DETERMINATION OF CAFFEINE CONTENTS IN COFFEE BEANS USING UV-VIS SPECTROPHOTOMETER

BY

ADAMU NEGERI

A THESIS SUBMITTED TO THE APPLIED PHYSICS PROGRAM

SCHOOL OF APPLIED SCIENCE

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MASTER IN PHYSICS

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Approval Page

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List of Abbreviations

ATR- Attenuated Total Reflectance

DCM- Dichloromethane

FTIR- Fourier Transform Infrared

FDA-Food and Drug Administration

HPLC- High Performance Liquid Chromatography

IOC-International Olympic Committee

IR-Infrared

NIR- Near Infrared

UV-Vis – Ultra-Violate Visible

Abstract

In this research work using UV-Vis spectrophotometer the molar decadic absorption coefficients and transitional dipole moment of pure caffeine in water and dichloromethane were obtained at 271.8 and 275.1 nm. The molar decadic absorption coefficient of caffeine in water and dichloromethane at these wavelengths are 1127.20 and 1021.13 m^2 mol^{-1} respectively. The integrated area under the curve of caffeine in water and DCM was (132.7 and 120.20) m^2 mol^{-1} respectively. The calculated values for the transitional dipole moment of caffeine in water and dichloromethane are (11.65 and 11.08) $x10^{-30}$ C m respectively; and the oscillator strength was obtained the values 0.22 and 0.18 in water and dichloromethane respectively. After characterizing caffeine in water and dichloromethane, fast and simple methods were developed that enable to quantify the content of caffeine in coffee beans. The methods helped in extracting caffeine from coffee dissolved in water by dichloromethane. The percentage of caffeine in green coffee bean in w/w % was found to be 0.95 ± 0.011 to 1.1 ± 0.006 using Uv-Vis spectrophotometer for the coffee samples.

Key words: Caffeine, Green Coffee, UV-Vis Spectrophotometer, Molar decadic absorption coefficient, Optical property.

1. Introduction

1.1. Background of caffeine

Caffeine is a naturally occurring alkaloid which is found in the leaves, seeds, or fruits of over 63 plant species worldwide. The most common sources of caffeine are coffee, cocoa beans, cola nuts and tea leaves and the worldwide consumption of products derived from these natural materials means that caffeine is one of the most popular and commonly consumed drugs in the world. Caffeine's popularity stems mainly from the fact that it is a pharmacological active substance and a mild central nervous system stimulant. It is generally agreed that there is little risk of harm when a person consumes less than 300mg of caffeine a day [1, 2]. However, at times of anxiety or stress, or pregnancy, the Food and Drug Administration recommends consumption of less than 200 mg a day.

Caffeine is classified as a main alkaloid with molecular weight of 194.19 g/mol that occurs as odorless, white crystalline appearance and bitter taste. Because of it is present in popular drinks and foods (including coffee, tea, cola beverages and chocolates), caffeine is the most widely consumed of all behaviorally active drug/food substance in the world [3, 4, 5, 6].

There is much speculation but only limited evidence of coffee consumption being linked to protective effects on human health. Literatures reveal coffee beans contain efficient water soluble antioxidants, such as chlorogenic acids, caffeic acids, ferulic acids, p-coumaroic acids, melanoids and alkaloids [7] and their content depends mainly on the coffee species, origin and degree of roasting [8, 9].

The total estimated global consumption of caffeine is 120,000 tons per year [10]. In human ranges individual persons take caffeine in different means of consumptions that can be estimated with average consumptions from 80 to 400 mg per person per day [11]. However, Food and Drug Administration (FDA) specifies that the maximum amount in carbonated beverages is limited to 0.02%. Therefore, the highest legal amount of caffeine allowed in 355 ml of soft drink is about 71 mg. International Olympic Committee (IOC) classified caffeine as a drug of abuse when present in human urine in a concentration more than 12 mg L^{-1} [12].

A moderate caffeine intake, 50-200 mg of caffeine per person per day is generally sufficient for a mild stimulation. However, excessive caffeine intake can lead to psychological, physiological and pharmacological effects [13]. Caffeine is considered as a psychoactive drug that causes dependence. It has a behaviorally effects that produces more rapid, clearer flow of thought and increases the capability of a greater sustained intellectual effort and more perfect ideas.

Caffeine is seen more as an aid to regulate sleep quality that cause insomnia of sleep effect. It affects the brain and results in elevated mood, decreased fatigue and increased attentiveness, probably aiding the person to think more clearly and work harder [1]. When taken before bed time, it can interfere with getting sleep or staying asleep. Exactly how caffeine will affect an individual and for how long depends on many factors including the amount of caffeine ingested, whether one is male or female, one's height and weight, one's age and whether one is pregnant or smoker [1, 14]. Caffeine can improve the performance of a wide variety of mental task directly and also indirectly by decrements in performance under suboptimal conditions of alertness [15].

Caffeine has many physiological effects in human beings such as fast heart rate, dieresis (excessive urination), nausea and vomiting. Over doses of caffeine may result in fever and cardiac arrest [16, 17]. It relaxes smooth muscles, including bronchial muscle, vascular and smooth muscles of the biliary in various species. It also can be stimulating the central nervous system that result in increase in intellectual activities of the body and gastric acid secretion [5, 18, 19]. In addition, caffeine interference with the uptake and storage of calcium by the sarcoplasmic reticulum. It also increases the respiratory rate and causes bronchodilator and stimulates lipolysis [17, 20].

The stimulatory action of caffeine involves antagonism of adenosine receptors which are present in brain, blood vessels, kidney, heart, the gastrointestinal tract and the respiratory hierarchy [20]. In addition, caffeine blocks the receptors and there by disorders the regulatory, function of adenosine and produces the stimulatory effect. The resulting effect in turn increases nerve activity that causes the hormone epinephrine, which leads to several effects such as high heart rate, increase blood pressure and release of glucose by the lever [21]. Caffeine acts as antagonistic effects on adenosine receptors or it can be stated that at lower dose of caffeine, adenosine receptors blockade but at higher dose of caffeine that are related to toxicity and depressant effects appear to exert their effect [15].

Caffeine also acts as antifungal [22], inhibition of phosphodiesterase [23], a selective phytotoxicin [24], stimulation of muscle contraction [25], chemostrilant towards certain insects [26] and alterations in glucose metabolism [27]. Caffeine was also found potentially to decrease the antibacterial effect of tetracycline hydrochloride and chloramphenicol [28]. This famous stimulant drug naturally acts in different pharmacological activities such as a pesticide that paralyzes and kills certain insects, larvae and veetles that feeds on plants [29]. Studies

have showed that caffeine at a concentration of 2.5 mg/ml is found to retard the growth of Escherichia coli, enterobacker, aero genes, proteus vulgaris and pseudomonas aeruginosa within a short time [30].

Due to physiological and psychological effects of caffeine, decaffeination is currently a popular method to minimize the caffeine content in various coffee beans. This process simply involves the use of solvents to extract caffeine. The currently available solvents for this purpose are chloroform, dichloromethane, ethyl acetate and supercritical carbon dioxide [31]. Dichloromethane is most effective and commonly employed for the extraction of caffeine in coffee beans [32].

Due to the wide spread consumption of caffeine, it is important to collect precise information of their content in foods. Therefore, a great variety of analytical techniques have been carried out for the determination of caffeine in coffee beans. The most frequently used methods are High Performance Liquid Chromatography [8, 9], Fourier Transforms Infrared [31, 33], Near Infrared Reflectance Spectrometer [8], Derivative Spectrometer [13], UV-Vis Spectrometer and Partial least squares [9, 34]. But caffeine content in coffee beans cannot be determined directly using UV-Vis spectrophotometer due to the matrix effect of UV absorbing substances [27].

However, recently the caffeine content of coffee beans has been reported using UV-Vis spectrometer by Beer-Lambert's law by extracting caffeine from a water solution using dichloromethane [9]. Caffeine absorption peek is seen at 271-276 nm [4]. The caffeine content of coffee beans is mainly expressed as a percentage of dry weight. The beans most cultivars of Arabic coffee contain up to 1.0% caffeine, but Robusta coffee contains about 2.0%

caffeine [13]. Since the over dose consumption of caffeine has health related problems described above, it is necessary to know the contents caffeine in beverages and coffee. Therefore, this research was to investigate the possibility of optical methods for the determination of caffeine in aqueous solution of green coffee beans by developing cost effective procedures.

1.2. Statement of the study

Caffeine is found in various kinds of foods and drinks that we consume in daily life [4]. It causes various physiological effects such as relaxation of bronchial muscle, stimulation of the central nervous system, gastric acid secretion and dieresis. And their concentration in vivo is a key mark for various disorders including heart disease, carcinogenesis, kidney malfunction and asthma [6].

On the other hand, chemical analysis of caffeine in coffee beans is also used as an additional tool for evaluating coffee quality. Higher caffeine contents associated with highest quality samples compared to other Arabic coffee samples have been reported [35]. Developing a system based on spectral information to assess the coffee quality and quantity parameters would bring economical benefits to the coffee industry by increasing consumer confidence in the quality of products. Therefore, establishing a rapid and cheap analytical method for the determination of caffeine in coffee beans has an important for a wide range of physiological effects on the human body and quality control.

The more recent methods of caffeine determination are based on the HPLC combined with several detection methods like ultraviolet-visible spectrophotometer, mass and IR spectrometry [9, 36]. However, most of these reported procedures involve relatively long retention times or require lengthy sample pretreatments. Spectrophotometric determination is preferred because of

fast, high accuracy and reproducibility of method, relatively cost effective, uses fewer reagents, easily performed and is less time consuming. [37]. But caffeine content in coffee beans cannot be determined directly using UV-Vis spectrometer due to the matrix effect of UV absorbing substances [27]. However, recently the caffeine content of coffee beans has been reported using UV-Vis spectrometer by Beer-Lambert's law by extracting caffeine from a water solution using dichloromethane [9].

Several studies have been conducted on the determination of caffeine contents in coffee beans. However, detail studies have not conducted on analysis of caffeine in coffee beans of Ethiopia, particularly western part of the country. Therefore, this research has intended to analyze the caffeine contents in Arabica coffee beans found in different parts of Ethiopia. By comparing their caffeine contents (quantitatively), their quality was determined.

1.3. Objective of the study

1.3.1. General objective

- The general objective of this research is to determine the optical transition properties of caffeine in different solvents and determine caffeine contents in coffee beans cultivated in western parts of Ethiopia.

1.3.2. Specific objectives

The specific objectives of this study are to:

- Calculate the oscillator strength of caffeine in water and dichloromethane.
- Calculate the molar-decadic absorption coefficient of caffeine in water and dichloromethane.

- Calculate the integrated absorption cross-section and transitional dipole moment of caffeine in water and dichloromethane.
- Determine the caffeine in raw coffee samples grown in Ethiopia particularly Wolega, Limu (Jimma), Sidamo and Yirgachefe.

2. Review of Related Literature

2.1. The nature of coffee

The name coffee probably originated from the name of the province' keffa' where shepherds from Abyssinia/Ethiopia discovered the coffee beans in the 6th century [38]. Then the Arabs introduced coffee from Ethiopia to Yemen during the 13th century, whereas the habit of drinking coffee was developed in the 15th century. This habit gradually spread to the rest of the world leading to the increased interest of some countries to produce coffee as a commodity on a large scale. The growth of popular coffee houses, which become favorite meeting places for both social and business purpose spread from the mid-17th century to other European countries including Austria, France, Germany, Holland and England. Coffee is now one of the most valuable commodity and primary source of foreign exchange to developing countries. Millions around the world earn their living from it [39].

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. The two most commercially important species of coffee are coffee Canephora (Robusta) and Coffee Arabica. These two species differ by appearance, origin, quality and flavor [40]. Coffee Arabica is a native to the southwestern highlands of Ethiopia. It is the most cultivated coffee species throughout the world. About 90% 0f the world's coffee production is coffee Arabica and 9% is Robusta. Coffee Arabica grown at higher altitude requires less rain and its beans have lower caffeine content than that of Robusta. It is mainly grown in central and southern America, East Africa and India. It is the most important foreign currency earner for more than 80 developing countries [41].

Ethiopia is the 3rd largest coffee producer in Africa after Uganda and Ivory Coast. Coffee grows in most part of Ethiopia particularly Oromia region and South Nation and Nationality (SNN) region comprises the largest coffee cultivated area. Wolega, Ilu Aba Bora, Bale, Guji, Jimma, East and West Hararge, Bench Maji, Sidamo, Gedeo/Yirgachefe, South and North Omo are the largest coffee cultivated area of Ethiopia [42]. Coffee Canephora widely grown in Western and Central Africa, Malaysia, Brazil and India. It is 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 fully body and a woody after test, which is useful in creating blends. It is grown at lower altitude [43].

2.2. Chemical composition of coffee

Coffee contains many biologically active substances [21]. The amounts and composition of these compounds are dependent on the types of coffee beans, the degree of maturation, the roasting and to some extent on environmental conditions and agricultural practices [44, 34].Among these active substances found in coffee beans caffeine, trigonelline, theobromine, theophylline, chlorogenic acid, quinic acid, caffeic acid and sucrose [45]. Because of its stimulating effect on the central nervous system, caffeine is the most widely known constituents of coffee. In addition, it is used in many beverages and drugs due to its pharmacological actions [46].

Caffeine (1, 3, 7-trimetyixanthine), is one of the main alkaloid found in various kinds of foods and drinks that we consume in daily life [3, 4, 5]. It is naturally found in leaves, seeds of fruits of 63 plant species. The most common sources of caffeine are coffee, cocoa beans, cola nuts and tea leaves [3]. The chemical formula for caffeine is $C_8H_{10}N_4O_2$ chemical structure is

shown in Fig.1. Pure caffeine occurs as odorless, white powder. It has a molecular weight of 194.19 g, melting point of un hydrate form178^{0}c, sublimating point of hydrate form 236 ^{0}c and PH values in the range of 6 to 9 [3, 36].

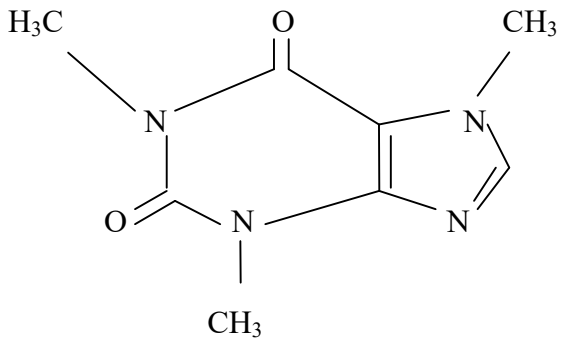

Fig.1: Chemical structure of caffeine

2.3. Physiological and psychological effects of caffeine

The effects of caffeine on human being depend on concentrations. Consuming high concentration of this compound causes various physiological and psychological effects such as relaxation of bronchial muscle, stimulation of the central nervous system, gastric acid secretion and diuresis [6, 18, 19, 47]. The increases in concentration of caffeine in vivo are also a key mark for various disorders including heart disease, kidney malfunction and asthma. Moreover, our sleeping habit, performance and concentration are modified by caffeine [4, 5, 6, 47].

Caffeine has a tendency of rapidly and completely absorbed from gastrointestinal tract within a short period of time and distributed in the body [32, 48]; however, it is not removed from the circulation until metabolized initially into paraxanthine, theophylline and theobromine then into derivative of uric acid and diaminourcil, which is eventually removed from the circulation. So

the plasma half life of caffeine in man, that is, the time required for its level to be diminished by 50 % as a result of biotransformation and excretion is 5 to 6 hour [32, 48].

On the other hand, besides the physiological and psychological effects of caffeine, the chemical analyses of caffeine in coffee beans have been used as an additional tool for evaluating coffee quality. It has been reported that higher caffeine contents is associated with less quality samples compared to other Arabic samples [35]. Due to the adverse effects mentioned previously, there is an increased demand for decaffeinating of coffee. About 10 % of the world production of green coffee and 20 % of the coffee import to some European countries have gone for decaffeination in coffee industry [32]. Decaffeination of coffee by chemicals in industry is expensive and it also changes the taste and aroma of coffee and results in reduction of cup quality of coffee [47].

Moreover, due to the wide spread consumption of caffeine and its potential on physiological, pharmacological and psychological effects, it is important for both health professionals and consumers to know the extract caffeine content in food. It is therefore important to precisely determine the caffeine content in different coffee types, as a way to assess their content in order to find a more precise relationship between the amounts of consumed caffeine and its physiological effects [32, 47]. Therefore, the aim of this study is to retrieve the information about content of caffeine in green coffee bean collected from various parts of Ethiopia despite the wide use of coffee in the country.

2.4. Methods developed for analysis of caffeine

Due to aforementioned facts, many chemical and physical methods have been developed for the determination of caffeine in coffee and other beverages. The most widely used methods for the determination of caffeine in beverages are UV-Vis spectrophotometer and partial least square [16], UV-Vis spectrophotometer [4, 9], luminescence [49], derivative spectrophotometer [13], HPLC [12, 15, 44], FTIR spectroscopy [5, 28, 30], near infrared reflectance spectrometry [8, 52, 53] Raman spectroscopy [54], capillary electrophoresis [47] are very commonly used techniques.

Spectrophotometer method is fast, simple, accurate, reproducible and inexpensive procedure as compared to other methods; however, it is not possible to determine caffeine directly in coffee beans by conventional UV-Vis absorption measurement due to the spectral overlap UV absorbing substances in the sample [9, 47]. The derivative spectrophotometer is relatively easy; but, it is not reliable for the small concentration of caffeine in samples. By HPLC methods, many caffeine contents were determined in various foods using different procedures since it provides the most reliable method. However, the use of expensive equipments and the demand for more operator attention prevents its applications in small industrial laboratories where only a few analyses are performed each day [13, 47].

Other methods such as FTIR, Raman and NIR reflectance spectrometry are equally versatile and powerful analytical tools for analyzing contents of caffeine in coffee [8, 53]. Moreover, they do not require expensive chemicals; however, such instruments are not available in most laboratories and involve huge capital costs. The qualitative and quantitative characterization of caffeine in coffee beans by UV-Vis spectrophotometer include the determination of the optical transition properties of caffeine and its composition in coffee beans using Beer-Lambert's law.

3. Materials and Methods

3.1. Chemicals and Samples

The chemicals that were used for the experiment are as follows. De-ionized water bought from (Ranchem, A.A., Ethiopia) and dichloromethane (RPE, France). Caffeine (Evan, England) and Arabic coffee samples were collected from Oromia Coffee Farmers Cooperative Union. The origin of the samples are Limu (Jimma), Sidamo, Wolega and Yirgachefe.

Fig.2: Coffee samples collected for the study

3.2. Apparatus and Instrumentation

Laboratory apparatus that were used during the experiment are listed as follows. UV-Vis spectrophotometer (serial no.AE 1408013) with a band width 4 nm was used for the electromagnetic absorption measurements of standard solutions of caffeine at room temperature and quartz cuvette (1cm) to hold the sample. And also for measurements of the mass, electronic balance (FA2104, China) with a good accuracy was used. In addition to these, different

instruments such as measuring cylinders with 50 ml, different types of beakers and filter paper (what-mann-1) to remove the particles, separator funnel (with 250 ml) to extract caffeine, magnetic stirrer with hot plate to stir, sieve (Fritsch-250 μm) to get a uniform texture were the apparatus that used during the experiment.

Fig.3: Magnetic stirrer, Analytical balance and Uv/vis spectrophotometer

3.3. Coffee Sample Preparation

Coffee sample preparation was performed according to the following procedures developed previously [9]. The raw coffee sample was ground and sieved to get a uniform texture. Then an accurately weighed 50 mg of coffee was dissolved in de-ionized water and stirred by a magnetic stirrer for 1 hr to remove caffeine easily from the solution. In addition, the solution was filtered by a filter paper to get rid of particles from the solution and the volume of the solution was measured [9].

3.4. Extraction of Caffeine from Coffee bean

In this research for liquid-liquid extraction, dichloromethane was used. Because, literature indicated that the current most widely used solvent for decaffeinating in coffee beans was dichloromethane [32]. Extraction was done according to the following procedures. The coffee solution prepared above (under coffee preparation) was mixed with dichloromethane by volume ratio (1: 1) for the extraction of caffeine from coffee. First, a mixture of the solution was stirred for 10 minutes. Then using separator funnel caffeine was extracted by dichloromethane from the solution. The extraction of caffeine was conducted four times for each sample. The volume of caffeine extracted by dichloromethane at each round was measured and stored in volumetric flasks. Finally, the absorbance of the solution was measured by a UV-Vis spectrophotometer. The absorbance versus wavelength measured by UV-Vis spectrophotometer was analyzed using origin 8 software.

3.5. Characterization of caffeine

For the characterization of caffeine, de-ionized water and dichloromethane were used as a solvent. The characterization of caffeine was done according to the following procedures. A 2.5 $x10^{-4}$ g of caffeine was dissolved in 15.0 cm^3 of de-ionized water and $2.3x10^{-4}$ g of caffeine was dissolved in 10 cm^3 dichloromethane. Stock solution of standard caffeine was prepared first for all measurements because it is widely recognized that dilute solutions are not as stable as concentrated one.

To determine the λ_{max} of absorption for standard caffeine in water and dichloromethane first the free spectral range of the solvents was determined by scanning the solvents over the wavelength range (200-500 nm).The λ_{max} for standard caffeine was obtained by scanning the

standard solution over the wavelength range (200-500 nm) and the result gave an absorption spectrum, which was characterized by a single intensive absorption band at 271.8 nm and 275.1 nm in water and dichloromethane respectively. To determine the percentage of caffeine in coffee beans calibration graph of absorbance versus concentration was constructed for the standard solution.

Then, molar decadic-absorption coefficient of caffeine in water and dichloromethane were respectively calculated from the equation of curves. The amount of caffeine in green coffee beans was also calculated using the given equation of line. In addition to this, integrated absorption cross-section, Oscillator strength and transitional dipole moment of caffeine in water and dichloromethane were respectively calculated.

4. Results and Discussions

4.1. The UV-Vis absorption of caffeine in Water and Dichloromethane

The UV-Vis absorption spectrum of caffeine in water is found to be in the region 243- 302 nm at room temperature. It is clearly shown in Fig.3 that the spectral intensity of caffeine drops to zero at wavelength greater than 302 nm, on the other hand, a new peak absorbance is noticed at a wavelength below 243 nm. This new spectrum is expected to be the peak absorbance due to the solvent. The peak absorbance of the solution is found to be A= 0.73 at the maximum wavelength λ_{max}= 271.8 nm. The peak absorbance for caffeine observed in these experiments was quite similar to those reported by [34]. The molar decadic absorption coefficient measuring the intensity of optical absorption at a given wavelength was calculated using Beer-Lambert's equation [48]. The molar decadic absorption of caffeine in water is computed and value of ε_{max}= 1127 m^2 mol^{-1} was obtained.

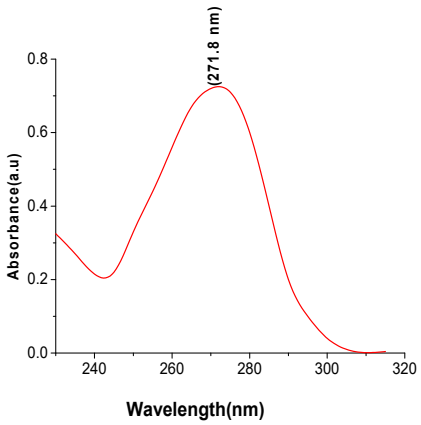

Fig. 4: UV-Vis Spectra of caffeine in water

Similarly, the UV-Vis absorbance spectra of caffeine in dichloromethane were found to be in region of 243- 301 nm. Its peak absorbance A= 0.93 was obtained at a maximum wavelength

75.1 nm. The molar decadic absorption coefficient of caffeine in dichloromethane, ε_{max}= 1021 m^2 mol^{-1} was obtained. Fig.5 shows the peak absorbance versus maximum wavelength of caffeine in dichloromethane.

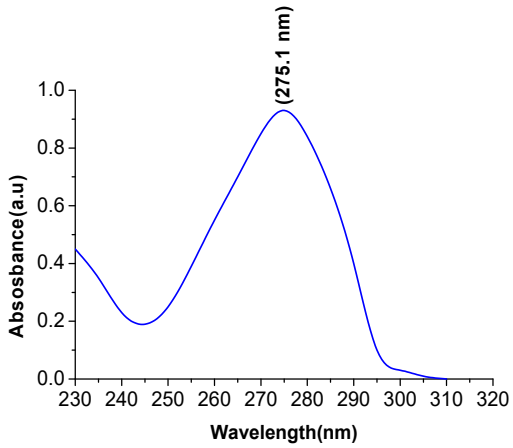

Fig.5: UV-Vis Spectrum of caffeine in Dichloromethane.

A linear function increase in the absorbance was observed as the concentration of caffeine was varied between 0.65 x 10^{-7} to 0.87x 10^{-7} mol cm^{-3} in water. A calibration curve for caffeine in water was constructed by plotting absorbance values as a function of analyte concentration. Each point of the calibration graphs was the average of four replicates. The molar decadid absorption coefficient obtained from equation of the relation was used for the quantitative determination of caffeine in coffee sample. The linear plot of absorbance versus concentration of caffeine in water was constructed in Fig.6 below.

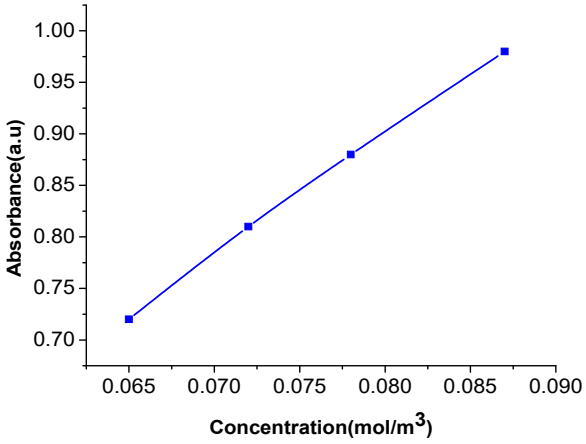

Fig.6: Concentration versus Absorbance of caffeine in Water

In order to establish the range in which linearity between absorption intensity and concentration exists for caffeine, different concentration of standard solutions of caffeine, ranging from 0.91x 10^{-7} to 1.18 x 10^{-7} mol cm^{-3} were prepared in dichloromethane. The linear relationship of absorbance and concentration in the concentration range was measured and the linear graph was constructed as shown in Fig.7

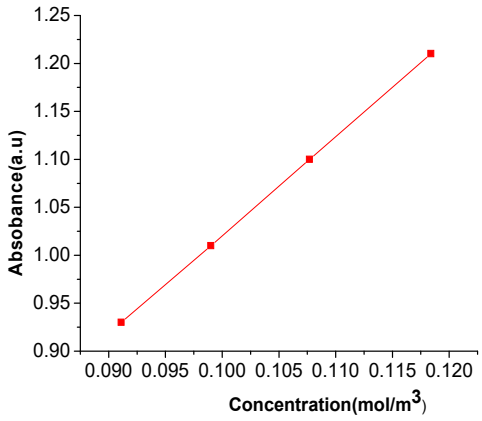

Fig.7 Concentration versus Absorbance of caffeine in Dichloromethane

4.2. Optical transition properties of caffeine in water and dichloromethane

From UV-Visible absorption spectra the optical transition properties of caffeine were calculated in solvents to compare the strength of transition. For incident light intensity I_o, propagating a distance l in the absorbing medium, the transmitted light intensity I can be described as:

$$I = I_0 e^{-a\lambda l} \quad (1)$$

where $a\,\lambda$ is the absorption coefficient**.**

The Beer-Lambert's law results directly from equation (1)

(Gunter and Tuan, 2003).

$$I = I_0 e^{-\varepsilon(v)cl} \quad (2)$$

Where $\varepsilon(v)$ is the molar decadic- absorption coefficient and c is the concentration of the absorbing compound. The Beer-Lambert law is usually expressed in its logarithmic form:

$$\ln\left(\frac{I0}{I}\right) = A = \varepsilon cl \quad (3)$$

where A is the dimensionless quantity called absorbance and I/I_0 transmittance (T).

The molar decadic-absorption coefficient measures the intensity of optical absorption at a given wavelength (Angew, 1969) was calculated from Equation (3) at λ_{max} for caffeine in water and dichloromethane. From Equation (1), the absorption coefficient is given by:

$$a\boldsymbol{\lambda} = \frac{1}{l} ln\,(\frac{Io}{I}) \quad (4)$$

The integrated absorption coefficient at which the sum of absorption coefficient for all frequencies in the band is expressed as:

$$at = \int a\lambda \mathrm{dv} \tag{5}$$

where v is the frequency.

The integrated absorption coefficient provides a measure of the inherent absorbing strength of atoms. It is independent of line function, which may vary with parameters like pressure, temperature and solute solvent interaction. The integrated absorption cross-section δt can be calculated by the following equation:

$$\delta t = \frac{1}{N} \int a\lambda dv \tag{6}$$

where N is the number density of the molecules.

The integrated absorption cross-section of caffeine can be found by recalculating the absorbance versus wave number using origin 8 software. The transitional dipole moment of the dissolved molecule, which is related the molar decadic-absorption coefficient by the integral absorption coefficient is calculated by the equation (Liptay, 1969):

$$I_A = \int \frac{\varepsilon(v)dv}{v} = \frac{\frac{1}{3}2h\pi 2Na}{\ln(10)co\varepsilon o}|\mu_{fi}|^2 = \frac{1}{3}S|\mu_{fi}|^2 \tag{7}$$

Where $S=2.9352 \times 10^{60}$ C^{-2} mol^{-1}. The transitional dipole moment of caffeine was found recalculating the absorbance versus number into $\frac{\varepsilon(v)}{v}$ versus v using origin 8 software along with equation (7). $\frac{1}{3}S|\mu_{fi}|^2$

The oscillator strength was considered the other useful parameter providing the intensity of transition; it expresses the relative strength of electron transition. Oscillator strength can be determined directly through absolute emission, absorption or dispersion measurement. In this research, the oscillator strength of caffeine in distilled water and dichloromethane was calculated by absorption measurements. It was related to the molar decadic absorption coefficient by the following equation [32]:

$$f = 4.32\text{x}10^{-9}\ \text{mol cm}^2\ \text{L}^{-1} \int \varepsilon dv \tag{8}$$

where ε is molar decadic absorption coefficient carrying the unit L $\text{mol}^{-1}\text{cm}^{-1}$ and wave number v, in cm^{-1}. In addition to this, the number density of caffeine in water and dichloromethane was calculated from the equation relating integrated absorption coefficient and oscillator strength as follows (Thorne, 1988):

$$\int a\lambda dv = 2.65\text{x}10^{-6}\ \text{N}f \tag{9}$$

Where N is number density in molecules cm^{-3}, $a\lambda$ in m^{-1} and v in Hz; and f is oscillator strength of the transition molecule.

Fig.8 shows the spectral of caffeine in water given as a function molar decadic absorption coefficient over wave number versus wave number ($\frac{\varepsilon(v)}{v}$ versus ν). The integrated area under this curve, I_A= 132.70 $\text{m}^2\ \text{mol}^{-1}$ was obtained by integrating from ν_1= 32000 cm^{-1} and ν_2= 42000 cm^{-1} and the transitional dipole moment of the dissolved molecule, which is related to the molar decadic absorption coefficient was calculated to be μ_{fi} =11.65x10^{-30} C m. The oscillator strength of caffeine in water was calculated and value f = 0.22 obtained.

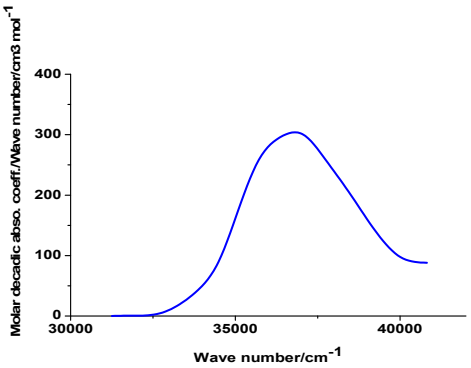

Fig.8: The graph of $\frac{\varepsilon(v)}{v}$ versus ν in water

Table 1: Optical transition properties of caffeine in water and dichloromethane

Solvent	Molar decadic absorption Coefficient (ε_{max}) in $m^2\ mol^{-1}$	Integrated absorption cross-section in cm mol^{-1}	Transitional dipole moment in C m	Oscillator strength
Water	1127.2± 2.9	132.70 $m^2\ mol^{-1}$	$11.65x10^{-30}$	0.22
Dichloro methane	1021.13± 0.66	120.20 $m^2\ mol^{-1}$	$11.08\ x10^{-30}$	0.18

In addition to this, the spectral of caffeine in dichloromethane as a function of molar decadic absorption coefficient over wave number versus wave number ($\frac{\varepsilon(v)}{v}$ versus ν) shown in Fig.9. The integrated absorption cross-section of caffeine under this curve I_A=120.20 $m^2\ mol^{-1}$was obtained by integrating from in the wave number ν_1=32000 cm^{-1} and ν_2=42000 cm^{-1}.

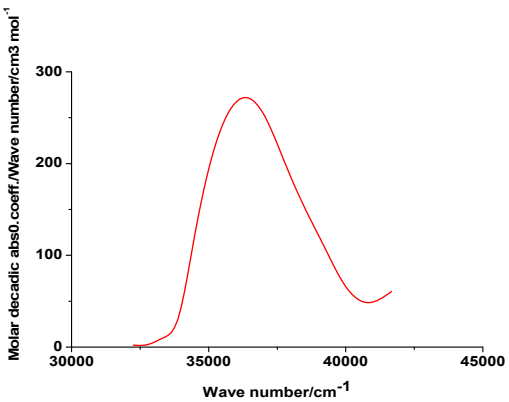

Fig.9: The graph of $\frac{\varepsilon(v)}{v}$ versus ν in dichloromethane

From the integral absorption cross-section value, the transitional dipole moment of caffeine in dichloromethane μ_{fi} =11.08 x10^{-30} C m was obtained. The calculated oscillator strength of caffeine in dichloromethane was found to be f = 0.18. .

4.3. Determination of Caffeine in Coffee Samples

A UV-Vis spectrophotometer method cannot be used directly for the determination of caffeine content in coffee beans owing to the matrix effect of UV absorbing substances in the simple matrix (Ortega-Burrales, 2002). Therefore, it is necessary to develop a method to overcome this difficulty. The method developed in these experimental activities is to first dissolve caffeine in water and then extract it, using dichloromethane as mentioned in the procedure part.

Hence, it is not suitable to determine the percentage of caffeine in coffee beans due to the overlapping of these interfering bands. In order to overcome this difficulty, coffee was first dissolved in water and caffeine extracted from the solution using dichloromethane.

Dichloromethane is the most commonly employed method for extraction of caffeine from green coffee beans (Radwan, 2007; Rofti, 1971). Many commercial products applied dichloromethane for decaffeinating the coffee beans due to its extraction efficiency 98-99 % [9, 32].

The developed Spectrophotometric method for determining caffeine in coffee has been tested for real sample. However, coffee contains a complex mixture of biologically active substances. Many studies imply that trigonelline, chlorogenic and caffeic acids are the primary interferents in the quantitative determination of caffeine in coffee sample using UV-Visible spectrophotometer. These acids show bands closer to the caffeine band. For removing these bands closer to caffeine peak, a suitable method has been developed. It was expected that complete extract of caffeine from coffee mixture could be affected with dichloromethane extraction. Because trigonelline, chlorogenic and caffeic acids being insoluble in DCM remained in the aqueous solution.

The samples were treated as described in the sample preparation section. The samples were collected from Oromia Farmers Coffee Union Limited Liability to get a coffee treated under the same conditions. All coffee is green and washed at the standard of the center. The samples were analyzed for caffeine content using dichloromethane. The results from the developed method are described below.

Studies of ultra-violate absorption spectra of dichloromethane extracts showed that all the spectra have maximum 274.8 nm which are similar to that of caffeine itself. UV-Vis spectra of caffeine extracted from coffee beans were found to be specific as no interfering peaks were observed during spectrophotometric measurement of caffeine in DCM as it was evidenced by the peak purity in Fig.10

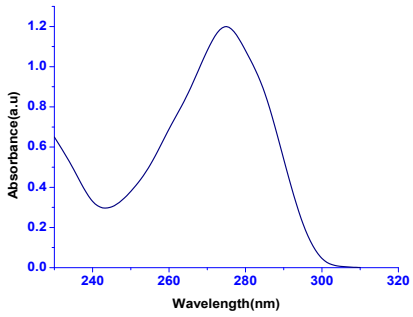

Fig. 10: Absorption spectra of caffeine in Coffee beans extracted by dichloromethane

The proposed method was applied to determine the caffeine content in coffee samples using dichloromethane. The results obtained from the proposed method were compared to the literature extraction method using dichloromethane extracts for analysis of caffeine in real sample. Table 2 summarizes the analytical results of the determination of caffeine in coffee samples collected different parts of Ethiopia.

Table 2: Results of caffeine extracted of from coffee in four replicates of each sample.

	Samples source			
	Limu	Sidamo	Wolega	Yirgachefe
Mass of coffee sample (mg)	50.30	50.25	50.10	50.34
Volume of extracts (ml)	22.20	22.33	21.63	22.50
Peak (λ_{max}) in nm	274.70	274.87	275.20	274.76
	275.20	274.92	275.00	275.40
	275.10	275.14	275.10	275.10
	275.3	274.65	275.25	274.74
Mean± *standard deviation*	275.08± 0.23	274.9± 0.17	275.2± 0.10	275.0± 0.27
Absorbance measured	1.22	1.12	1.201	1.020
	1.26	1.40	1.273	1.310
	1.34	1.26	1.265	1.240
	1.36	0.970	1.281	0.9510
Mean± *standard deviation*	1.30± 0.06	1.19± 0.11	1.26± 0.03	1.13± 0.15
Calculated caffeine mass (mg)	0.517	0.479	0.481	0.479
	0.514	0.484	0.507	0.568
	0.570	0.525	0.530	0.526
	0.563	0.418	0.549	0.471
Mean± *standard deviation*	0.541±	0.477±	0.517±	0.511±
Percentage of caffeine (w/w %)	1.03	0.95	0.960	0.952
	1.02	0.960	1.01	1.13
	1.13	1.04	1.06	1.10
	1.12	0.830	1.03	0.94
Mean± *standard deviation*	1.1± 0.006	0.95± 0.011	1.01 ± 0.003	1.0± 0.013

The results of this method were compared with the results of the same published analytical methods that have been applied for the determination of caffeine in coffee sample. Compared with the literature methods, the present method showed outstanding advantage of simplicity of instrument, faster, relatively cost effective and environmentally friendly.

Using the proposed methods the percentage of caffeine in coffee beans for four samples was determined. This is simple for comparing the percentage of caffeine contents for samples collected from Oromia Coffee Farmers Cooperative Union to get a coffee treated under the same condition. All coffees are green and washed at the standard of the center. The mean percentage of caffeine in coffee seeds investigated using UV/Vis spectrophotometer for four independent measurements is 1.1± 0.006% for Limu, 1.0± 0.003% for Wolega, 1.0± 0.013% for Yirgachefe and 0.95± 0.11% for Sidamo. The literature report [35] indicated that the highest caffeine content observed in Arabic coffee beans was 1.23± 0.06% and the lowest was 0.96± 0.01%. Moreover, it has also proposed by Illy that the percentage of caffeine for Arabic coffees was on the average less than 1.5%. In addition, the percentage of caffeine in Arabic coffees obtained HPLC reported [9] show that it is 0.90 to 1.10%. Using derivative spectrophotometer it was also reported [13] that the percentage of caffeine in coffee seed was 1.36± 0.03%. Therefore, the results from the present method are in good agreement with those obtained by previously reported.

Table 3: Comparisons of results for the determination of caffeine current method and published methods.

Origin of samples	Percentage of caffeine found in (w/w %)			
	This study results	Literature reports by		
		Dervative spectrophotometer	UV-Vis spectrophotometer	HPLC
Limu	1.10 ± 0.006	1.36± 0.03 [13]	0.96 ± 0.01 to 1.23 ± 0.06 [35]	0.90 t 1.10 [9]
Sidamo	0.95 ± 0.11		1.01 ± 0.04 to 1.19 ± 0.02 [9]	
Wolega	1.01 ± 0.003			
Yirgachefe	1.0 ± 0.013			

5. Conclusions

The UV-Vis Spectrophotometric method has been developed for the determination of caffeine in coffee samples. The proposed method is simpler, rapid, cheap, highly sensitive for the determination of caffeine content of coffee and also environmentally friendly than most of previously reported methods. Moreover, chemicals and equipments necessary to carry out the analysis by proposed methods are those which are available in most common laboratories. Furthermore, literature methods were mainly applied to determine caffeine using liquid-liquid extraction; most of them were not applied to aqueous extracts for the determination of caffeine in coffee. The current method provides a new technique for the determination of caffeine in coffee sample using aqueous extracts with good precision and accuracy.

The percentage of caffeine in green coffee bean was also determined by using previously developed UV-Vis spectrophotometric method. The caffeine content of green coffee bean in % w/w was found between 0.95 ± 0.11 and 1.1± 0.006 using UV-Vis spectrophotometer for the coffee sample. According to the study results, coffee with high caffeine content was Limu and is quality coffee because, higher caffeine contents associated with highest quality samples compared to other Arabic samples have been reported [44]. These differences might be due to the agricultural practices, altitude of the coffee origin and the degree of maturation since it was reported [34, 35, 44] that the amounts and composition of compound in coffee are dependent on the types of coffee beans, the degree of maturation, the roasting, and to some extent on environmental conditions and agricultural practices.

To make the result more reliable, the experiments were repeated four times and the average values were taken. The results obtained using these methods are comparable with the results reported by literatures. In addition to this, the optical properties of pure caffeine and caffeine content of real coffee were analyzed and the results agreed with the literature values of other analytical methods. Moreover, the current methods developed on UV-Vis spectrophotometer are relatively easy, fast, cheap and highly sensitive for the determination of caffeine in coffee beans and environmentally friendly.

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