ORIGINAL RESEARCH PAPER

UV/VIS SPECTROMETER DETERMINATION OF CAFFEINE IN GREEN COFFEE BEANS FROM HARARGHE, ETHIOPIA, USING BEER-LAMBERT'S LAW AND INTEGRATED ABSORPTION COEFFICIENT TECHNIQUES

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Abstract: A total of fifteen samples of green coffee (*Coffea arabica L.*) beans from the major producing region of Hararghe Ethiopia were studied using UV-Vis spectrometer measurement caffeine quantitative analysis from coffee beans. The number density of caffeine in green coffee beans has been reported using Beer-Lambert's law and integrating absorption coefficient technique. Our results obtained using integrated absorption and Beer-Lambert's law has a good agreement and we observed a maximum difference of 10.4 %. Based on their low caffeine concentrations among the samples collected were found in Jarso coffee. Coffee beans from the Harar Aboker were characterized by higher concentrations of caffeine. The determined concentration for caffeine in coffee beans (% w/w) ranged 0.601 % to 0.903 %. The concentrations of the caffeine varied significantly, depending on the geographical origin of the beans. The concentrations of caffeine in coffee collected from in Hararghe region were noticeably lower than their counterpart (1.0 - 1.2 %) grows in the other parts of Ethiopia.

Keywords: absorbance, arabica coffee, caffeine, molar decadic absorption coefficient, oscillator strength

INTRODUCTION

Caffeine is an alkaloid of the methylxanthine family found in many foods and beverages, including coffee, tea, soft drinks and chocolate [1, 2]. Coffee drinking based on epidemiological and experimental studies has shown various physiological effects such as relaxation of bronchial muscle, stimulation of the central nervous system through its adenosine antagonist action, gastric acid secretion and dieresis [3], but in high doses caffeine consumption can be associated with the following symptoms: nervousness, anxiety, restlessness, insomnia, gastrointestinal upset, tremors, tachycardia and psychomotor agitation [4]. Even low doses of caffeine, such as those found in decaffeinated coffee, can have psychoactive properties [5]. The stimulating effect of caffeine is not only exploited in nutrient technology but also in cosmetics and pharmaceuticals, which accounts for the economic importance of this particular additive [6]. Coffee's most studied component, caffeine, varies substantially depending on the coffee species, method of bean-roasting and beverage preparation. The widespread natural occurrence of caffeine in a variety of plants undoubtedly played a major role in the long-standing popularity of caffeine-containing products, especially coffee [7, 8]. The coffee tree is a tropical evergreen shrub (genus *Coffea*) which grows in the area between the Tropics of Cancer and that of Capricorn. The dominant commercial species of coffee are Arabica (*Coffea arabica L.*) and Robusta (*Coffea canephora Pierre*). Arabica beans are more expensive and valuable because of their preferable flavor in comparison to Robusta beans [9 – 11]. As suggested by its name, Robusta coffee trees are more robust, a high - yielding and disease - resistant tree standing up to 12 meters tall that grows best in warm, humid climes, and less climate-specific than Arabica trees [12, 13]. Robusta coffee contains higher amounts of antioxidant compounds, caffeine, and soluble solids, thus resulting in added body and increased yield. Arabica coffee is superior quality coffee and sells for a higher price; however Robusta does not give rise to the highest quality coffee [12, 14]. Majority of coffee produced around eight countries in Southern and Central America, the Caribbean, Africa and Asia. Robusta grows in Western and Central Africa, South-East Asia, and, to some extent, in Brazil, while Arabica, originated in the Ethiopian highlands, which accounts for three-quarters of world production, grows in Ethiopia, Latin America, India, and, to some extent, Indonesia [12].

For caffeine quantitation, chromatographic methods (HPLC) are still regarded as state-of-the-art [15 – 17]. However, the desire for shorter analysis times drives many analytical methods for caffeine determination in the most varied matrices [5], amongst them: fluorescence spectroscopy [6], near-infrared reflectance spectroscopy (NIRS) [10, 18]; liquid chromatography-mass spectrometry [19]; gas chromatography-mass spectrometry/flame ionization detection (GC-MS/FID) [20]; ion chromatography (IC) [21]; Gas chromatography equipped with a sensitive nitrogen–phosphorous detection system (GC-NPD) [22]; Fourier transform-infrared spectrophotometry (FT-IR) [23]. However, UV-Vis spectrophotometry method which is available in most laboratories is easy, fast and cheap for the determination of the caffeine contents in Arabic coffee beans [3].

Due to the widespread consumption of caffeine and its potential physiological and pharmacological effects, it is important for both health professionals and consumers to know the exact 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 [7, 25]. Despite the wide use of coffee in Ethiopia there is a lack of extensive studies addressing the issues related to the caffeine content of coffee occurring in Haraghe, Ethiopia. Therefore, the aim of this study was to retrieve the information about content of caffeine in green coffee bean collected directly from the farmers in various parts of Haraghe region, Ethiopia using Beer-Lambert's law and integrated absorption coefficient technique. Determination of caffeine content was conducted by means dichloromethane extraction using UV-Vis spectrophotometric methods. The peak height measurements based on Beer-Lambert's law and integrated absorption coefficient techniques were used to study the concentration and number of molecules $\cdot cm^{-3}$ of caffeine present in green coffee beans.

MATERIALS AND METHODS

Chemicals and samples

Fifteen Arabica bean coffees were collected from Hararghe region (Figure 1) in eastern part of Ethiopia. The collection areas were Chiro (Dingetie and Gewugewu), Gurache, Harar (Harar Aboker, Harar Zuria 1and Harar Zuria 2), Dire Dawa, Jarso, Kobo, Kurfachele, Girawa, Gorumute, Hirna (Hirina Zuria 1 and Hirina Zuria 2) and Langie. The chemical reagents used for standard solution preparation and caffeine extraction from coffee were Dichloromethane (sigma Aldrich, Germany) and doubly distilled water (Haramaya University, Ethiopia). For each run (15 samples), three repetitions of samples were included to monitor the reproducibility of our results. These repetitions were carried out by going to the places three times within six months to collect coffee samples from already chosen coffee farm land.
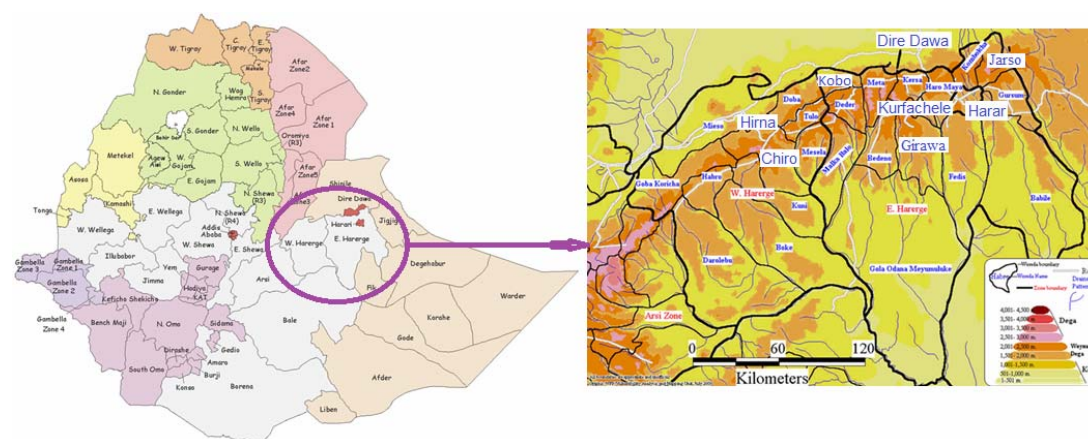

Figure 1. *Coffee bean Collection Sites for the present study in Hararghe area, Oromia regional state, Ethiopia*

Instrumentation

All absorption spectra were obtained using a double-beam spectrophotometer (Lambda 9-UV-Vis-NIR, PerkinElmer, USA) with 1 nm resolution in a 1 cm path length quartz cell. The excitation light source was Deuterium and Tungsten arc lamp. The spectra obtained by computer interfaced with spectrometer.

Standard solution preparation

From caffeine (Figure 2) stock solution, 0.56 mg of pure caffeine was added into 50 mL dichloromethane and de-ionized water, then by taking six volumetric flasks of 25 mL the caffeine solution was further diluted with various concentration of caffeine solution in dichloromethane or de-ionized distilled water. After preparing the desired concentration the caffeine solutions were stirred with magnetic stirrer for 30 min. The absorbance versus wavelength of the solution was measured using UV-Vis spectrometer. These procedures were repeated three times for the same concentrations then the mean value was taken. Finally, from the absorbance the molar decadic absorption coefficient and transitional dipole moment of caffeine in dichloromethane and water were obtained.
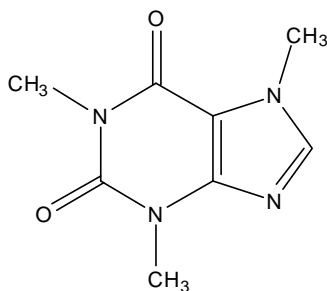

Figure 2. *Caffeine (1, 3, 5-trimethylxanthine)*

Coffee sample preparation

Green coffee beans were milled and pulverized using a coffee grinder. Approximately 50 mg of grounded coffee was weighed out on a three decimal place analytical balance. Then the grounded coffee was dissolved in 25 mL of distilled water. The solution was stirred for one hour using magnetic stirrer and heated gently to 60 °C to remove caffeine easily from the solution. In addition the solution was filtered by a glass filter to get rid of particle from the solution. It had been observed that many interfering matrices were extracted with water than dichloromethane. The efficiency of dichloromethane to extract caffeine from coffee beans is 98 - 99 % and caffeine is more soluble in dichloromethane (140 mg·mL^{-1}) than it is in water (22 mg·mL^{-1}) [3, 25]. To extract caffeine from water, the coffee solution prepared above (under coffee sample preparation) was mixed with 25 mL dichloromethane for the extraction of caffeine from coffee. First, a mixture of the solution was stirred for 15 min. Then, using a separatory funnel caffeine was extracted by dichloromethane from the solution. The extraction of caffeine was proceeded 3 times with 25 mL dichloromethane at each round. The contents of caffeine in the 4th extract were found negligible. The total content of caffeine in coffee samples was found from the spectrum of combinations of the three extractions.

RESULTS AND DISCUSSION

Calibration curve of pure caffeine in dichloromethane

The molar decadic absorption coefficient of caffeine in water and dichloromethane was obtained by measuring the intensity of the absorption of a series of concentration of caffeine in dichloromethane and water. Caffeine concentration of (0.01341 - 0.05801 mol·m^{-3}) and (0.00178 - 0.01482 mol·m^{-3}) was prepared in water and dichloromethane, respectively, for calibration curves. From the analysis of calibrations of dichloromethane (Figure 3), good linear relationships ($R = 0.99908$) were observed for a wide concentration range, which is convenient for the determination of caffeine in coffee beans. The molar decadic absorption coefficient measuring the intensity of optical absorption at a given wavelength was calculated using Beer-Lambert's equation (1) [26, 27].

$$A = \varepsilon \cdot c \cdot l \tag{1}$$

where A is absorbance, ε is the molar decadic absorption coefficient, l is a distance in the absorbing medium and c is the concentration of the absorbing compound.

The molar decadic absorption coefficient of caffeine in water and dichloromethane were calculated based on the Beer-Lambert's law and obtained value of 1143 m^2·mol^{-1} and 1051 m^2·mol^{-1}, respectively.
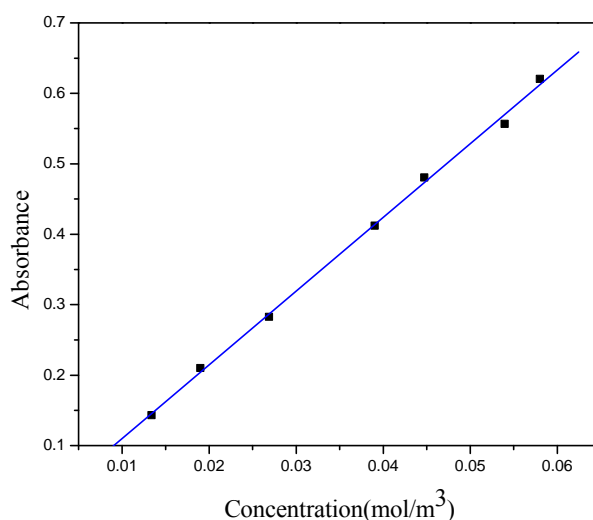

Figure 3. *Absorbance versus concentration graph for caffeine in dichloromethane*

Optical Transition Property of caffeine

The absorption spectrum of pure caffeine in water and dichloromethane were measured using UV-Vis spectrometer in the region of 210 - 365 nm at room temperature. The maximum absorption peak of dichloromethane was obtained at 277 while in water it occurred at 270 nm. As it can be seen in Figure 4 and 5 there was another absorption in

both solvent approximately below 241 nm as a result of solvent absorption in mentioned region.
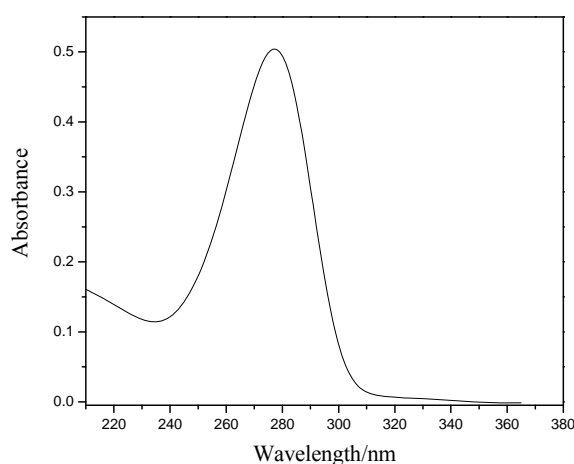

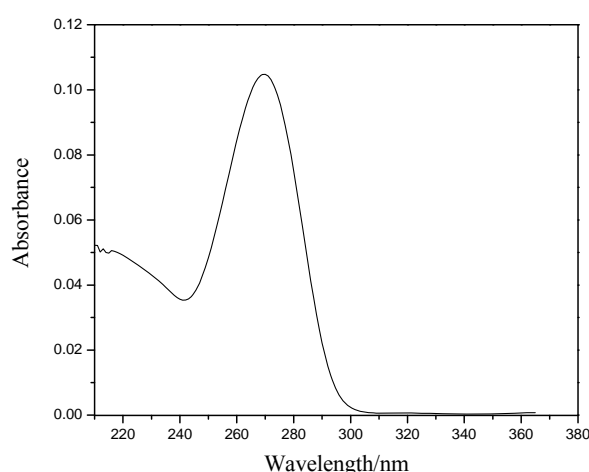**Figure 4.** *UV-Vis spectra of caffeine in dichloromethane*

Figure 5. *UV-Vis spectra of caffeine in water*

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) [3, 28]:

$$I_A = \int_{band} \frac{\varepsilon(\overline{v})}{\overline{v}} d\overline{v} = \frac{1}{3} \frac{2h\pi^2 N_a}{\ln(10)c_o \varepsilon_0} \left|\mu_{fi}\right|^2 = \frac{1}{3} s \left|\mu_{fi}\right|^2 \tag{2}$$

where: $S = 2.9352 \times 10^{60}\,C^{-2}\,mol^{-1}$,

μ_{fi} - transition dipole moment.

The transitional dipole moment of the dissolved molecule of caffeine in dichloromethane and water was calculated using the given equation. The absorbance's

versus wavelength of caffeine in dichloromethane was recalculated according to equation (2) as a function of molar decadic absorption coefficient over wave number versus wave number using Origin 7 software. As it can be seen in Figure 6 from area under the spectrum, integrated absorption cross-section of caffeine was calculated in the wave number regions of 29500 - 43500 cm^{-1}.
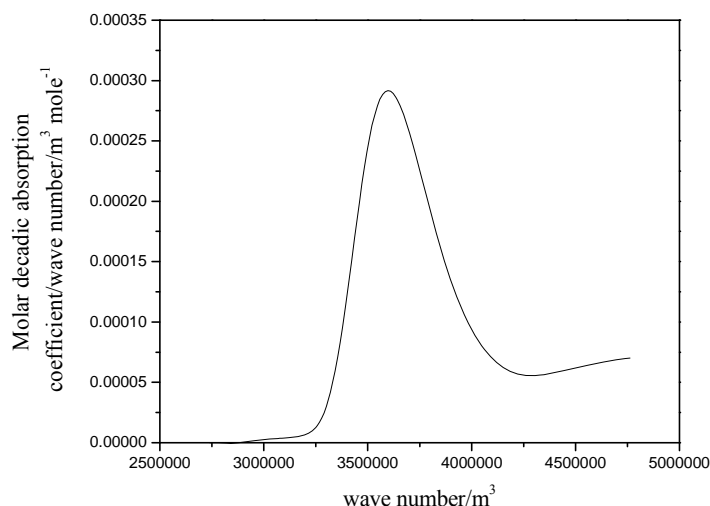

Figure 6. *Molar decadic absorption coefficient per wave number versus wave number of caffeine in dichloromethane*
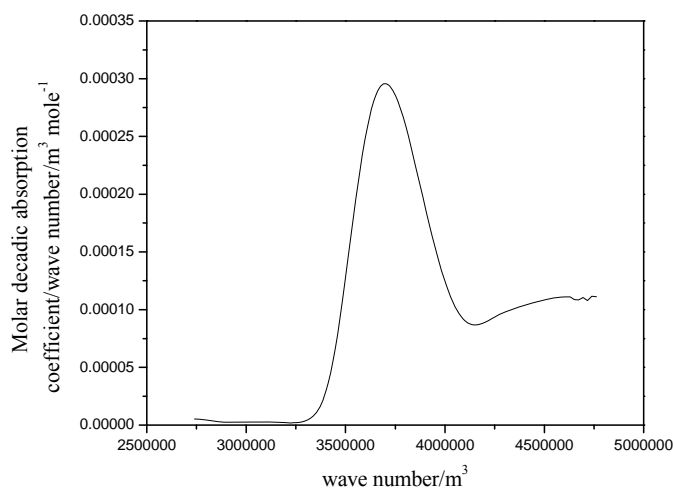

Figure 7. *Molar decadic absorption coefficient per wave number versus wave number of caffeine in dichloromethane*

The integrated area of caffeine in dichloromethane equals 182.422 $m^2 \cdot mol^{-1}$ was obtained by integrating between 29500 cm^{-1} and 43500 cm^{-1}. Similarly, the integrated area under the same range of curve for caffeine dissolved in water equals

228.046 m^2·mol^{-1} was obtained. Consequently, using equation (2) and the result obtained for the area under the curve for molar decadic absorption over wave number versus wave number the transitional dipole moment of caffeine in dichloromethane and water 13.655 × 10^{-30} Cm and 15.267 × 10^{-30} Cm was obtained, respectively.

The other most important parameter which provides the relative strength of electron transition is the oscillator strength. It is one of the fundamental quantities in analytical spectroscopy. In practice, it determines the sensitivity of a given atomic resonance line and needs to be accurately known if one to relate the magnitude of the absorption signal to its concentration: Oscillator strength related to molar decadic absorption coefficient by the equation (3) [26]:

$$f = 2.303 \frac{mc_0^2}{N_a \pi e^2 n} \int \varepsilon(\overline{v})d\overline{v} = 4.32 \times 10^{-9} \, mol \, cm^2 L^{-1} \int \varepsilon(\overline{v})d\overline{v} \qquad (3)$$

where: ε carries the unit L·mol^{-1}·cm^{-1} and the wavenumber in cm^{-1}.

Similarly, the oscillator strength using equation 3 was obtained by integrating molar decadic absorption versus wave number from 29500 cm^{-1} to 43500 cm^{-1}. The oscillator strength of caffeine in dichloromethane and water 0.3027 L·mol^{-1}·cm^{-1} and 0.3889 L·mol^{-1}·cm^{-1} was obtained, respectively.

Integrated absorption coefficient of pure caffeine in dichloromethane

Beer-Lambert's law based on peak absorbance was the most popular method to investigate the concentration of the targeted molecule in a sample. Recently, integrated absorption techniques becomes alternative methods instead of Beer-Lambert's law to determine the number density of caffeine in coffee beans. The integrated absorption coefficient (a_t) which is the sum of absorption coefficient (a_λ) for all frequencies in the band is expressed as equation (4). The absorption coefficient is the absorbance divided by the optical path length using equation (5). [29 − 31]:

$$a_t = \int a_\lambda d\overline{v} \qquad (4)$$

$$a_\lambda = \frac{A}{l} = \frac{1}{l}\log\frac{I_\lambda^0}{I_\lambda} \qquad (5)$$

Equation (4) related the integrated absorption coefficient with the number density for the Gaussian shaped spectrum. To determine the number density of caffeine in coffee the calibration curve correlating the integrated absorption coefficient with the corresponding number density of pure caffeine in dichloromethane was constructed. As it can be seen in Table 1 a series of concentration (0.01341-0.05801 mol·m^3) of caffeine dissolved in dichloromethane were prepared. The spectrum area of each sample was plotted against the number density to generate the calibration curve using Origin-7 software. The calibration equations are ($Y = 12.35 + 7.27494 \times 10^{-14} X$, $R = 0.99934$, SD = 29.94, N = 7) where Y is the area of absorption coefficient of caffeine in cm^2 and X is concentration for caffeine dissolved in dichloromethane.

Table 1. *Number density of caffeine dissolved in dichloromethane and the corresponding peak absorbance, integrated absorption coefficients, oscillator strength, and integrated absorption cross-section*

Peak of absorbance	Number density of caffeine [cm^{-3}]	Integrated absorption Coefficients, [cm^{-2}] ($\int a_\lambda d\overline{v}$)	Oscillator strength [cm/molecules]	Integrated absorption cross-section [cm/molecules]
0.14307	8.19488×10^{15}	592.836200	2.72990×10^{-8}	7.23423×10^{-14}
0.21013	1.20360×10^{16}	919.964610	2.88432×10^{-8}	7.64345×10^{-14}
0.28262	1.61881×10^{16}	1231.94103	2.87175×10^{-8}	7.61015×10^{-14}
0.41227	2.36143×10^{16}	1748.40295	2.79396×10^{-8}	7.40399×10^{-14}
0.48066	2.75316×10^{16}	2001.88643	2.74386×10^{-8}	7.27123×10^{-14}
0.55663	3.18831×10^{16}	2388.08191	2.82646×10^{-8}	7.49012×10^{-14}
0.62029	3.55295×10^{16}	2601.07016	2.76260×10^{-8}	7.32088×10^{-14}

Integrated absorption cross-section (σ_t) characterizes the photon-capture area of a molecule and oscillator strength can also calculated by relating the integrated absorption coefficient with the number density corresponds with the Gaussian shaped absorption spectrum according to equation (6) [26, 29]. The integrated absorption cross-section and oscillator strength were calculated from the following relations

$$\sigma_t = \frac{1}{N} \int a_\lambda \overline{v} \tag{6}$$

$$\int a_\lambda d\overline{v} = 2.65 \times 10^{-6} \, Nf \tag{7}$$

where: N is number density in molecules·cm^{-3}, a_λ in m^{-1} and f is oscillator strength of the transition molecule.

For measuring integrated absorption cross-section and oscillator strength of caffeine solutions in a similar concentration ranges (0.01341 - 0.05801 mol·m^{-3}) and in the regions of 17680 - 25406 cm^{-1} using equation (7) and (8) were measured. The absorbance versus wavelength measured by spectrophotometry was re-calculated into absorption coefficient versus wave number using Origin 7 software. From the area under the spectrum the mean (±S.D) value of integrated absorption cross-section and oscillator strength of caffeine in dichloromethane was obtained and it was $7.44 \times 10^{-14} \pm 0.17 \times 10^{-14}$ and $2.78 \times 10^{-8} \pm 0.064 \times 10^{-8}$ respectively.

Number density of caffeine in green coffee bean

In addition to caffeine spectrum there are interfering band from other coffee components (such as chlorogenic acid related compound) extracted by dichloromethane [3]. This effect can be observed in the spectral bands of caffeine in coffee bean dissolved by dichloromethane as shown in Figure 8; as seen from the Figure, caffeine spectra interfere with other compounds in coffee bean. To avoid the matrix associated with other coffee components the following equation (8) of Gaussian function was used by fitting to the experimental data.

$$y = y_0 + Ae^{-\left(\frac{x-x_c}{w}\right)^2} \tag{8}$$

where: y_0 - represents the minimum point,

A - is amplitude,

x_c - is the central wavelength,

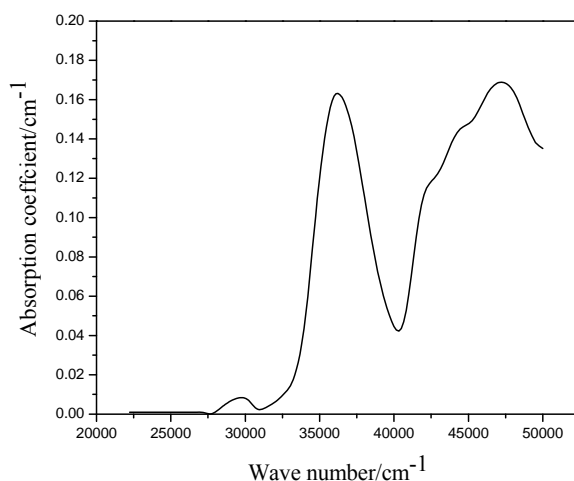w - is full width at half maxima of the equation.

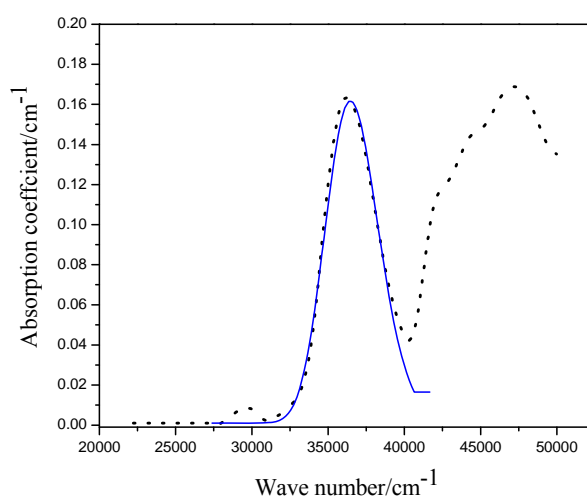**Figure 8.** *UV-Vis spectra of caffeine extracted by dichloromethane before Gaussian fitting*

Figure 9. *UV-Vis spectra of caffeine dissolved in dichloromethane - the Gaussian function (—) and the dot (.....) show plotted absorption coefficient versus wave number*

In this work the extraction of caffeine from coffee solution performed for three rounds with dichloromethane until the spectrum of caffeine becomes flat, when seen under UV-Vis spectrometer. However, the amount of caffeine extracted at each round of extraction is not equal. Figure 10 shows absorbance versus wavelength of caffeine extracted by dichloromethane solution from water at three rounds of extraction. In the fourth extraction, the content of caffeine was found negligible.
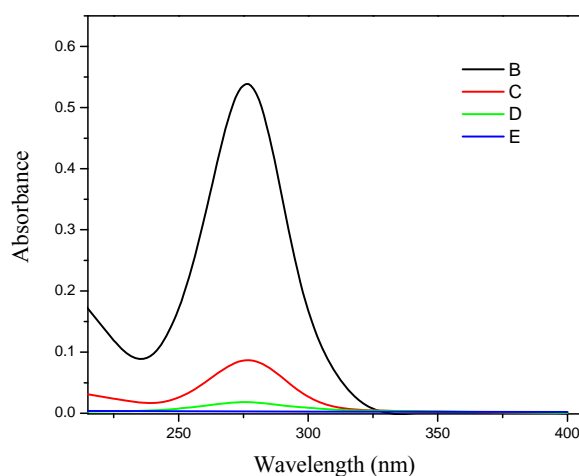
Figure 10. *The over lapped spectra of caffeine for different round of extraction from water solution using dichloromethane*
(B) first round, (C) second round, (D) third round, (E) fourth round

The number densities of caffeine in coffee beans were calculated by fitting the Gaussian function to the spectra of absorption coefficient versus wave number of caffeine extracted from coffee solutions. As it can been seen from Figure 10 the number density was calculated from the area under Gaussian function in the frequency region of 27397 - 47619 cm^{-1} using equation (4). Therefore using extraction with dichloromethane followed by Gaussian fit, the percentage of caffeine in coffee beans for fifteen samples was determined. The samples were collected from west and east Hararghe Ethiopia directly by the researchers from the farm lands. All coffees are green (Figure 8) and washed thoroughly to get rid of the dirt.

The calculated number density in terms of integrated absorption coefficient ranged from $(0.99205 - 1.79190) \times 10^{16}$ cm^{-1} as shown in Table 2. On the other hand, using Beer-Lambert's law a similar results of caffeine content in green coffee $(1.07111 - 1.86729) \times 10^{16}$ cm^{-1} were obtained. The caffeine content levels in green coffee beans calculated in terms of integrated absorption coefficient and Beer-Lambert's law were presented in Table 2. Our results obtained using integrated absorption and Beer-Lambert's law have a good agreement and we observed a maximum difference of 10.4 %. Streit et al., suggested that spectrally integrated absorption are more robust parameters than peak values [30]. Of all the samples tested, Harar Aboker coffee had the highest mean percentage of caffeine content and Jarso green coffee had the least mean percentage of caffeine.

Table 2. *The percentage of caffeine in coffee beans calculated by integrated absorption coefficient and Beer–Lambert's law (n = 3)*

Coffee samples	Peak of Absorbance (mean±SD)	Number density based on integrated absorption (mean ± SD) $\times 10^{16}$ [cm^{-3}]	Number density based on Beer–Lambert's law [cm^{-3}] (mean ± SD)$\times 10^{16}$cm^{-3}	Percentage of caffeine (w/w %) (mean ± SD)
Chiro (Dingetie)	0.224±0.001	1.17232±0.00483	1.28304±0.00584	0.60820±0.00277
Chiro (Gewugewu)	0.262±0.002	1.37627±0.00861	1.50070±0.00952	0.71138±0.00544
Gurache	0.294±0.001	1.58106±0.00412	1.68399±0.00502	0.79826±0.00254
Harar Aboker	0.326±0.002	1.79190±0.00799	1.86729±0.00899	0.90285±0.00601
Harar Zuria 1	0.274±0.002	1.49635±0.00819	1.56944±0.00931	0.75884±0.00538
Harar Zuria 2	0.248±0.001	1.33098±0.00399	1.42051±0.00507	0.67336±0.00231
Dire Dawa	0.245±0.002	1.25738±0.00787	1.40333±0.00973	0.67852±0.00499
Jarso	0.217±0.001	1.15975±0.00431	1.24295±0.00491	0.60098±0.00214
Kobo	0.187±0.001	0.99205±0.00401	1.07111±0.00489	0.51789±0.00231
Kurfacheli	0.308±0.001	1.66251±0.00397	1.76418±0.00522	0.83628±0.00301
Girawa	0.251±0.001	1.36413±0.00412	1.43770±0.00574	0.68151±0.00273
Goru Mute	0.232±0.002	1.22220±0.00812	1.32887±0.00972	0.64252±0.00651
Hirina Zuria 1	0.271±0.001	1.46594±0.00422	1.55225±0.00512	0.75053±0.00295
Hirina Zuria 2	0.263±0.001	1.39586±0.00501	1.50643±0.00601	0.72837±0.00313
Langie	0.261±0.002	1.39083±0.00812	1.49497±0.00899	0.72284±0.00673

The caffeine content for all coffee samples was observed below 1.0 % which ranged from 0.60098 to 0.90285. As it can been seen from Figure 11 Harar Aboker, Kurfachele coffee bean has a magnificent yellowish color and in terms of price relatively expensive. On the other hand Jarso, Dingeti and other coffee bean with low caffeine content were relatively cheap in terms of cost and the color of the bean is green. In conclusion, the coffee bean very demanded by the consumers due to their high quality has high caffeine content while those with less demanded in the market has low caffeine content.

Figure 11. *Coffee samples obtained from*
a) Harar Aboker, b) Chiro (Dingetie), c) Jarso

The caffeine concentrations depend principally on the genus or variety of coffee, the geographical origin (agro-ecology) and the caffeine extraction method from coffee beans. Furthermore, the caffeine contents determined in this work was compared with the caffeine contents of Arabica coffee beans reported by similar analytical techniques.

Caffeine concentrations in Arabica coffee beans between 0.9 % and 1.2 % (w/w) have been reported by Petracco [32]. Similar results have been obtained by other authors for Arabica green coffee bean between 1.0 - 1.2 % (w/w) for several varieties was obtained in western and southern parts of Ethiopia [3]. Therefore, the concentrations of caffeine in coffee collected from in Hararghe region were noticeably lower than their counterpart grows in the other parts of Ethiopia and worldwide. To our knowledge this article reports the first extensive investigation and comes to the conclusion of Hararghe coffee could be considered as naturally decaffeinated coffee. Now a day having naturally decaffeinated coffee is a blessing since it becomes one of the most important world commodity. Therefore, producing naturally decaffeinated coffee would generate a huge amount of foreign currency- a burden for one country.

CONCLUSIONS

In this study, the possibility of using both Beer–Lambert's law and integrated absorption coefficient techniques as efficient method for the determination of the concentration of caffeine in green coffee beans has been demonstrated. In addition, the transition dipole moment, the oscillator strength and integrated absorption cross-section of pure caffeine were analyzed and the results agree with the literature values of other analytical methods. UV-Vis spectrophotometer method was successfully applied for the determination of the caffeine content in green coffee beans extracted by dichloromethane from Hararghe region of Ethiopia. In this research a significant variations in the concentration of caffeine were observed depending on the geographical origin of the coffee beans. Among the fifteen coffee varieties from Hararghe, Harar Aboker coffee can be considered as high source of caffeine originated from the region. On the other hand the coffee varities selling with relatively cheap value such as Jarso, Chiro (Dingetie) have low caffeine content. To make the result more reliable, the experiments were repeated three times and the average values were taken. The result of the experiment indicates that the concentration of caffeine in coffee beans (% w/w) was ranged from 0.601 % to 0.903 %. However, the caffeine content obtained by other authors for green coffee bean were between 1.0 - 1.2 % (w/w) in western and southern parts of Ethiopia. Therefore, from this research work the Hararghe coffee varieties could be considered as naturally decaffeinated coffee.

ACKNOWLEDGMENTS

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