STANDARD PROCEDURE TO DETERMINE THE PERCENTAGE OF CAFFEINE IN COFFEE SEEDS BY UV/VIS SPECTROPHOTOMETER

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By

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In the Memory of My Beloved father Belay Gemta (1930-1997 EC)

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Content

List of figures

Abstract

In this work using UV/Vis spectrophotometer the electronic transition properties of caffeine are investigated. The molar decadic absorption coefficients of caffeine in water and dichloromethane are 1112 m^2 $mole^{-1}$, 1010 m^2 $mole^{-1}$ respectively obtained. From the experimental results the transitional dipole moment of caffeine in water $11.50x10^{-30}$ C m and in dichloromethane $10.80x10^{-30}$ C m respectively calculated. In addition, fast and simple methods are developed that enable to quantify the contents of caffeine in coffee seeds. The methods are extracting caffeine from coffee solution by dichloromethane and apply Gaussian fit to remove the possible interferences with the caffeine spectra. The results obtained using our techniques are compared with HPLC and found to be similar. The methods have a good application for identifying decaffeinated coffee seeds which are discovered in Ethiopia.

Key words: caffeine, extract, absorbance, UV/Vis spectrophotometer.

1. Introduction

Coffee is a tropical plant, which grows between tropical cancer and tropical Capricorn [1-2]. It belongs to member of large Rubiaceae family in which it constitutes the Coffea genus [1]. There are many species in Coffea. The two main species familiar in the world at the present time are Coffea arabica and Coffea canephora [1]. Others species of coffee are being cultivated less extensively, although they are still found in certain countries for various reason.

The different species of the Coffea genus have very diverse appearance and behaviors. This applies to their development up to adult stage starting from the sub-shrub stage. The characteristic of their branches leaves flowers fruits and beans are very disparate [1, 3].

It was also mentioned that there are species that dropped leaves annually with on set of the dry season and other are every green that hold leaves for three or more years [3]. The color of species also varies from yellow and dark green to bronzed and purple green. The size of a plant also range from small woody shrubs most of them all bushes or small to medium sized trees and there are a few that characteristically large forest trees. Some species have while flowers some pink or almost purplish and some creamy to yellowish. The variation among beans were much less marked than the measured different found among trees or bushes, leaves, branches or other vegetative features.

Coffee requires a very specific environmental condition for cultivation [2]; Temperature, rainfall, sunlight, wind, and soil are all important. But the requirements vary according to the varieties. Arabica coffee in equatorial regions successfully cultivated only at altitude 1200-1500 m but at higher latitude it is grown in lower laying regions. The mean annual temperature is about 18-22 ^{0}C and rainfall averaging 1100-1500 mm per year. A dry season of 2 to 3 month is necessary to prepare for flowering that starts as soon as the rains return. On the other hand Robusta needs a distinctly warmer climate with a mean annual temperature of 22-27 °C Hence the cultivation confined to low lying regions not exceeding 700-800 m in altitude. Robusta needs more rainfall than arabica but it withstands well a fairly long dry period of the order of 3 month [6].

Moreover, generally coffee prospers in region where rainfall is between 1500-1800 mm per year with a pattern consisting of a few months (3 to 4) with little rain or even relative drought corresponding to dormant that precedes the main flowering period [1].

Coffee tree requires a deep permeable soil of good structure containing enough organic matter and a favorable water balance [3]. It also reported one ideal coffee soil would be a deep slightly acid, well drained loam and it should be rich in nutrients especially potash with an ample supply of humus [7]. The pH of good coffee soil is 6.8 to 7.0 in the upper soil and about 5.5 for sub soil. In Ethiopia the pH soil growing Coffea arabica is about 5.4 to 6.0 [8].

Today coffee is produced in some 80 countries spread over 4 continents: Asia, Africa, America, and Australia [8]. Brazil is the leading producing country in the world followed by Vietnam Colombia and Indonesia [8].

Ethiopia is the third largest coffee producing in Africa after Uganda and Ivory cost. It comprises 2.5 percent of the world coffee trade [36]. Today coffee is growing in most regions of Ethiopia. The biggest area share is concentrated in Oromia and Southern regions, mainly Wollega, Illubabour, Jimma, Benchi maji, Sidamo, Gedeo, Guji, East and West Hararge, South and North Omo. The rest is distributed in other minor coffee producing areas such as Gambella and Amhara region [36]. In Ethiopia it is estimated to be large hectares of area are suitable for coffee production [37]. But at the present the area covered by coffee is is very small compared to suitable area available for coffee production. Similarly the production is low which 3.2 million bags on average are for 24 years.

Coffee is one of the most important commodities in volume next to petroleum in the world. It amounts 14 billion US dollar annually [4]. It provides employment for some twenty million people. More than 50 developing countries 25 of them in Africa depend on coffee as their foreign exchange earner [36].

Coffee has been Ethiopian commodity for over 500 years [36]. Now it accounts about 5% of gross national product, more than 60% of the foreign exchange earning, and 42% of taxes from foreign trade and around 25% of the entire population of the country directly or indirectly depend on coffee for livelihood [36].

Coffee is a well-loved drink. Several characteristics of coffee make it good to man. The stimulating effect of its aromatic beans has made the crop desirable in way that ordinary food product do not satisfy. It's habitually drink early in the morning also gives good refreshment with all meals and because of its taste make the most important place as a beverage. Moreover the golden color, its inimitable bouquet, the delightful fragrance, the miscibility with additives such as cream and sugar and sometimes spices and certain alcoholic liquors, welcomed warmth,

the strength derived from its mild and good stimulus that suffuse the person who had a cup. It is also the best beverage, which stimulate with harmless effect. That is why the medical men mentioned that coffee is a good stimulant both for physiologically and psychologically than either Tobacco or alcohol [3].

On the other hand Ethiopia is the only coffee producing country in Africa with a traditional coffee drinking culture. This coffee ceremony is a daily ritual performed by native Ethiopian women, they get together in neighbor's home to share news and nourish friend ships [37].

According to the recent studies drinking a moderate amount of coffee (two to four cups per day) lower the risk of colon cancer by amount of 25%, gallstones by 45%, cirrhosis of liver by 80%, and Parkinson's disease by 50-80%. Other benefits include 25% reduction in on set of attacks among asthma suffers and at least among a large groups of female nurses tracked over many years fewer suicides. Some studies also indicated that coffee contains four times the amount of cancer fighting antioxidants as green tea [5]. On the other hand a chlorogenic acid which is found in coffee beans also lowered the increase in blood glucose by 15-20% [9]. Further more the risk of type-2 diabetes decreased with higher coffee consumption due to chlorogenic acid [10].

On the contrary heavy coffee drinking causes a sleep disturbance and less evidence nervousness, further more, if the morning coffee was stopped the habitual of coffee drinker's experiences headache and irritation [15].

Coffee is a complex mixture of neutriceutricals. The constituents of coffee have been known for over half of century [12]. In order of abundance typical value for water-soluble constituents are phenol polymers 8%, polysaccharides 6%, chlorogenic acids 4%, and minerals 3%, water 2%, and caffeine 1%, organic acids 0.5%, sugar 0.3%, lipids 0.2% and aroma 0.1%. Further more it also reported [3] that a sample collected from eighty widely separated plantation that include both robusta and arabica species found that the water soluble substances from the sample averaged 28.3% which ranging from 24.2% to 31. %.

The chemical composition of green coffee depends on species and variety, to less extent other factors such as agricultural practices, degree of maturation and storage condition [4]. On roasting there are considerable changes as the more labile components are degraded and the more reactive compound interact to form complex products. Roasting process greatly increase the

chemical complexity of coffee. The aroma of green coffee contains some 250 different volatile molecular species whereas the roasting coffee gives rise to more than 800 [12].

Generally coffee is one of the most altered foods during the process from the point of view the range of compound formed. And this is reflected in changes in sensory characteristics. During coffee brew there is also a large change in composition since water-soluble components are extracted. A similar effect is observed in industrial extraction process to prepare instant coffees. There are also minor changes in composition observed during any additional process such as decaffeinating [4].

There are some biological active substances contained in the coffee, which have a particular interest. Their amount and composition in the coffee brew dependent on the type of coffee bean and on the degree of roasting [13]. Among the active substances caffeine (1, 3, 7 trimethylxanthine) with the chemical formula $C_8H_{10}N_4O_2$ [14] is one of the main constituents of coffee.

The amount of caffeine in different parts of coffee tree have been determined by a number of chemists the caffeine contents for robusta ranged from 1.97 to 2.6 percent with on an average of 2.29 and for arabica about 1.47 percent [3]. On the other hand [12] has got the caffeine content for robusta which is 2.4 to 2.8 percent by weight and arabica which is not exceeding 1.5 percent by weight.

The main physiological effects of caffeine are stimulates the central nerve system, this result in increase intellectual activity and learning ability [15, 16]. It also an effect on the cardio vascular system by relaxation of the smooth muscle of blood vessels and an increase in heart out put [16]. Caffeine also has an effect of gastric acid secretion [15, 17], that is why people preferred it after meal for digestion. It also added to dietary supplements for weight loss since it increases the caloric burning rate [16-17].

On the other hand caffeine has a negative effect with calcium absorption [16]. As study indicated women who drink caffeinated product lose more calcium through urine and tend to have less dense bones than non-caffeinated drinkers. Hence they affected by osteoporosis problem. Further more caffeine is rapidly and completely absorbed from gastrointestinal tract with in a short period of time and distributed in the body but, it is not removed from the circulation until metabolized initially in to paraxanthine, theophylline and thyobromine then into derivatives of uric acid and diaminourcil, which can eventually removed from the circulation, so

the plasma half of caffeine in man that means the time required for its level to be diminished by 50% as a result of biotransformation and excretion is 5-6 hour. If a peak plasma caffeine concentration 5-10 μM the effect at this level generally restricted to increase in sleep latency, an enhancement of alertness during fatigue. At peak plasma level of 15-30 μM the effect may include mild anxiety, respiratory stimulation, cardio vascular effect, dieresis, and increase gastric secretion. And a symptom of acute toxicity may appear when the level is 150-200 μM. These may include severe restlessness and excitement leading to mild delirium, muscular tension and twitching and cardio vascular disturbance such as tachycardia [4].

After drinking a cup of coffee, caffeine is distributed through out the body. As it is similar to substances present in the tissue, as a result of its potent physiological activity caffeine can alter our behavior it affects our sleeping habit. Generally resulting in insomnia and hyperactivity, task-oriented performance, attention, and concentration may be modified by caffeine at higher dose we also expect a toxic, which leads to death. Hence dues to the above mentioned problems people start to seek a decaffeinated coffee. Decaffeinated coffee retains its aroma and taste despite the process, which are necessary to remove the caffeine [18].

The first successful extraction of caffeine from coffee beans was achieved by a German chemist, Runge in 1820 [18]. His friend the poet Goethe had suggested that Runge analyses the constituents of coffee to discover the cause of his insomnia, and the history of decaffeinated coffee began.

Commercial decaffeination of coffee was commenced in Germany early in the twentieth century and a little later in USA. Although for many years sales were fairly static, consumption of decaffeinated coffee has more recently shown an increase, some 10% of world production of green coffee now being treated in this way. In some European countries as much as 20% of the coffee import may go for decaffeination [4].

Basically decaffeination process involves treating the green coffee beans with a solvent, then removing the caffeine-laden solvent from the beans. The three types of decaffeination method in commercial use today are - 1. Chemical solvent 2. Supercritical 3. Water and caffeine free extracts.

In the chemical solvent decaffeination the green bean are treated with steam under pressure this treatment swells the beans increasing their surface area and making the caffeine easier to remove. The next stage is extraction of caffeine by a solvent. Ideally the solvent should

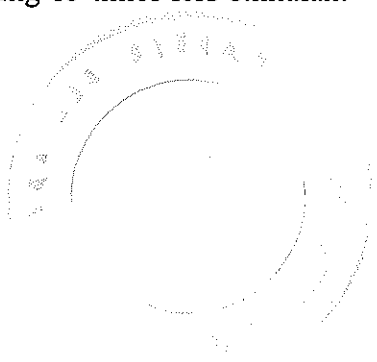
remove caffeine selectively with out affecting the coffee in any other way. After decaffeination only a minute traces of solvent are left in the coffee. The solvents current uses are methylene chloride (dichloromethane) and ethyl acetate they are generally recognized as safe.

The other is decaffeination by a supercritical gas, in this method at temperature above their critical point under pressure; gases behave rather like liquids and can be used as solvent. Supercritical carbon dioxide is used as a selective solvent for caffeine. It is applied to steamed green coffee at temperature about 70 °C and at high pressure. The caffeine is separated from the gas by rinsing or by adsorption and the gas recirculated. In this method the wax layer of the coffee bean is retained and nothing but the caffeine is removed.

The last one is method-using water and caffeine free extract, in this way the green beans are immersed in water and the resulting extract passed over activated carbon to remove the caffeine by adsorption. The caffeine free mixture is then added to the partially dried coffee beans before they are fully dried and roasted. The method results in the loss of some other water-soluble components of coffee such as carbohydrates and chlorogenic acids [18]. Among the decaffeination methods mentioned above methylene chloride extraction retains the most flavor but leaves a dry taste in the mouth, both water and carbon dioxide extraction blur the flavor of the beans and ethyl acetate adds a sweat fruit of flavor [19].

On the other hand scientists from Nara Institute of science and Technology in Japan created transgenic plant with a 70 % reduced caffeine contents. The work was under taken on Coffea canephora plants. The researchers designed to repress a gene, which activated one of the three enzymes, involved in caffeine biosynthesis [20]. Three N-methyltransferase enzymes are involved in caffeine biosynthesis in coffee plants caXMT1, caMXMT1 (theobromine synthase), and caDXMT1 (caffeine synthase), which successively add methyl groups to xanthosine in converting it in to caffeine [20]. The construction of transgenic coffee plants of a gene encoding theobromine synthase (caMXMT1) is repressed by RNA interference (RNAi) [20].

Decaffeination of coffee by chemicals changes the taste and aroma of coffee. Moreover risks of chemicals are involved in this process so for this and others scientists start to search naturally decaffeinated plants. Accordingly Brazilian researchers bred 3000 Ethiopian coffee plants as part of the Programme to produce low caffeine strains. They found three bushes, all derived from the same plant that were virtually caffeine free, containing 15 times less stimulant



than commercial strains [21]. The discover hope, it will yield a cheap flavor some alternative to the artificial decaffeinated coffee already on the market.

Due to the above-mentioned and others, today the demand for decaffeinated coffee is highly increased in the world particularly in Europe and USA. On the other hand Brazilian scientists discovered a naturally decaffeinated plant, which is a modified one than the artificially decaffeinated coffee already on the market. But the origin of this natural decaffeinated plant is Ethiopia. There fore, in order that the country to get a high foreign currency from decaffeinated coffee plant it intended to develop a means of determining the caffeine content of coffee seeds with a simple and fastest way.

It has been reported that many analytic methods have been developed for the determination of caffeine [22]. For determination of caffeine in beverages various analytical techniques including, derivative spectrophotometer, polarography, GC, and HPLC have been reported [22].

Spectroscopic techniques are interest due to the recent development of powerful optical instruments, which permits the detection of numerous substances at low concentration [23]. More over it is relatively easy fast and cheap for the determination of the caffeine content of coffee [22].

Even though the method of using optical techniques (methods) for identification of contents of caffeine in coffee may be economical, time saving, suitable for on line monitoring, due to rapid development of new technologies based on diode array fiber optic and charged coupled device it also cheaper than relative to other instruments no standard methods is set for the quantitative determination caffeine from coffee seeds. Further more as to our knowledge there is no standard techniques report on UV absorption in quantitative determination of caffeine in coffee seeds.

There for the general objective of this paper is to set up a standard procedure for determination of caffeine contents in coffee seeds by using optical methods particularly by UV/Vis spectrophotometer. And the specific objectives are Specify the spectral regions of caffeine in water dichloromethane chloroform, determine molar decadic absorption coefficient, transitional dipole moment of caffeine in water and dichloromethane, set up simple and fast methods for determine caffeine contents in coffee seeds.

2. Electromagnetic Radiation and Its Interaction with Matter

2.1 The Macroscopic Maxwell Equations

All macroscopic aspects of the static and dynamics of the electromagnetic field in the presence of the material media are described by Maxwell's equations [24]. These equations are the most fundamental description of electric and magnetic field [25]. The differential form of these axioms in the international system of units (SI) given by

$$\vec{\nabla} \cdot \vec{D} = \rho \tag{2.1}$$

$$\vec{\nabla} \cdot \vec{B} = 0 \tag{2.2}$$

$$\vec{\nabla} \times \vec{E} = -\frac{\partial \vec{B}}{\partial t} \tag{2.3}$$

$$\vec{\nabla} \times \vec{H} = \frac{\partial \vec{D}}{\partial t} + \vec{J} \tag{2.4}$$

For a given position vector $\vec{r}$ (m) and time t(s) the Maxwell's equations couple the electric displacement vector $\vec{D}$ (C/m^2), the charge density ρ (C/m^3), the magnetic induction $\vec{B}$ (T), the electric field strength $\vec{E}$ (V/m), the magnetic field strength $\vec{H}$ (A/m) and total electric current density $\vec{J}$ (A/m^2). Equation (1) relates the electric charge distribution to the dielectric displacement, (2) precludes the existence of magnetic monopoles, (3) describes the creation of an electric field by time-varying magnetic induction and (4) the creation of magnetic field by an electric current. The charge density ρ and the current density $\vec{J}$ may be regarded as the source of the electromagnetic fields.

When an electric field applied to dielectric medium composed of polarizable molecule, it acquires an average dipole moment $\vec{p}$ proportional to the local electric field $\vec{E}$ [26]. If there are N such molecule per unit volume the macroscopic polarization $\vec{P}$ is given by

$$\vec{P} = N\vec{p} = \chi_e \varepsilon_0 \vec{E} \tag{2.5}$$

where ε_0 permittivity in vacuum which is given by $1/(4\pi\varepsilon_0) = 8.9874 \cdot 10^9\ \mathrm{Nm^2C^{-2}}$, χ_e electric susceptibility and $\vec{P}$ electric dipole moment per volume. Material media response is expressed by the so-called constructive or material relations.

$$\vec{D} = \varepsilon_0 \vec{E} + \vec{P} \tag{2.6}$$

$$\vec{B} = \mu_0 \vec{H} + \mu_0 \vec{M} \tag{2.7}$$

using equation (2.5) for $\vec{P}$, for the dielectric displacement results

$$\vec{D} = \varepsilon_0 \vec{E} + \chi_e \varepsilon_0 \vec{E} \tag{2.8}$$

$$\vec{D} = \varepsilon_0 \vec{E}(1 + \chi_e) \tag{2.9}$$

Where $1 + \chi_e = \varepsilon_r$. Since $\varepsilon = \varepsilon_r \varepsilon_0$ equation (2.6) can be written in terms of equation (2.9)

$$\vec{D} = \varepsilon \vec{E} \tag{2.10}$$

Similarly in the presence of magnetic materials, the applied magnetic field induces a density $\vec{J}$ of magnetization current and hence a magnetization $\vec{M}$ in the materials [26]. Therefore $\vec{B}$ becomes

$$\vec{B} = \mu_0 \vec{H} + \vec{M} \tag{2.11}$$

Where $\vec{M} = \mu_0 \chi_m \vec{H}$. Using this equation (2.11) becomes

$$\vec{B} = \mu_0 \vec{H}(1 + \chi_m) \tag{2.12}$$

With $\mu_r = 1 + \chi_m$ and $\mu = \mu_0 \mu_r$ equation (2.12) can be rewritten as

$$\vec{B} = \mu\vec{H} \tag{2.13}$$

where $\vec{M}$ the magnetization is vector (magnetic dipole moment per unit volume induced by magnetic field) μ_0 permeability in vacuum $(\mu_0 = 4\pi \times 10^{-7} NA^{-2})$ and μ_r is the relative permeability, χ_m magnetic susceptibility.

2.2 Electromagnetic Wave

The energy of a photon is transported in the form of electromagnetic wave [24]. The equations of electromagnetic wave result from Maxwell's equations [25, 26].From equation (2.3)

$$\vec{\nabla} \times \vec{E} = -\frac{\partial \vec{B}}{\partial t} \tag{2.14}$$

applying the curl operation to both sides of equation (2.14) it becomes

$$\vec{\nabla} \times \vec{\nabla} \times \vec{E} = -\nabla \times \frac{\partial \vec{B}}{\partial t} = -\frac{\partial}{\partial t}(\vec{\nabla} \times \vec{B}) \tag{2.15}$$

using the general identity of vector calculus $\vec{\nabla} \times \vec{\nabla} \times \vec{E} = \vec{\nabla}(\vec{\nabla} \cdot \vec{E}) - \nabla^2 \vec{E}$ and by substituting to the left hand side of equation (2.15)

$$\vec{\nabla}(\vec{\nabla} \cdot \vec{E}) - \nabla^2 \vec{E} = -\frac{\partial}{\partial t}(\vec{\nabla} \times \vec{B}) \tag{2.16}$$

Again for $\vec{B} = \mu_0 \vec{H}$ and for current density zero equation (2.4) becomes

$$\vec{\nabla} \times \frac{\vec{B}}{\mu_0} = \frac{\partial \vec{D}}{\partial t} \tag{2.17}$$

Using general identity equation (2.23) becomes

$$\vec{\nabla}(\vec{\nabla}\cdot\frac{\vec{B}}{\mu_0}) - \nabla^2\vec{B}/\mu_0 = \varepsilon_0\frac{\partial}{\partial t}(\vec{\nabla}\times\vec{E}) \tag{2.24}$$

Since $\vec{\nabla}\cdot\vec{B} = 0$ from equation (2.2) equation (2.24) becomes

$$\nabla^2\vec{B} = \mu_0\varepsilon_0\frac{\partial^2\vec{B}}{\partial t^2} \tag{2.25}$$

$$\nabla^2\vec{B} - \mu_0\varepsilon_0\frac{\partial^2\vec{B}}{\partial t^2} = 0 \tag{2.26}$$

Equation (2.22) and (2.26) are the wave equations for electromagnetic field in vacuum. They are the simplest, indeed the only equations having a solution undamped vector waves traveling in three dimensions at any frequency and constant velocity [26]. Using the method of separation of variables the solution of the equation (2.22) and (2.26) becomes

$$\vec{E}(\vec{r},t) = \vec{\varepsilon}E_0\exp -i(\omega t - kz) \tag{2.27}$$

$$\vec{B}(\vec{r},t) = \vec{\varepsilon}B_0\exp -i(\omega t - kz) \tag{2.28}$$

where k is magnitude of wave vector. Since electric and magnetic fields are real, observable quantities [26], for electromagnetic wave moving along positive z direction equation (2.27) and (2.28) becomes the following, for the phase shiftsδ.

$$\vec{E}(\vec{z},t) = E_0\cos(\omega t - kz + \delta) \tag{2.29}$$

$$\vec{B}(\vec{z},t) = B_0\cos(\omega t - kz + \delta) \tag{2.30}$$

Equation (2.21) is a wave equation for propagating in a material media. In this case for a wave propagating in $+z$ direction in a weakly absorbing medium the possible solution for the electric field is [25]

$$\vec{E}(z,t) = \vec{\varepsilon} E_0 \exp(-\alpha z)\cos(\omega t - kz) \tag{2.31}$$

where α is the natural absorption coefficient and $k = 2\pi n/\lambda$ is the magnitude of wave vector. Both α and k are related to the imaginary and real parts of the linear susceptibility $\chi^{(1)}(-\omega;\omega)$, and to the refractive index n and the speed of light in the medium by the following equations

$$\alpha = \frac{\omega}{c}\mathrm{Im}\left\{\chi^{(1)}(-\omega;\omega)\right\} \tag{2.32}$$

$$n = \sqrt{1 + R_e\left\{\chi^{(1)}(-\omega;\omega)\right\}} \tag{2.33}$$

$$k = \frac{\omega}{c} = \frac{n\omega}{c_0} = \frac{2\pi n}{\lambda_0}$$

Here, $c_0 = 2.9979 \cdot 10^8\ \mathrm{m\,s^{-1}}$ is the speed of light in vacuum and λ_0 the vacuum wavelength. For non-absorbing medium or far from the resonance $\chi^{(1)}$ is a real quantity and related to a property n, which is commonly known as the refractive index of the material.

The real and imaginary parts of susceptibility are in certain cases coupled through Kramers-Kronig relations such as

$$R_e\left\{\chi^{(1)}(\omega_{eg})\right\} = \frac{2}{\pi}\int_0^\infty \frac{\omega\,\mathrm{Im}\,\chi^{(1)}(-\omega;\omega)}{\omega_{eg}^2 - \omega^2}d\omega \tag{2.34}$$

where ω_{eg} is the frequency of maximum absorption. The equation holds for $\chi^{(1)}(-\omega;\omega)$ and thus relates the absorption coefficient (imaginary part) to the refractive index (real part) of the medium. For a static field the frequency is zero ($\omega = 0$) the susceptibility is expressed as $\chi^{(1)}(0;0)$. $\chi^{(1)}(0;0)$ is related to the relative permittivity or dielectric constant of the medium ε_r as follow

$$\chi^{(1)}(0;0) = \varepsilon_r - 1 \tag{2.35}$$

and can be determined from permittivity measuremen

2.3 Time dependent Perturbation Theory

If a system has initially a wave function ψ_i and energy E_i and the final state ψ_f and E_f the new Hamiltonian $\hat{H}$ due to perturbation is the sum of unperturbed $\hat{H}^{(0)}$ and the perturbing $H^{(1)}$ Hamiltonians [24, 31]. Therefore

$$\hat{H} = \hat{H}^{(0)} + \hat{H}^{(1)} \tag{2.36}$$

if the perturbation is due to an electric field the change in Hamiltonian due to this field can be

$$\hat{H}^{(1)} = \hat{\vec{\mu}} \cdot \vec{E} \tag{2.37}$$

where $\hat{\vec{\mu}}$ and $\vec{E}$ represent the dipole operator and the electric field respectively. The rate of transition probability of the system dP_{fi}/dt is expressed through the Fermi Golden rule [24] as

$$\frac{dP_{fi}}{dt} = \frac{1}{6\varepsilon_0 \hbar^2} \left|\mu_{fi}\right|^2 \rho(\nu_{fi}) . \tag{2.38}$$

The rate of transition depends on transition dipole moment and energy density of radiation. It is also expressed in terms of Einstein's coefficient of induced absorption (B_{fi}) [24]. Hence

$$\frac{dp_{fi}}{dt} = B_{fi}\rho(\nu_{fi}) \tag{2.39}$$

where B_{fi} is the Einstein's coefficient for the induced absorption and is expressed as

$$B_{fi} = \frac{1}{6\varepsilon_0 \hbar^2} \left|\mu_{fi}\right|^2 \tag{2.40}$$

2.4 Comparision of Rate Probability with Experimental Quantities

Now we relate the rate of probability (microscopic molecule) to the molar decadic absorption coefficient (macroscopic). Since the square of electric field is directly proportional to intensity of the light [25], using equation (2.31).

$$I(z) \propto \left\langle \left(E(z,t)\right)^2 \right\rangle_T \tag{2.41}$$

$$I(z) \propto \left\langle \left(E_0 \exp(-\alpha z)\cos(\omega t - Kz)\right)^2 \right\rangle_T \tag{2.42}$$

$$\propto E_0{}^2 e^{-\alpha z} \tag{2.43}$$

This implies that

$$I(z) = I_0 e^{-\alpha z} \tag{2.44}$$

But the absorption coefficient α related to the molar absorption coefficient κ and molar decadic absorption coefficient $\varepsilon(\tilde{\nu})$ [30-31] as follow.

$$\alpha = \kappa c \tag{2.45}$$

$$\alpha = \ln(10)\varepsilon(\tilde{\nu})c \tag{2.46}$$

So equation (2.44) can be rewrite in terms of equation (2.45) and (2.46) as (when z=l)

$$I(z,\tilde{\nu}) = I_0 e^{-\kappa c l} = I_0 e^{-\ln(10)\varepsilon(\tilde{\nu})cl} \tag{2.47}$$

For very diluted and short path length the above equation becomes

$$I = I_0(1 - \varepsilon c l \ln(10)) \tag{2.48}$$

Differentiate both sides of equation (2.48) we have

$$-dI = I_0 \varepsilon c \ln(10) dl \tag{2.49}$$

we can also express c as follow

$$c = \frac{N}{N_a} \tag{2.50}$$

where N is number of molecules and N_a Avogadro numbers, therefore equation (2.49) becomes

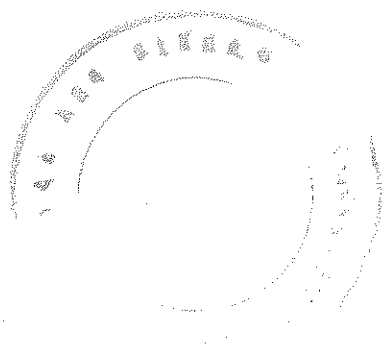
$$-dI = \varepsilon \frac{N}{N_a} I_0 \ln(10) dl \tag{2.51}$$

dP/dt is the rate of probability for a single molecule changes as a result of absorption of radiation under perturbing effect of electric field of radiation then $P_{fi} N dl$ is the number of molecules excited in a layer dl with an energy absorption $h\nu_{fi}$ So the loss in intensity can be written as follow [30-31].

$$-dI = \frac{dP_{fi}}{dt} N h\nu_{fi} dl \tag{2.52}$$

Comparing equation (2.51) an experimental quantities with equation (2.52) the quantum expression, the rate of probability can be expressed as (when $I_0 = \rho c$)

$$\frac{dP_{fi}}{dt} = \frac{\varepsilon(\tilde{\nu}) c \rho \ln 10}{N_a h\nu_{fi}} \tag{2.53}$$



In order to obtain the rate of probability of transition for the entire absorption band one has to integrate over the entire frequency range. Further by assuming ρ to be constant through out the band it results the following expression for the rate of transition probability dP_{fi}/dt

$$\frac{dP_{fi}}{dt} = \frac{c\rho \ln 10}{N_a h\nu_l} \int \frac{\varepsilon(\nu)}{\nu} d\nu = \frac{1}{6\varepsilon_0 \hbar^2} \left|\mu_{lm}\right|^2 \rho \tag{2.54}$$

Rearranging the above equation the transitional dipole moment therefore expressed as follow

$$\left|\mu_{fi}\right|^2 = 3\frac{2\ln(10)c_0\varepsilon_0}{N_a 2\pi^2} h \int \frac{\varepsilon(\tilde{\nu})}{\nu} d\tilde{\nu} \tag{2.55}$$

The factor 3 in the above equation is due to the orientation averaging of the transitional dipole in isotropic medium.

The total intensity of the band obtained by measuring $\varepsilon(\tilde{\nu})$ in the regions of absorption and usually determine by integrating the area under the graph. So the integrated absorption coefficient due to transition [33] becomes.

$$I_A = \int_{band} \frac{\varepsilon(\nu)}{\nu} d\nu = \int_{band} \frac{\varepsilon(\tilde{\nu})}{\tilde{\nu}} d\tilde{\nu} = \frac{1}{3} \frac{2h\pi^2 N_a}{\ln(10)c_0\varepsilon_0} \left|\mu_{fi}\right|^2 = \frac{1}{3} S \left|\mu_{fi}\right|^2 \tag{2.56}$$

where

$$S = 2.9352 \times 10^{60} C^{-2} mole^{-1}$$

3. Materials and Methods of the Experiment

In this chapter, laboratory apparatus, Instruments, chemicals and experimental procedures will be discussed.

3.1 Apparatus and Instruments

The laboratory apparatus used for the experiment listed as follow. Beakers, measuring cylinders, pipettes, volumetric flasks, spatula, magnetic stirrer with hot plate, funnel, separatory funnel, glass filter, quartz cuvete and 25μm sieve. In addition, different instruments were used. The types and their specification will be discussed here. Different electronic balances for measuring mass of coffee (SA 120, number 14510, a division 10^{-4} g , BOULDER company), for measuring mass of water and dichloromethane solution (SM 1600, with a division of 10^{-2} g, by Sauter company), the UV/Vis spectrophotometer with a model SPECTRONIC® GENESYS™ 2PC, wavelength range 200-1100 nm, wavelength accuracy ±1 nm, absorbance (photometric) accuracy ± 1 nm percent and slit width 2 nm. The spectrophotometer was interfaced with personal computer, which is used to be operated by WinSpec™ application soft wares.

3.1.1 UV/Vis Spectrophotometer

In this section the components and working principles of UV/Vis spectrophotometer will be discussed. Generally Spectrophotometer is used to measure the absorption or emission of radiation using a photo detector. The spectrophotometer used in the 200-800 nm regions are known as UV/Vis spectrophotometer, however the modern extends to IR regions. The main components of the spectrophotometer are shown in block diagram below.

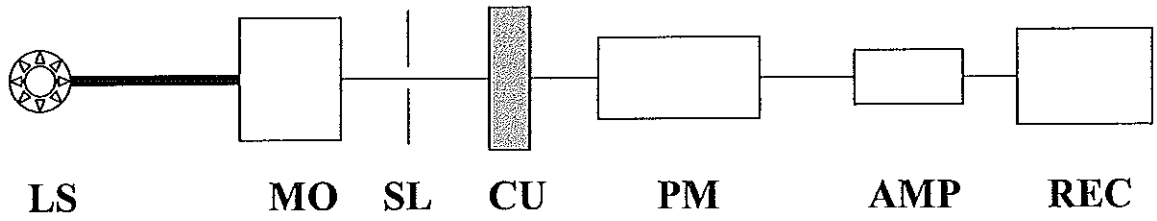


Fig. 3.1: Block diagram of spectrophotometer (LS light source, MO monochromator, SL slight width, CU cuvete, PM photo multiplier, AMP amplifier, REC recorder)

In fig 3.1 the major components of the spectrophotometer are shown. In the diagram LS represents the light sources. There are two types of radiation sources in the instrument to the entire UV to near IR region. The hydrogen and deuterium lamps are the most common sources of ultra violet radiation and the tungsten filament lamp is the source of visible and near infrared radiation. The light from the lamp has passed through monochromator MO that selects a certain part of continuous radiation. The monochromator contains an entrance slit, a collimating lens, dispersing device, a focusing lens and an exit slit. A radiation of only a particular wavelength leaves the monochromator through an exit slit SL. A quartz cuvete CU is a container to hold a sample solution. It is transparent through out the measured wavelength range. An incident light passes through the cuvette. The light passes through the sample holder detected by photomultiplier PM. The photomultiplier converts the photon energy to electrical current. This electrical signal quantitatively related to the radiation power transmitted through the sample in the cuvette and amplified in amplifier AMP and finally reaches the recorder REC where the results are recorded digitally in a PC attached to the instruments.

3.2 Chemicals

The chemicals used for the experiment are presented and described in the following. Different solvents distilled and de-ionized water, dichloromethane (dichloromethane, 99.6 % A.C.S, reagent, ALDRICH), chloroform (Content/assay(GC) 99.0-99.4 %, Riedel-Detlaen) caffeine produced by Evans pharmaceutical industry company (England) and coffee seeds were brought from Ethiopian coffee and tea quality and liquoring center. The coffee seeds washed at the standard of export. The coffee samples brought from Bench maji, Gediyo yergacheffe, west wellega (Ayera guliso), Mezenger (godare), Jimma (Gomma limu) Fig-below shows molecular structure of caffeine

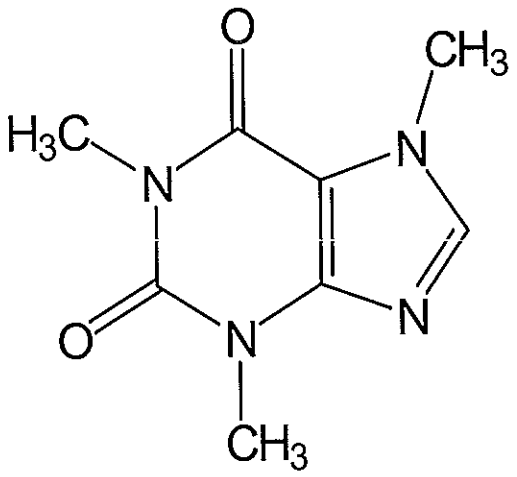


Fig. 3.2: Molecular structure of caffeine.

3.3. Measuring Physical Quantities

The physical quantities measured here are the absorbance versus the wavelength using PC interfaced to spectrophotometer. The application soft ware was Win Spec™ that has been designed to operate with spectrophotometer. It contains different application programs such as absorbance, %transmittance, concentration, absorbance difference, absorbance ratio, kinetic, multiple wavelength, and scan application. In this experiment the measurement of the program were run according to the following procedures. Select the measurement mode enter the

appropriate wavelength to specify the initial and end wavelength range (200 -500 nm) with medium (600 nm min^{-1}) scan speed. Then a clean cuvete is filled with a pure dichloromethane placed in the cell holder, the cover closed and press RUN to store the base line data. Finally the samples put in to the instrument and read the data displayed on the screen in the form of spectra. For data analysis the spectra was converted to ASCII format.

3.4 Methods of the Experiment

In determining the percentage of caffeine in different coffee seeds, using UV/Vis two main steps are necessary. The first step is set up calibration using pure caffeine and the other is set up experimental procedure to determine the concentration caffeine in coffee seeds. For the calibration, pure caffeine and dichloromethane were used. The calibration performed according to the following procedures. Caffeine and dichloromethane weighed. The caffeine dissolved in dichloromethane and stirred for 1 hour using magnetic stirrer. Using the molecular weight of caffeine and the above measured quantities the concentration c of caffeine determined. The absorbance measurement of this concentration was undertaken using UV/Vis spectrophotometer. From the absorbance, value obtained the molar decadic absorption coefficient of caffeine determined using Lambert- Beer's law.

On the other hand, in determining the caffeine concentration in coffee seeds, two main procedures are found to be necessary. These are the preliminary and extraction procedures implemented.

3.4.1 Preliminary Procedures (Grinding green coffee by mortar and pestle)

The ground coffee is sieved by micro sieve which has a scale of 250 μm to get a uniform texture About 50 mg, sieved coffee is measured and added to 25 ml of de-ionized water. Slowly heating the solution of coffee powder and stirred by magnetic stirrer for an hour

3.4.2 Procedures for Extraction of Caffeine from Coffee Dissolved in De-ionized Water (Liquid-Liquid Extraction)

The procedures that are used to extract caffeine from coffee dissolved in de-ionized water performed according to the following:

1. 25 ml dichloromethane is added to a coffee solution prepared under procedure (3.4.1) and stirred again by magnetic stirrer for 10 minutes
2. The above mixture added to a separator funnel. Since the density of water and dichloromethane is not equal dichloromethane, extract caffeine from water solution.
3. The dichloromethane, which has caffeine stored in a volumetric flask. The peak absorbance of this solution has been observed under spectrophotometer. Here rinse the cuvette at least two times is necessary to be run with the first standard
4. The above steps (1-3) repeated for six times until the caffeine spectrum disappeared when observed by UV/Vis spectrophotometer

The extracted solution stored in a volumetric flask, finally measured and the volume of the solution determined as

$$V_{sol} = \frac{m_{sol}}{\rho_{dic}} \tag{3.1}$$

The peak absorbance (A) of solution is measured. Using equation Beer-Lambert's the absorbance expressed as

$$A = \varepsilon \, c l \tag{3.2}$$

but

$$c = \frac{m_{caf}}{MV} \tag{3.3}$$

Therefore, the mass of caffeine in terms of equation (3.2-3.3) rewrite as

$$m_{caf} = \frac{AMV}{\varepsilon l} \tag{3.4}$$

The percentage of caffeine in coffee seeds calculated as follow. The mass of coffee measured under 3.4.1 and mass caffeine calculated above combine together and becomes

$$\%\text{Caffeine} = \frac{m_{caf}}{m_{co}} \times 100\% \tag{3.5}$$

4. Results and Discussion

In this chapter, electronic transition properties of caffeine investigated in water and dichloromethane are discussed. From experimental results, the molar decadic absorption coefficient, transitional dipole moment of caffeine in water and dichloromethane are also calculated. Moreover, technical procedures developed to determine the caffeine contents in coffee seeds using UV/Vis would also be presented.

4.1 UV/Vis absorbance of Caffeine in Water and Dichloromethane

In this section the electronic transition properties of caffeine in water and dichloromethane were investigated. A caffeine of mass 5.03×10^{-4} g dissolved in 23.07 cm^3 of water and absorbance of this solution was measured. The UV/Vis absorption spectrum of caffeine in water found to be in the region of 243 -302 nm. It is clearly shown in fig 4.1 that the spectral intensity of caffeine drops to zero at wavelength greater than 302 nm, on the other hand a new peak absorbance 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=1.224 at the maximum wavelength λ_{max} =272 nm. The position at which the maximum peak absorbance for caffeine obtained in these experiments is quite similar with the one obtained by [4]. The molar decadic absorption coefficient measures the intensity of optical absorption at a given wavelength [33] were calculated using equation (3.2). The molar decadic absorption coefficient of caffeine in water is computed and value of ε_{max} =1112 m^2 $mole^{-1}$ is obtained. The transitional dipole moment of the dissolved molecule, which is related to the molar decadic absorption coefficient by the integral absorption coefficient, was calculated using equation (2.56). Fig 4.2 shows the spectra of caffeine in water given as a function of $\varepsilon(\tilde{\nu})/\tilde{\nu}$ versus $\tilde{\nu}$. The integrated area under this curve $I_A = 129.48$ m^2 $mole^{-1}$ was obtained by integrating from $\tilde{\nu}_1$ =33,000 $cm^{-1}$to $\tilde{\nu}_2 = 41{,}000$ cm^{-1} therefore, the transitional dipole moment of caffeine in water μ_{fi}= 11.50×10^{-30} C m were obtained.

Similarly, Caffeine of mass 3.05×10^{-4} g is dissolved in 15.19 cm^3 of dichloromethane. The UV/Vis absorption spectra of caffeine in dichloromethane found to be in the region of 243-301 nm. The characteristic of the band observed at the two ends similar with that of water. Peak absorbance A=1.043 was obtained at maximum wavelength λ_{max} = 274.7 nm. The molar decadic absorption coefficient of caffeine in dichloromethane ε_{max} = 1010 m^2 $mole^{-1}$ is determined. Fig 4.3 shows the peak absorbance versus maximum wavelength of caffeine in dichloromethane. And fig 4.4 below shows the graph of $\varepsilon(\tilde{\nu})/\tilde{\nu}$ versus $\tilde{\nu}$ in dichloromethane. The integrated area under this graph I_A=114.16 m^2 $mole^{-1}$ in the wave number between $\tilde{\nu}_1$=33,000 cm^{-1} to $\tilde{\nu}_2$=41,000 cm^{-1}. From the integral absorption value the transitional dipole moment of caffeine in dichloromethane determined to be μ_{fi}=10.80×10^{-30} Cm. Since ε_{max} of caffeine is high both in water and dichloromethane this clearly indicate that caffeine is due to $\pi \rightarrow \pi^*$ transition.

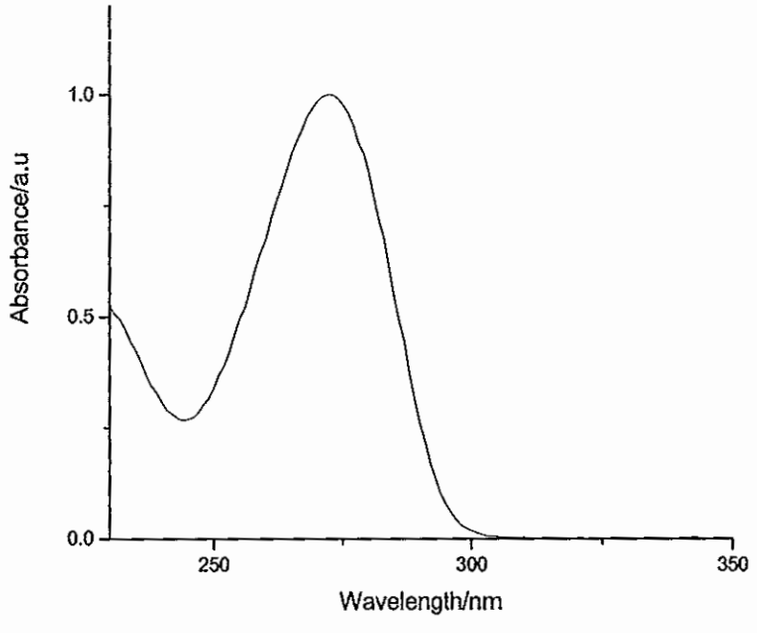


Fig. 4.1: UV-Vis spectra of caffeine in water.

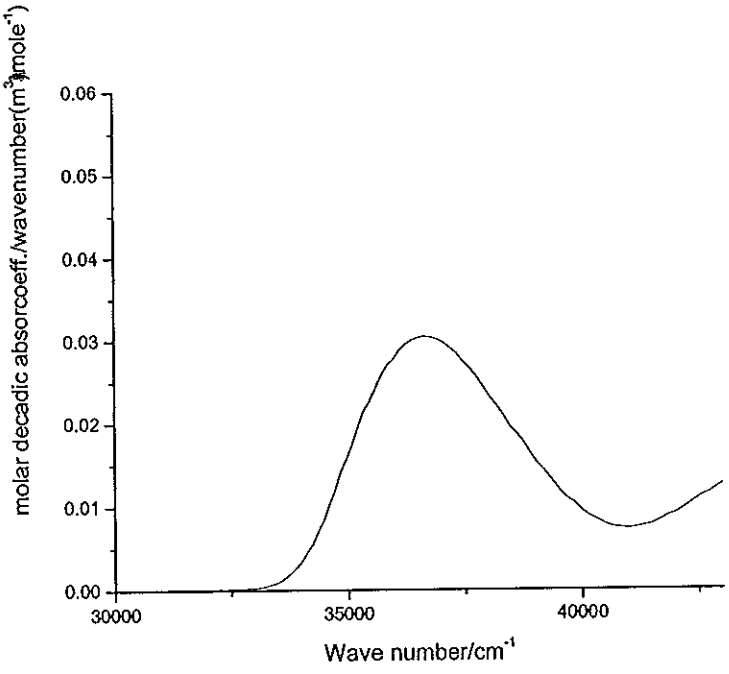


Fig. 4.2 Molar decadic absorption coefficient divided by wave number versus wave number of caffeine in water.

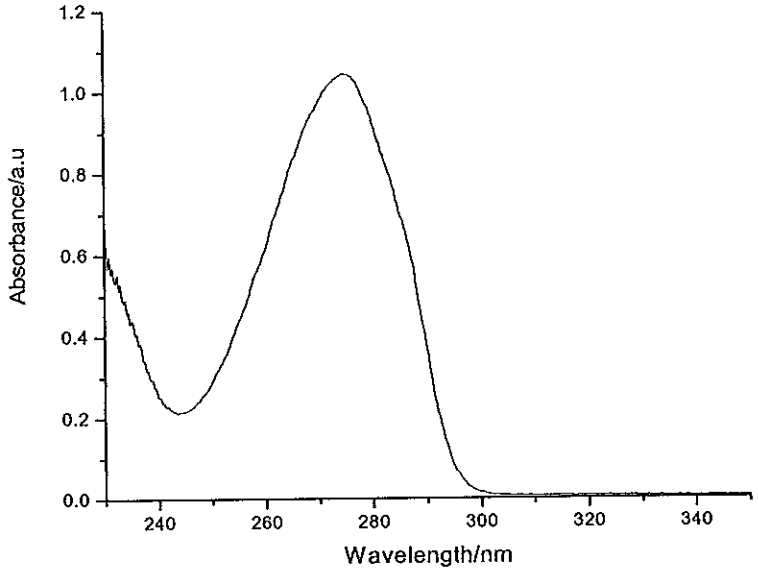


Fig. 4.3: UV-Vis spectra of caffeine in dichloromethane.

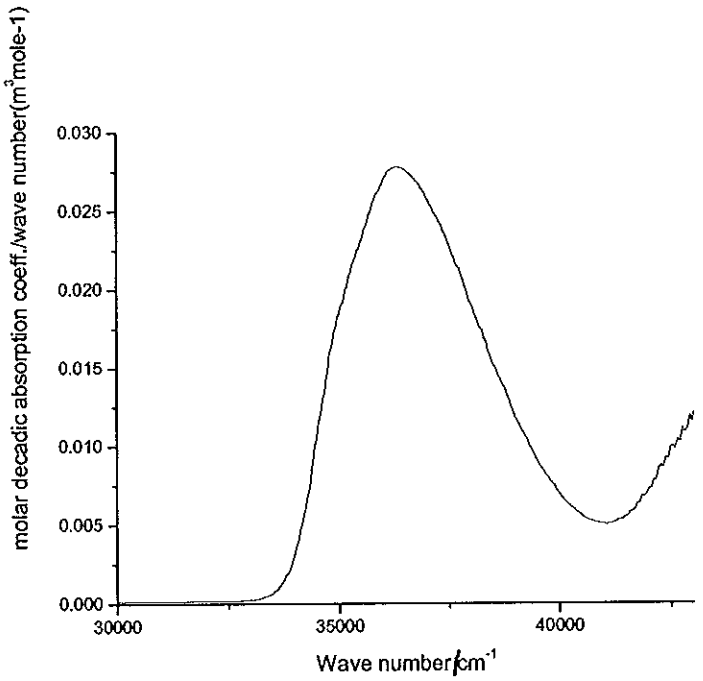


Fig. 4.4: Molar decadic absorption coefficient divided by wave number versus wave number in dichloromethane.

Fig 4.5 shows the UV/Vis absorbance versus wavelength of caffeine in chloroform. The absorption spectra of caffeine in chloroform were recorded between 243-299 nm and maximum peak absorbance, A=0.353 observed at wavelength λ_{max}=275.5 nm. This value is similar to the corresponding value reported by [4]. A similar character was observed with water at the two ends of spectral regions except a peak shift of 3 nm to higher wavelength in chloroform. Fig 4.6 shows the normalized spectra of caffeine in water, dichloromethane and chloroform respectively. It is observed from the obtained results that the shape of absorption bands of caffeine found to be identical in water, dichloromethane, and chloroform respectively with a slight change in a maximum wavelength of caffeine in three solvents. The changes in the position of absorption bands are mainly due to the dipole moment of the molecule in the ground state and the change in the dipole moment during excitation process [33].

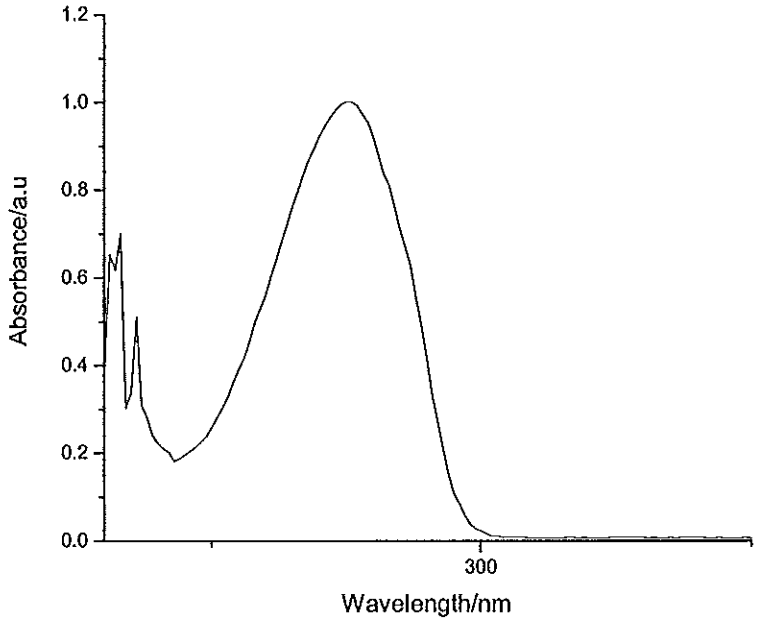


Fig 4.5: UV/Vis spectra caffeine in chloroform

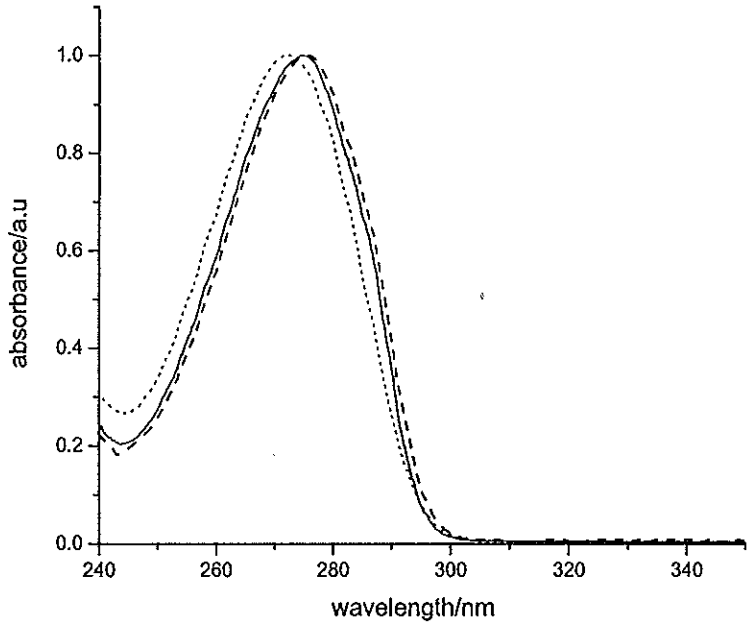


Fig 4.6The normalized spectra of caffeine in different solvents. (---) In chloroform, (....) in water and (—) in dichloromethane

4.2 Technical Procedures Developed to determine The Caffeine Contents in Coffee Seeds by UV/Vis

A UV/Vis spectrophotometer method cannot be used directly for the determination of caffeine in coffee seeds owing to the matrix effect of UV absorbing substances in the simple matrix [22, 34]. This effect can clearly be seen in the spectral bands of caffeine in coffee seeds (figs 4.7-4.8) dissolved in water and chloroform. Although water and chloroform are good solvents for dissolving it is observed that caffeine spectra interfere with other unknown compounds. So this spectrum is not suitable to determine the percentage of caffeine in coffee seeds due to the overlapping of these bands.

Inorder to overcome this difficulty coffee was first dissolved in water and extracted using dichloromethane to remove the interferences. The extraction was made six times until the spectrum of caffeine becomes a flat when seen under UV/Vis spectrophotometer. Figs 4.9a-f below show peak absorbance versus wavelength of caffeine spectra extracted using dichloromethane from water solution at different stages. It is clearly shown in the figures that the concentration of caffeine at each stage is different the size of peak absorbance also varies. From the results obtained high amount of caffeine concentration were extracted in the first stage of extraction. Moreover as seen from the graph almost no caffeine peak were seen after third extraction so it is possible to say that more than 95 % of caffeine has been extracted up to third round of extraction. Fig 4.9a shows the first stage of extraction, fig 4.9b the second stage of extraction, fig 4.9c the third stage of extraction, fig 4.9d the fourth stage of extraction, fig 4.9e the fifth stage of extraction and fig 4.9f the sixth stage of extraction. The comparison of caffeine peaks at each stages are shown in fig 4.10 the spectra of all stages are overlapped no peak has been observed for fourth, fifth and sixth stages respectively.

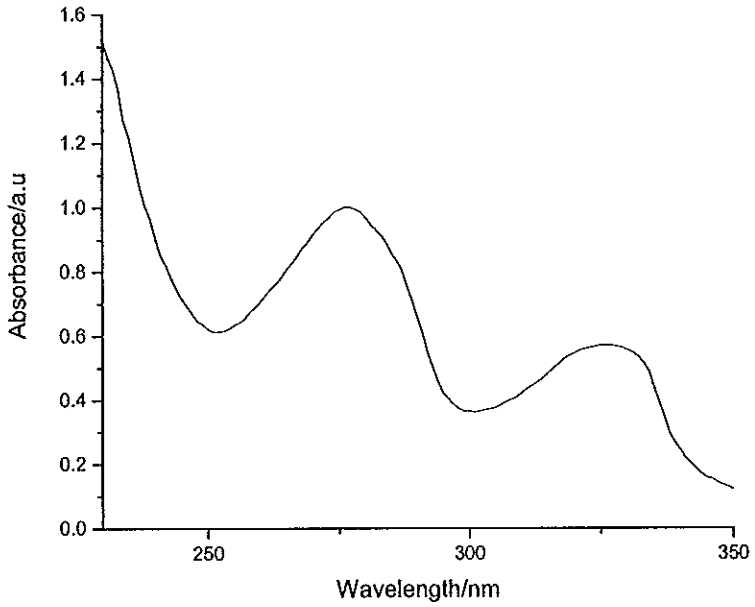


Fig 4.7 UV/Vis spectra of caffeine in coffee in water

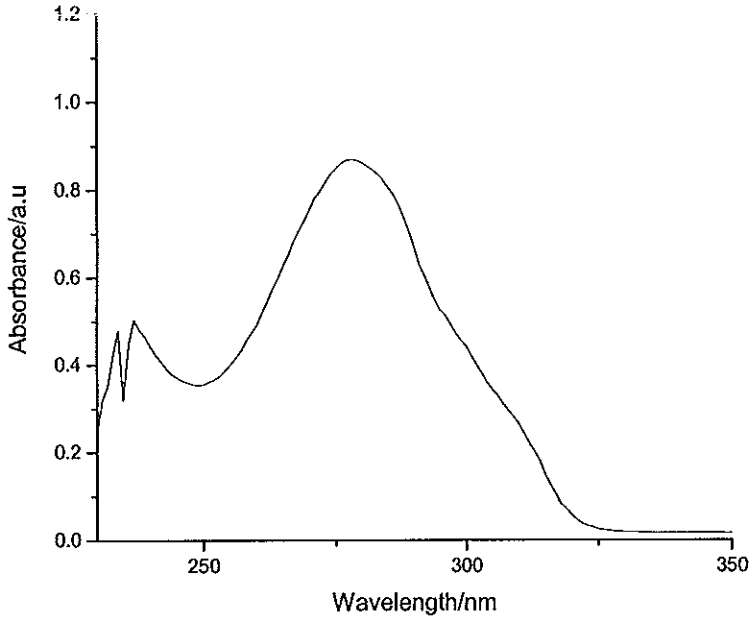


Fig. 4.8: UV/Vis spectra of caffeine in coffee in chloroform.

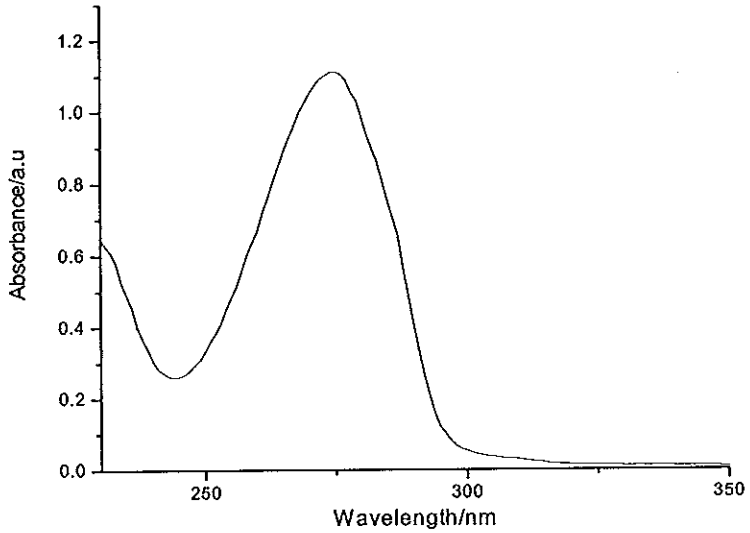


Fig 4.9a UV/Vis spectra of caffeine for first stage of extraction.

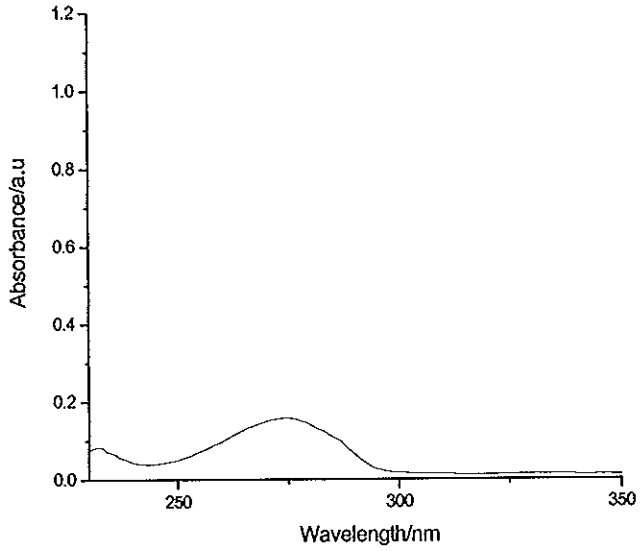


Fig. 4.9b: UV/Vis spectra of caffeine for second stage of extraction.

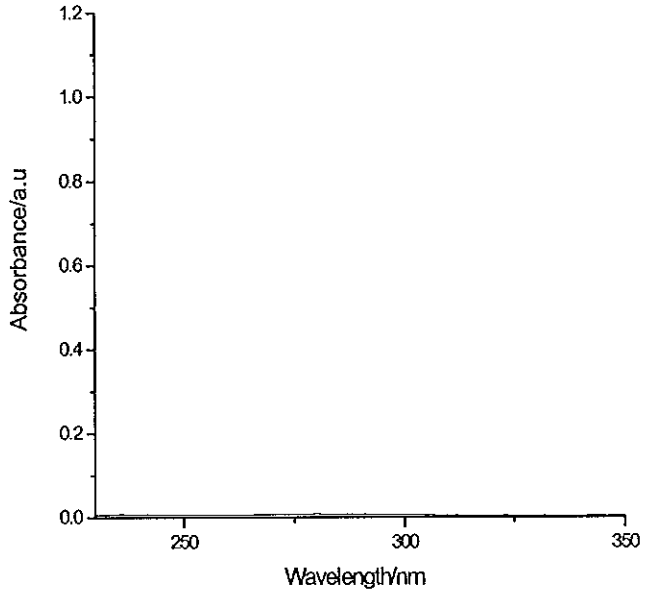


Fig. 4.9e: UV/Vis spectra of caffeine for fifth stage of extraction.

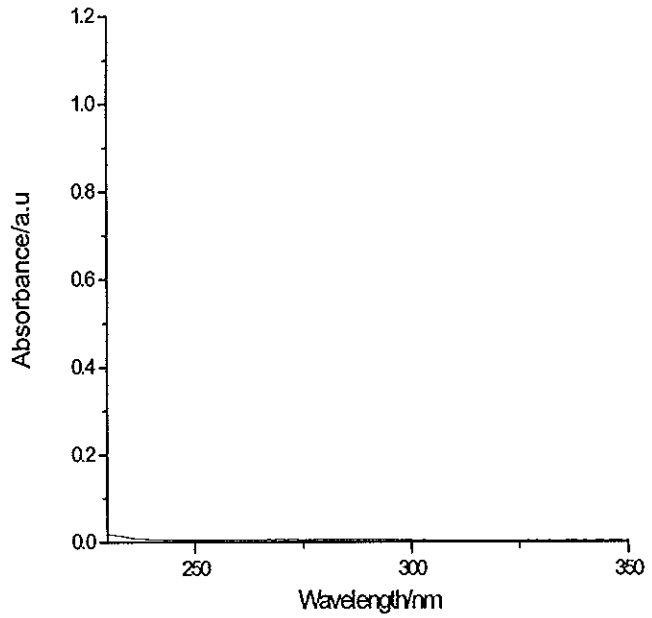


Fig. 4.9f: UV/Vis spectra of caffeine for sixth stage of extraction.

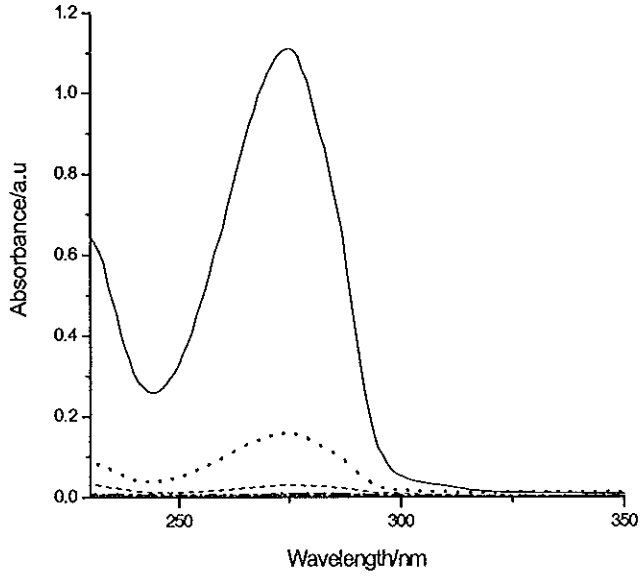


Fig. 4.10: The over lapped spectra of caffeine for different stages (—) for first stage of extraction, (...) for second stage of extraction, (----) for third stage of extraction, (– · – ·) for fourth stage of extraction, (– ·· – ··) for fifth stage of extraction and (·····) for sixth stage of extraction.

The extraction techniques could not completely remove the possible interferences with caffeine spectra. Therefore, extracting qualitative and quantitative information from the spectra composed of unresolved bands is impossible. To eliminate the effect of interference we have applied Gaussian fit. It is also assumed that the shape of interferences spectra have a Gaussian function. Fig 4.11 shows the spectra of caffeine in coffee seeds extracted by dichloromethane before Gaussian fit while fig 4.12 shows the spectra of caffeine with Gaussian fit and fig 4.13 the spectra of caffeine after best Gaussian fit is subtracted. As it seen in fig 4.13 the shape of the spectra after fitting exactly similar with the shape of spectra of pure caffeine dissolved in dichloromethane. Further to compare this spectra with pure caffeine the two spectra were overlapped. Fig 4.14 shows the normalized spectra of pure caffeine and the spectra after Gaussian fit, as it observed in the figure below the two spectra exactly fitting to each other. Therefore using extraction followed by Gaussian fit it is possible to get a good spectrum of caffeine which could possible to determine the concentration of caffeine in coffee seeds.

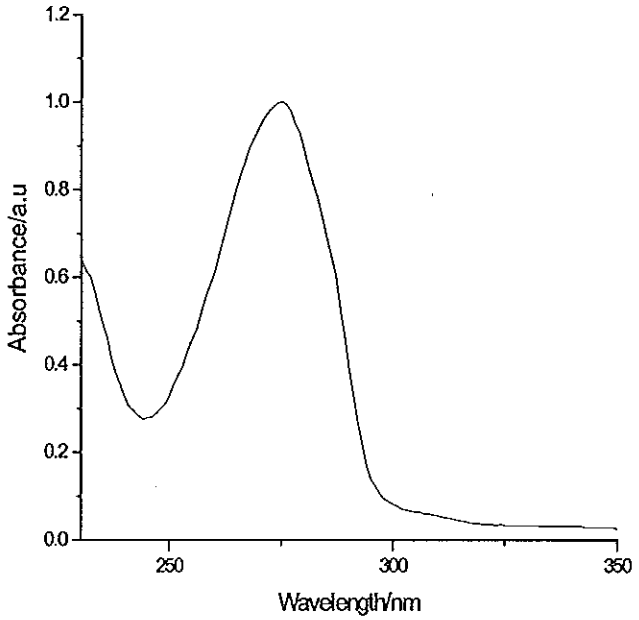


Fig. 4.11: UV/Vis spectra of caffeine before Gaussian fitting.

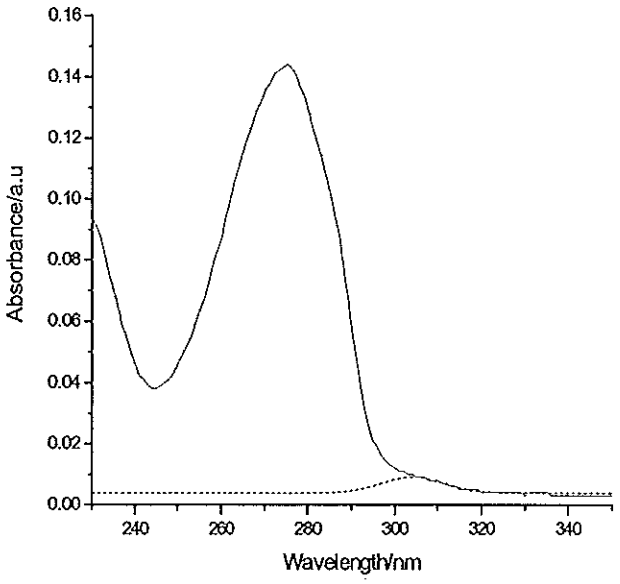


Fig. 4.12: The UV/Vis (—) spectra of caffeine in coffee and (...) Gaussian fit.

6. References

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