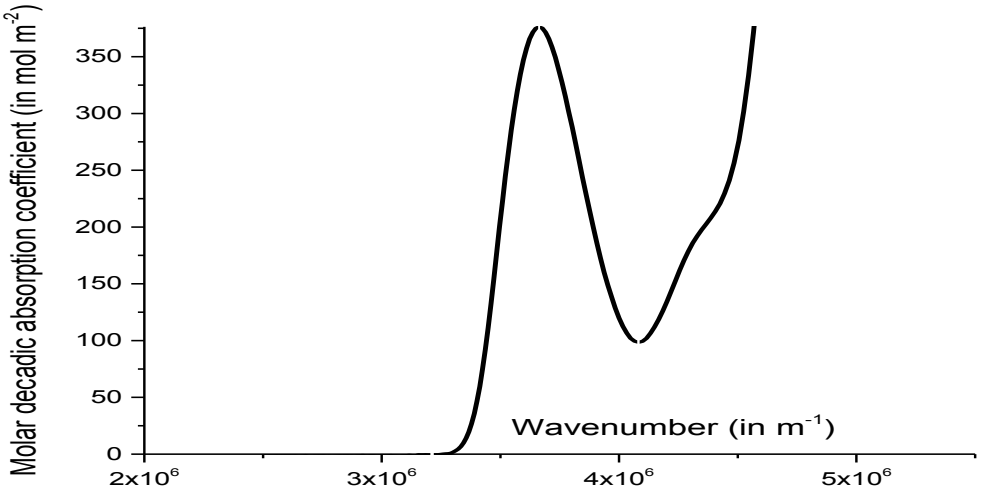

Fig 4.8;- UV/Vis spectra of caffeine solution in distilled water

The value of the above optical & quantum mechanical transition properties, number density, Einstein B coefficient and transition dipole moments of caffeine dissolved in DCM & distilled water by BLL expressed below in table 4.1 & 4.2 respectively.

Table 4.1:- Optical & quantum mechanical transition properties, number density, Einstein B coefficient and transition dipole moments of caffeine dissolved in DCM by BLL for corresponding concentration & absorbance.

Concentration (C in mol/m^{-3})	Absorbance (A in arbitrary unit)	Integrated area (I_A in m^2/mol)	Transitiondipole moment (μ_{21}in10^{-30}cm)	Einstein Bcoefficient (in S^{-1})	Integrated absorption coefficient (a_t in m^{-1})	Number density of caffeine (N in m^{-3})	Absorption cross-section (δ_t in m^2)	Integrated value of $\int \varepsilon(\tilde{\nu})d\tilde{\nu}$ (in m/mol)	Oscillator strength (f)
1.84×10^{-2}	0.28	183.37	13.69	3.17×10^{80}	1.23×10^{7}	1.61×10^{13}	0.766×10^{-6}	6.71×10^{8}	0.290
3.28×10^{-2}	0.48	173.94	13.33	3.01×10^{80}	2.11×10^{7}	2.87×10^{13}	0.737×10^{-6}	6.44×10^{8}	0.278
5.52×10^{-2}	0.71	158.16	10.71	1.94×10^{80}	3.22×10^{7}	4.82×10^{13}	0.668×10^{-6}	5.83×10^{8}	0.252
7.36×10^{-2}	0.89	110.64	10.63	1.91×10^{80}	3.01×10^{7}	6.43×10^{13}	0.470×10^{-6}	4.09×10^{8}	0.177
9.20×10^{-2}	1.09	117.12	10.94	2.03×10^{80}	4.06×10^{7}	8.04×10^{13}	0.506×10^{-6}	4.41×10^{8}	0.191
1.10×10^{-1}	1.30	132.37	11.63	2.29×10^{80}	5.49×10^{7}	9.65×10^{13}	0.570×10^{-6}	4.97×10^{8}	0.215

Table 4.2:- Optical & quantum mechanical transition properties, number density, Einstein B coefficient and transition dipole moments of caffeine dissolved in distilled water by BLL for corresponding concentration & absorbance.

Concentration (C in mol/m^{-3})	Absorbance (A in arbitrary unit)	Integrated area (I_A in m^2/mol)	Transition dipole moment (μ_{21} in 10^{-30}cm)	Einstein B coefficient (in S^{-1})	Integrated absorption coefficient (a_t in m^{-1})	Number density of caffeine (N in m^{-3})	Absorption cross-section (δ_t in m^2)	Integrated value of $\int \varepsilon(\tilde{\nu})d\tilde{\nu}$ (in m/mol)	Oscillator strength (f)
1.76×10^{-2}	0.08	49.61	7.12	8.58×10^{79}	3.21×10^{6}	1.54×10^{13}	0.208×10^{-7}	1.82×10^{8}	0.079
6.87×10^{-2}	0.27	45.65	6.83	7.89×10^{79}	1.16×10^{7}	6.00×10^{13}	0.193×10^{-6}	1.69×10^{8}	0.073
1.37×10^{-1}	0.51	44.75	6.76	7.73×10^{79}	2.27×10^{7}	11.96×10^{13}	0.190×10^{-6}	1.66×10^{8}	0.072
2.06×10^{-1}	0.77	43.87	6.70	7.59×10^{79}	3.34×10^{7}	17.98×10^{13}	0.186×10^{-6}	1.62×10^{8}	0.070
2.75×10^{-1}	1.02	43.43	6.66	7.51×10^{79}	4.41×10^{7}	24.07×10^{13}	0.183×10^{-6}	1.60×10^{8}	0.069
3.43×10^{-1}	1.29	43.94	6.70	7.59×10^{79}	5.56×10^{7}	29.95×10^{13}	0.186×10^{-6}	1.62×10^{8}	0.070

4.5. Optical and quantum mechanical Transition Properties of Caffeine by integrated absorption technique (IAT)

From UV-visible absorption spectroscopy by using IAT, we can calculate the optical & quantum mechanical transition properties (integrated absorption coefficient, integrated absorption cross-section, oscillator strength) & number density of caffeine in DCM and distilled water after deconvolution of each spectrum bands of Gaussian fit of Gaussian function to improve matrix related to chloregenic acid. Since in IAT all calculations performed on the calculated area of the

spectrum band after Gaussian fit of each bands & IAT is independent of the line function, which may vary by parameters like pressure, temperature, concentration of the solute & solute-solvent interaction. These make integrated absorption technique different from Beer Lambert's technique. Then we can calculate the optical & quantum mechanical transition properties with the same procedure as we calculated in BLL, but after deconvolution of the spectrum by Gaussian fit of Gaussian function.

For deconvoluted bands of spectrum after Gaussian fit for caffeine molecules dissolved in DCM and distilled water, integrated absorption coefficient [Fig 4.9(b) & 4.10(b)] in the wave number regions 2,500,00m^{-1} to 4,100,00m^{-1}, integrated absorption cross-section, oscillator strength [Fig 4.11 (a & b)] in the region of 3,200,000 m^{-1} – 4,100,000 m^{-1} and the number densities are given in table 4.3 & 4.4.

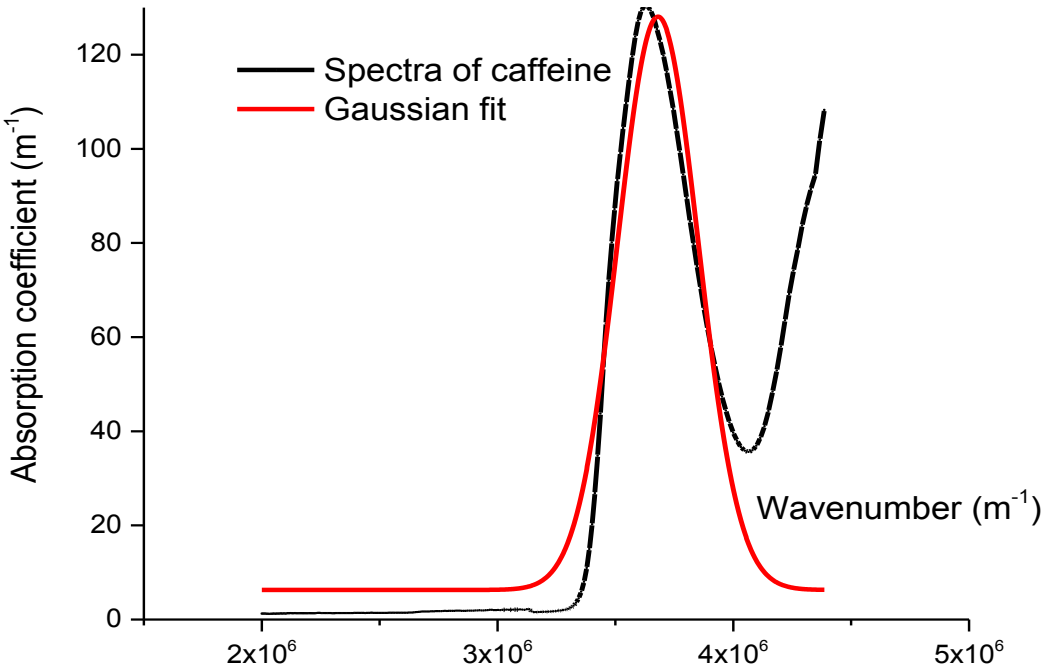

Fig 4.9(a);- Gaussian fit to the spectra of absorption coefficient Vs wavenumber of caffeine in DCM

As the standard concentration of caffeine in DCM & distilled water increases the absorbance, integrated absorption coefficient and number density are increased. On the other hand as concentration of caffeine in DCM & distilled water increases the transition dipole moment, Einstein B coefficient, integrated absorption cross-sections and oscillator strengths are decreased in both solvents of the two methods.

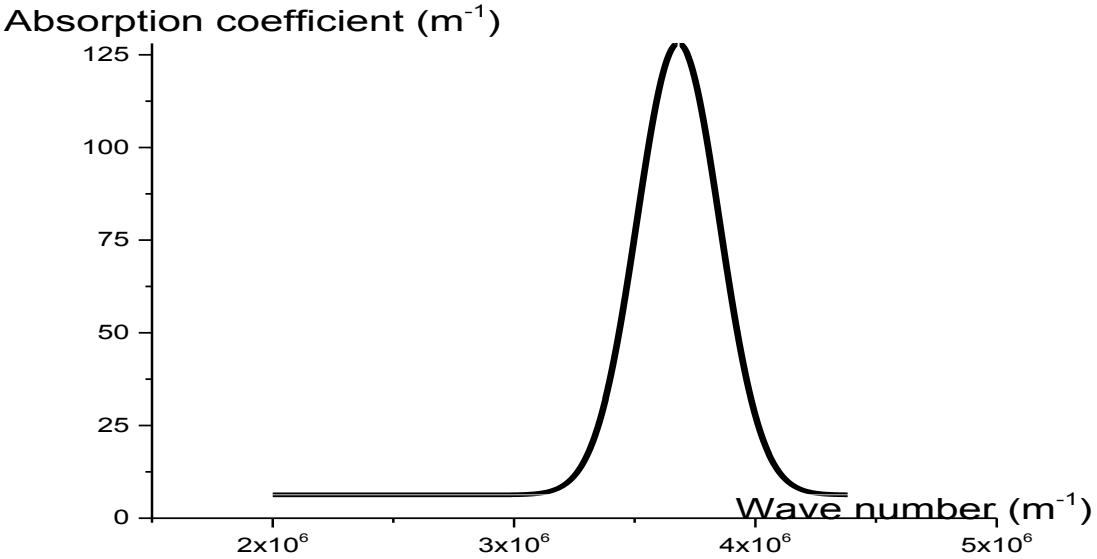

Fig 4.9(b):- Absorption coefficient versus wave number of caffeine i DCM after Gaussian fit

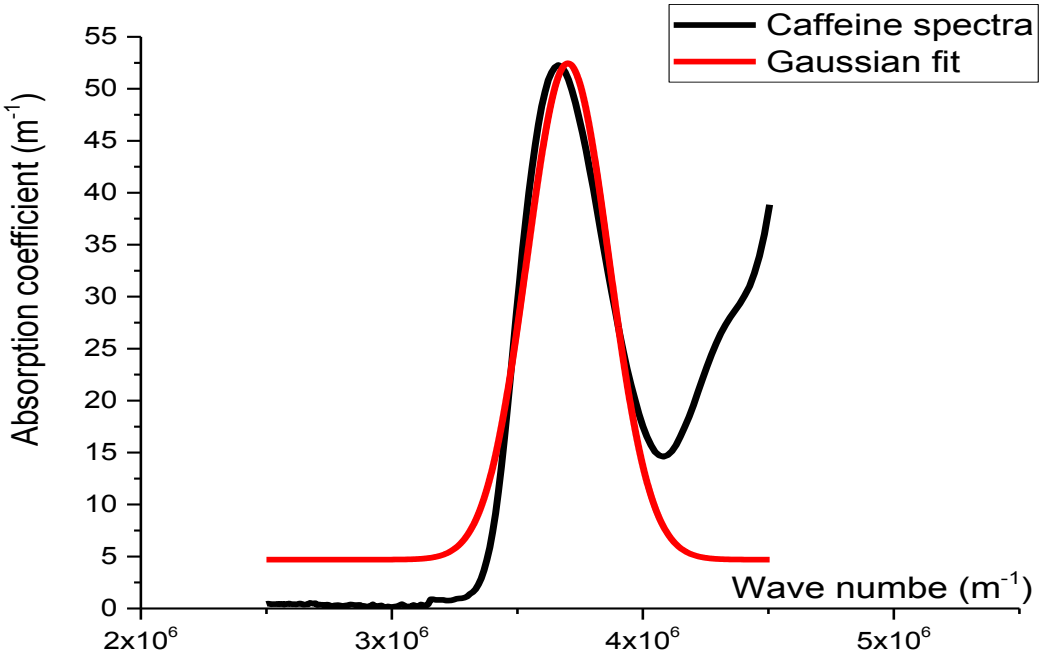

Fig 4.10(a):-the Gaussian function fited to the spectrum band of Absorption coefficient versus Wave number of caffeine in distilled water

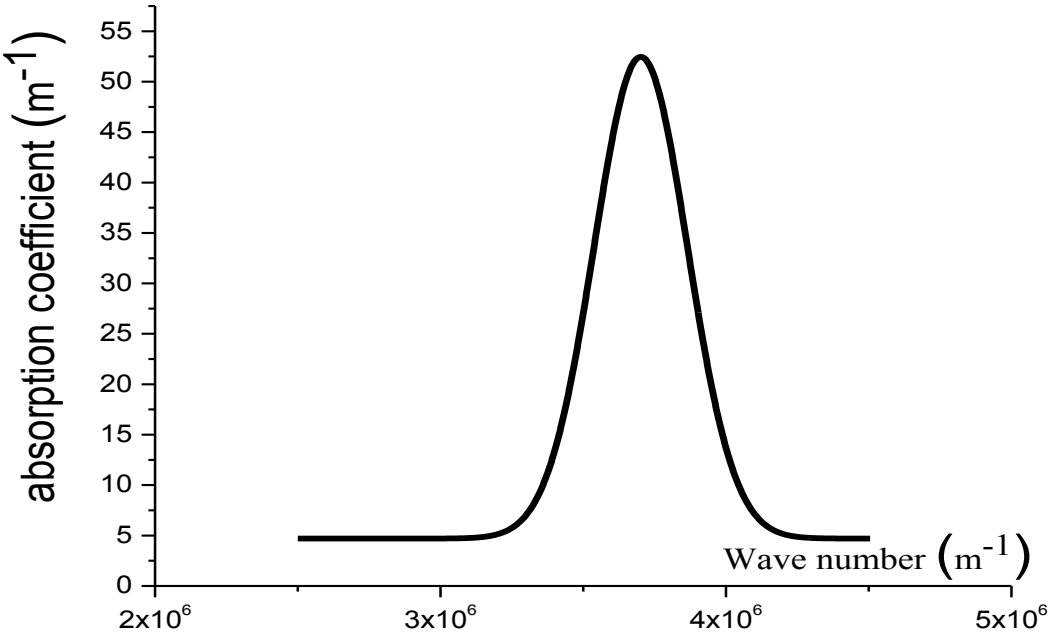

Fig 4.10(b):- After Gaussian fit of the spectrum for caffein in distilled water

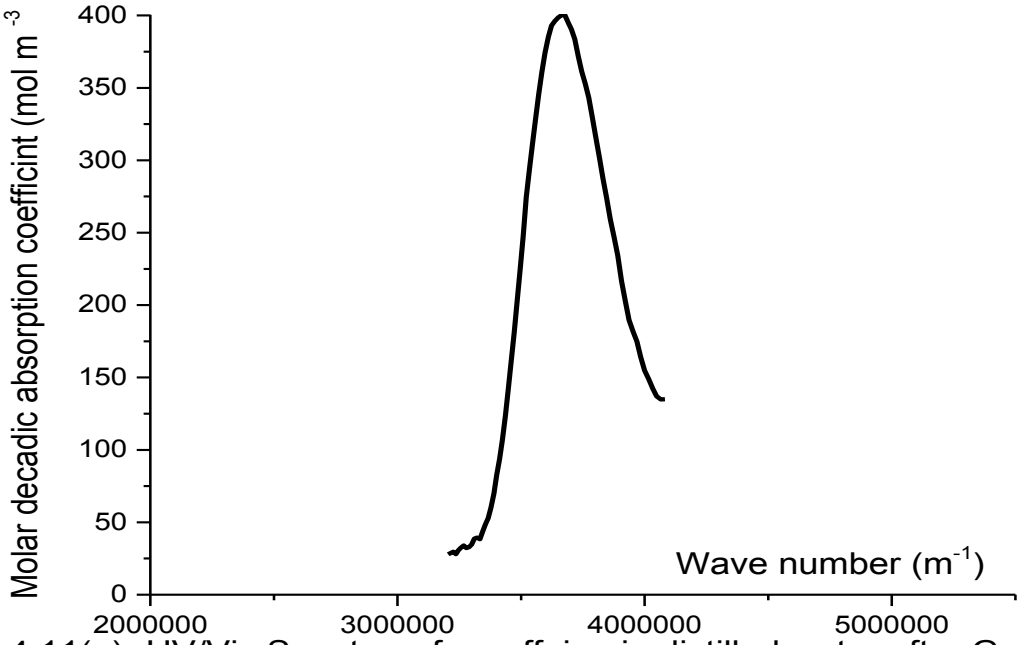

Fig 4.11(a):-UV/Vis Spectrum for caffeine in distilled water after Gaussian fitt

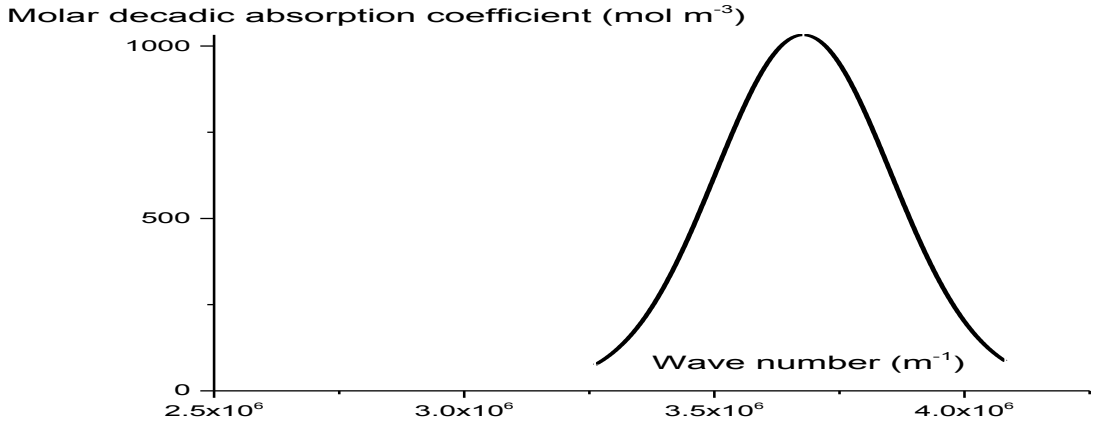

Fig 4.11(b):-UV/Vis spectrum for Caffeine in DCM after Gaussian fitt

Table 4.3:- Optical & quantum mechanical transition properties, number density, Einstein B coefficient and transition dipole moments of caffeine dissolved in DCM by IAT for corresponding concentration & absorbance.

Concentration (C in mol/m^2)	Absorbance (A in arbitrary unit)	Integrated area (I_A in m^2/mol)	Transition dipole moment (μ_{21} in 10^{-30}C m)	Einstein B coefficient (in S^{-1})	Integrated absorption coefficient (a_t in m^{-2})	Number density (N in m^{-3})	Integrated absorption cross-section (δ_t in m^1)	Integrated value of $\int \varepsilon(\tilde{\nu})d\tilde{\nu}$ (in /mol)	Oscillatorstrengh (f)(unit less)
1.84 x10^{-2}	0.28	183.37	13.69	3.17 x10^{80}	1.24 x10^{7}	1.59 x 10^{13}	0.77 x 10^{-6}	6.81x10^{8}	0.294
3.28 x10^{-2}	0.48	173.94	13.33	3.01 x10^{80}	2.12 x10^{7}	2.84 x 10^{13}	0.74 x 10^{-6}	6.51 x10^{8}	0.281
5.52 x10^{-2}	0.71	158.16	10.71	1.94 x10^{80}	3.29 x10^{7}	4.94 x 10^{13}	0.66 x 10^{-6}	5.82 x10^{8}	0.251
7.36 x10^{-2}	0.89	110.64	10.63	1.91 x10^{80}	3.03 x10^{7}	6.55 x 10^{13}	0.45 x 10^{-6}	4.04 x10^{8}	0.175
9.20 x10^{-2}	1.09	117.12	10.94	2.03 x10^{80}	4.03 x10^{7}	7.98 x 10^{13}	0.50 x 10^{-6}	4.41 x10^{8}	0.191
1.10 x10^{-1}	1.30	132.37	11.63	2.29 x10^{80}	5.47 x10^{7}	9.61 x 10^{13}	0.56 x 10^{-6}	4.97 x10^{8}	0.215

Table 4.4:- Optical & quantum mechanical transition properties, number density, Einstein B coefficient and transition dipole moments of caffeine dissolved in distilled water by IAT for corresponding concentration & absorbance.

Concentration (C in mol/m^2)	Absorbance (A in arbitrary unit)	Integrated area (I_A in m^2/mol)	Transition dipole moment (μ_{21} in 10^{-30}C m)	Einstein B coefficient (in S^{-1})	Integrated absorption coefficient (a_t in m^{-2})	Number density (N in m^{-3})	Integrated absorption cross-section (δ_t in m^1)	Integrated value of $\int \varepsilon(\tilde{\nu})d\tilde{\nu}$ (in m/mol)	Oscillator strength (f)
1.76 x10^{-2}	0.08	49.61	7.12	8.58 x10^{79}	2.68x10^{6}	1.46 x 10^{13}	0.184x10^{-6}	1.60x10^{8}	0.069
6.87 x10^{-2}	0.27	45.65	6.83	7.89 x10^{79}	1.09x10^{7}	5.88 x 10^{13}	0.185 x10^{-6}	1.62x10^{8}	0.069
1.37 x10^{-1}	0.51	44.75	6.76	7.73 x10^{79}	2.18x10^{7}	11.80 x 10^{13}	0.185 x10^{-6}	1.61x10^{8}	0.069
2.06 x10^{-1}	0.77	43.87	6.70	7.59 x10^{79}	3.25x10^{7}	17.78 x 10^{13}	0.183 x10^{-6}	1.59x10^{8}	0.069
2.75 x10^{-1}	1.02	43.43	6.66	7.51 x10^{79}	4.32x10^{7}	23.72 x 10^{13}	0.182 x10^{-6}	1.59x10^{8}	0.069
3.43 x10^{-1}	1.29	43.94	6.70	7.59 x10^{79}	5.45x10^{7}	29.63 X 10^{13}	0.184 x10^{-6}	1.61x10^{8}	0.069

4.6. Number density of caffeine in medium roasted coffee beans

The number densities of caffeine in coffee beans were calculated by fitting the Gaussian function to the spectra of absorption coefficient versus wave number of caffeine Fig 4.12(a & b) and to the spectra of molar decadic absorption coefficient versus wave number of caffeine extracted from coffee solutions Fig 4.14. From the area of Gaussian function fitted to the spectra in Fig 4.12(a & b) and Fig 4.14, the number density of caffeine was calculated in the frequency region of 2,500,000-4,100,000m^{-1} using eq (2.19). The Number density of caffeine molecule extracted from roasted coffee beans based on BLL and IAT given in table 4.5 below.

4.7. Determination of Caffeine concentration in Roasted Coffee Beans by Beer Lambert's Law (BLL) and integrated absorption technique (IAT)

In this work by UV/Vis absorption spectroscopy a direct measurement of roasted Ethiopian export standard coffee samples were impossible owing to the matrix effect of UV/Vis absorbing. This effect is clearly seen in (Fig 4.13) for spectrum band of roasted coffee beans dissolved in distilled water. To resolve the interfering substances of this work the extraction of caffeine from roasted coffee solution performed for three rounds with equal amount 25ml of DCM solutions. However, the extraction technique still could not completely remove the possible interference of caffeine spectra and it is impossible to obtain the qualitative and quantitative information from unresolved band of spectrum. The peak of this interference bands observed below wavelength of 246 nm and above 312 nm as shown in Fig 4.12(a) for caffeine extracted from roasted coffee beans. These peaks are due to the interference of solvent effect and chlorogenic acid.

To eliminate this interference Gaussian function given in Equation (3.11) was fitted to the spectrum of caffeine using non-linear curve fitting by origin 2015 software. Fig 4.12(a) shows the spectrum of caffeine extracted from roasted coffee beans by DCM and Gaussian function fitted to this spectrum for Absorbance versus wavelength overlap together and to eliminate another peaks due to interference we deconvoluted the spectrum as it shown in fig 4.12(b). After the inferences eliminated, the shape and peak of the two spectra are the same. Therefore, it can concluded that the applications of experimental and computational methods enable to determine the concentration of caffeine in roasted coffee beans using UV-Vis absorption spectroscopy of this study. By this method, the concentrations of caffeine in roasted Ethiopian export standard coffee bean samples were determined. The mean percentage of caffeine concentration in roasted coffee beans calculated for three independent measurements by Beer Lambert's Law & Integrated absorption techniques are given in Table (4.5).

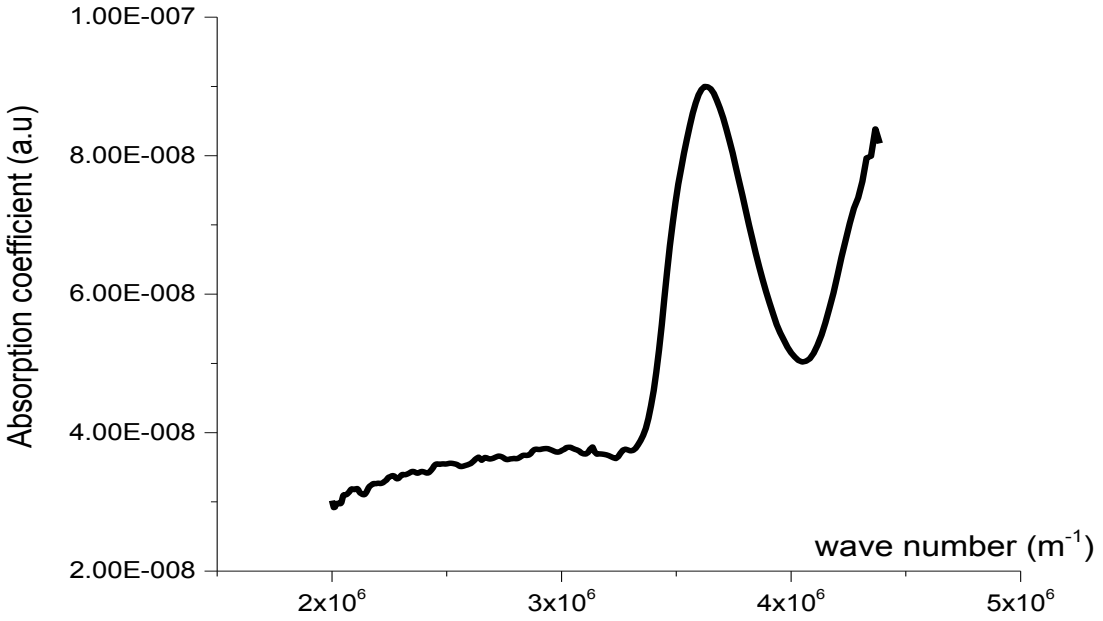

Fig 4.12(a);- UV/Vis spectrum of caffeine extracted by DCM from roasted coffee before Gaussian fitt

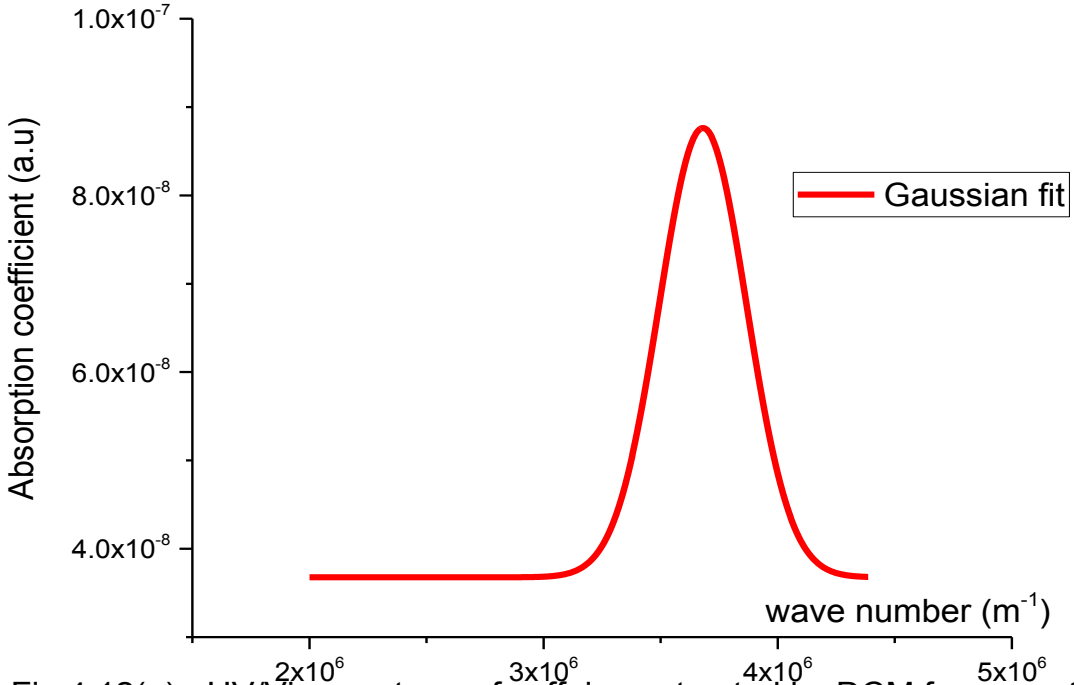

Fig 4.12(a);- UV/Vis spectrum of caffeine extracted by DCM from roasted coffee after Gaussian fitt

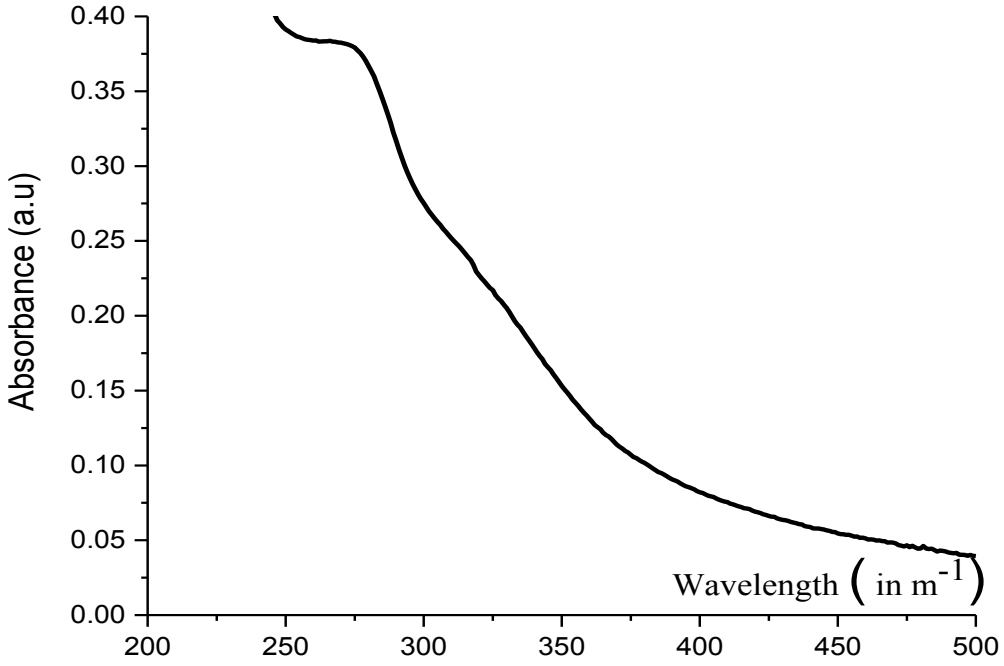

Fig 4.13:- UV/Vis spectra of medium roasted coffee disolved in distilled water

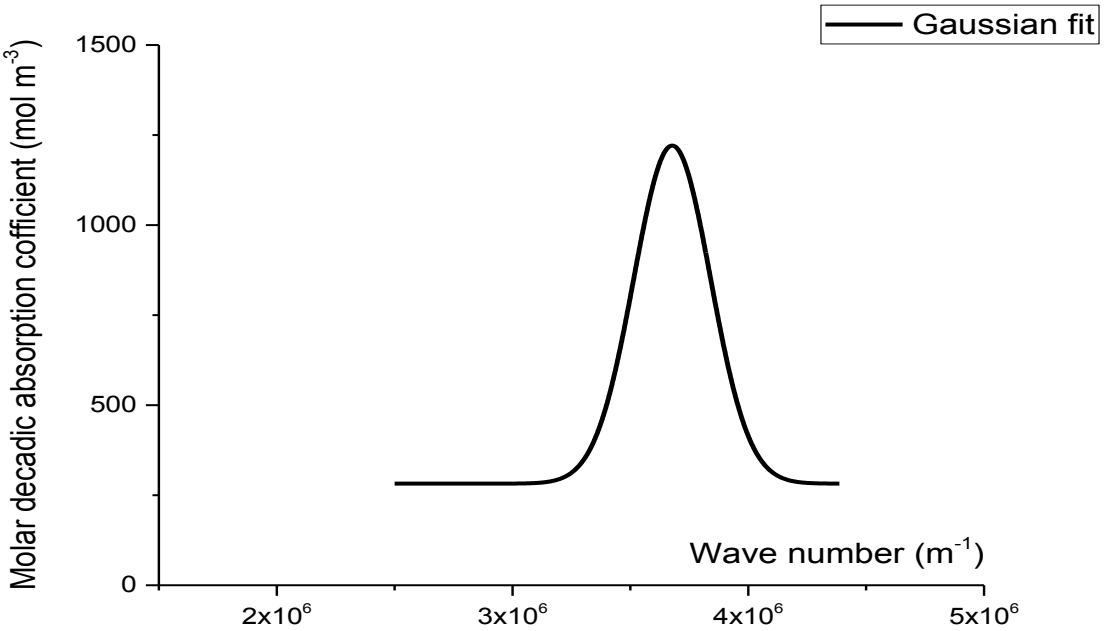

Fig 4.14:- UV/Vis Spectra of caffeine extracted from coffee Beans

Table 4.5:- Number density and Weight by weight (w/w) concentration of Caffeine measured in Ethiopian export standard Roasted Coffee Beans by UV-Vis Spectroscopy in the BLL & IAT.

Samples	Maximum Absorbance (a.u)	Number density of caffeine in roasted coffee (in $10^{13}m^{-1}$)		Percentage of Caffeine concentration in roasted coffee (w/w %)	
		By BLL	By IAT	By BLL	By IAT
Bebeka	1.04	8.001	8.346	2.40	2.38
Harer	0.69	5.160	6.470	1.58	1.51
DJimmah	0.90	7.290	7.213	2.01	1.96
Limmu	0.60	4.236	4.564	1.35	1.34
Nekempti	0.78	6.010	6.808	1.76	1.71
Sidama unwashed	0.77	12.417	15.375	1.76	1.72
Sidamw washed	1.12	3.666	3.735	2.51	2.48
Teppi	1.12	7.580	7.241	2.51	2.50
Yirgacheffe	0.90	6.538	7.032	2.03	1.98

The other basic parameters describing any individual absorption band is a bandwidth at half-maximal intensity, defined as follows (Antonov and Nedltcheva, 2000)

$\Delta\nu_{1/2} = \nu_1 - \nu_2$, where ν_1, ν_2 are initial and final frequencies at half of intensity maximum

In electronic spectroscopy especially in organic molecules, the transition observed in UV-visible region is $\pi^* \leftarrow \pi$. Thus, for pure caffeine the electronic type transition is $\pi^* \leftarrow \pi$ and this transition is the cause for absorption. It measures the anti-bonding character of the excited state. The anti-bonding character is a function of inter nuclear distance and in some cases the change of the bandwidth can indicate the change of inter nuclear distance. In this research, the bandwidth at half maximum (FWHM) of caffeine calculated in DCM and roasted coffee beans are presented in Table 4.6 from the graph of absorption coefficient versus wave number. Here we observed that the bandwidth at FWHM of pure caffeine in DCM decreases as the concentration increases.

Table 4.6:- the bandwidths of pure caffeine in DCM and caffeine extracted from roasted coffee.

Pure caffeine concentration in DCM (mol m^{-3})	Bandwidth at (Fwhm) of pure caffeine in DCM (m^{-2})	Samples of coffee	Bandwidth at (Fwhm) of caffeine extracted from coffee
1.84×10^{-2}	1.09×10^{6}	Bebeka	1.20×10^{6}
3.28×10^{-2}	0.81×10^{6}	Harer	2.28×10^{6}
5.52×10^{-2}	0.90×10^{6}	DJimmah	1.59×10^{6}
7.36×10^{-2}	0.42×10^{6}	Limmu	0.95×10^{6}
9.20×10^{-2}	0.41×10^{6}	Nekempti	2.52×10^{6}
11.0×10^{-2}	0.40×10^{6}	Sidama unwashed	2.49×10^{6}
		Sidamw washed	1.03×10^{6}
		Teppi	0.41×10^{6}
		Yirgacheffe	0.44×10^{6}

The percentage for caffeine concentration of Ethiopian export standard roasted coffee bean sample varies from 1.35% -2.51 % in Beer Lambert's Law and from 1.34% - 2.50% in integrated absorption techniques. Our results obtained by using integrated absorption technique and Beer-Lambert's laws had a good agreement. Of all sample tested Tepi and Sidama (washed) coffee samples had the highest percentage of caffeine concentration, but Limu coffee sample had the least percentage of caffeine concentration. From these samples based on Ethiopian coffee export standard and liquoring board standardization Tepi and Limu are in the same category, which were in washed & Harer was in another category, which was in unwashed. Sidama coffee that was of the same origin with different category had different concentration of caffeine. Sidama unwashed had caffeine concentration of 1.76% in BLL & 1.72% in IAT and sidama washed had caffeine concentration of 2.51% in BLL & 2.48% in IAT.

Furthermore, the caffeine concentration varies from 1.34% to 2.51% of medium roasted at 215^0C in fifteen minute of Ethiopian export standard Arabica coffee samples determined by this work were in a good agreement with the caffeine concentration reported by various analytical techniques in literatures. The amount of caffeine concentration by UV-Vis spectrophotometer in medium roasted Vietnam Robusta coffee bean reported to be 3.591% [43]. By using UV/Vis-spectrophotometer & HPLC reported that the amount of caffeine in Arabica coffee was about 3.312%[42]. Other studies also have reported that caffeine content increased during the roasting process from 0.96% to 1.26% [40] & from 2.04% to 2.515% and it was higher in the medium roasting level[40]. By using UV-Vis spectrophotometer that coffee Arabica in light roasted coffee concentration is 2.24%, in the medium roasted coffee concentration is 2.47% and in the medium roasted Cherry coffee concentration is 2.52% reported [42]. The caffeine content reported for Ethiopian Arabica coffee concentration ranges from 0.46% to 2.82% by HPLC[43]. For caffeine, content in Ethiopian coffee samples by using UV/Vis spectrophotometer analysis was from 0.90% to 1.27% [22] and by HPLC it was from 1.10% to 2.90% for 68 samples[44] which our work is found in this range.

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5. CONCLUSIONS

In this work we determined optical and quantum mechanical parameters of caffeine for nine samples of export quality Ethiopian coffee by UV/Vis-absorption spectroscopy. The integrated absorption technique has advantage that it is independent of line function. The effects of line broadening and shift in peak intensity due to, temperature and pressure variation, solute-solvent interaction and a finite slit width of UV-Vis spectroscopy do not affect the intensity of the absorbing molecules. Therefore, the technique is best for evaluating the UV-Vis absorption intensity; moreover, it is also sensitive, precise, and accurate for determining caffeine in coffee beans. In addition, the optical transition & quantum mechanical properties (oscillator strength, dipole moment, integrated absorption cross-section and peak absorption cross-section) of pure caffeine analyzed and the results agree with other workers and other analytical methods. The optical transition & quantum mechanical properties calculated in dichloromethane & distilled water are the intrinsic ability of caffeine molecules to absorb light and they are proportional to the intensity of transition. UV-Vis spectroscopy method applied successfully for the determination of caffeine concentration in medium roasted coffee beans extracted by dichloromethane from nine washed and unwashed samples of Ethiopian export standard coffee. In thesis, a significant variation in the concentration of caffeine was observed depending on the geographical origin and category of the coffee beans of Ethiopia. In case of category among the fife washed coffee samples Tepi & sidama coffee beans had high caffeine concentration and Limu coffee bean had the list caffeine concentration, but among four unwashed coffee samples, DJimmah coffee had the highest caffeine concentration & Harer coffee had the list caffeine concentration. On the other hand, in the case of geographical origin of coffee varieties Tepi's coffee had the highest concentration of caffeine & Limu's coffee had the list concentration of caffeine. Both Tepi & Limu coffee samples that have highest and lowest concentration of caffeine respectively found in washed coffee brands.

Generally the average values were taken for highest and lowest values of caffeine concentration for medium roasted Ethiopian export standard coffee samples percentage (% w/w) are 2.51% in BLL & 2.50% in IAT for Tepi and 1.35% in BLL & 1.34% in IAT for Limu. These of caffeine concentration results of Ethiopian export standard coffee are in a good agreement with literatures.

In this thesis we had different concentration of caffeine from the same origin but from different category of coffee sample that is 2.50% for washed Sidama and 1.72% for unwashed Sidama. This difference is because of the category difference of coffee that originated from the same place. Thus, we recommended that there should be further research investigation to be done based on category difference using UV/Vis absorption spectroscopy.

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APPENDICES

Appendix A: Raw data for absorbance measurement of standard caffeine in DCM

Working solution(V in ml)	1	2	3	4	5	6
Volume of solution in dichloromethane(V_1 in ml)	25	25	25	25	25	25
Concentration(C_1 mol m^{-3})	1.84×10^{-2}	3.28×10^{-2}	5.52×10^{-2}	7.36×10^{-2}	9.2×10^{-2}	1.104×10^{-1}
Absorbance (A in a.u) at 276nm	0.28264	0.48467	0.70854	0.89721	1.09016	1.30409

Table (3.1):- The Concentration and peak absorbance at 276nm wavelength for working solutions of standard caffeine in DCM

Appendix B: Raw data for absorbance measurement of standard caffeine in water

Working solution(V in ml)	1	4	8	12	16	20
Volume of solution in distilled water (V_1 ml)	30	30	30	30	30	30
Concentration of solution(C_1 in mol m^{-3})	1.762×10^{-2}	6.87×10^{-2}	13.73×10^{-2}	2.06×10^{-1}	2.746×10^{-1}	3.43×10^{-1}
Absorbance (A in a.u) at 273nm	0.07761	0.26543	0.51115	0.77233	1.02628	1.29181

Table (3.2):-The Concentration and peak absorbance at 273nm wavelength for working solutions of standard caffeine in distilled water

Appendix C: Raw data for absorbance measurement of caffeine extracted from coffee by DCM

Samples	Bebeka	Harer	DJimma	Limmu	Nekempti	Sidama(unwashed)	Sidamw(washed)	Teppi	Yirgach-effe
Concentration In mol m^{-3}	1.04348	0.69139	0.89881	0.60313	0.77841	0.7749	1.12464	1.11749	0.89996
Absorbance	0.8497	0.0563	0.07319	0.04911	0.06339	0.0631	0.9158	0.091	0.07328

Table 3.3:- Coffee samples with its concentration and maximum absorbance at 276nm wavelength.