

Full Length Research Paper

Determination of integrated absorption cross-section, oscillator strength and number density of caffeine in coffee beans by the integrated absorption coefficient technique

Abebe Belay* and A. V. Gholap

Addis Ababa University, Science Faculty, Physics Department P. O. Box -1176, Addis Ababa, Ethiopia.

Accepted 8 October, 2009

The integrated absorption cross-section of caffeine in water and dichloromethane, in the wave number regions of $20,000 - 39,062 \text{ cm}^{-1}$ at room temperature, were $(4.44 \pm 0.18) \times 10^7$ and $(4.32 \pm 0.11) \times 10^7 \text{ L mol}^{-1} \text{ cm}^{-2}$, respectively. The corresponding calculated values for oscillator strength of caffeine in water and dichloromethane were 0.19×0.01 and 0.18 ± 0.01 , respectively. In addition, the number densities of caffeine in different coffee beans were reported using integrated absorption coefficient technique. The number density of caffeine calculated in different coffee varieties were $(3.53 - 4.76) \times 10^{-5} \text{ mol L}^{-1}$. The new applied technique has an advantage over other techniques in that it provides more information about the nature of the absorbing molecules and establishes accurate evaluation of the UV-Vis absorption intensity.

Key words: Caffeine, number density, integrated absorption coefficient, integrated absorption cross-section, oscillator strength.

INTRODUCTION

Coffee contains many biologically active substances (Minamisawa et al., 2004; Yukawa et al., 2004). 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 (Clarke and Macarae, 1985; Minamisawa et al., 2004). Among the active substances, caffeine (1, 3, 7-trimethylxanthine) is one of the main constituents of coffee. The consumption of these compounds in high concentration can cause various physiological and psychological effects. It stimulates the central nervous system, gastric acid secretion and diuresis (Yukawa et al., 2004). Moreover, caffeine modifies our sleeping habit, performance and concentration (Najafi et al., 2003). The International Olympic Committee classified caffeine as a drug of abuse when present in human urine in a concentration more than 12 mg L^{-1} (De Aragao et al., 2005; L-Martinez et al., 2003).

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 (Clarke and Macarae, 1985; Bouhsain et al., 1999; Paradkar and Irudayaraj 2002). Dichloromethane is most effective and commonly employed for the extraction of caffeine in coffee beans (Clarke and Macarae, 1985; Belay et al., 2008). The safety of this solvent has been tested in animals and humans. It has been studied and reviewed by scientific authorities. It also has an advantage of low boiling points, and therefore can be used at low temperatures. However, dichloromethane has also a potential danger under certain circumstances. It can cause faintness, dizziness and head ache if inhaled at a high concentration.

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 (Minamisawa et al., 2004; Casal et al., 2000), Fourier transforms infrared (Bouhsain et al.,

*Corresponding author. E-mail-abebealem2004@yahoo.com.
Tel: +251 911712766.

1999; Paradkar and Irudayaraj, 2002), near infrared reflectance spectrometer (Huck et al., 2005), derivative spectrometer (Alpdogan et al., 2002) and UV-Vis spectrometer and partial least squares (L-Martinez et al., 2003; Belay et al., 2008).

By conventional UV-Vis spectrometer methods, direct determination of caffeine in coffee beans is impossible owing to the matrix effects of the UV absorbing substances. 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 (Belay et al., 2008). The results obtained using Beer-Lambert's law are satisfactory and reproducible at room temperature; however, when there are temperature and pressure variations, or the concentration of solute in the solution is high, or a reaction field exists due to the solute and the solvent, line broadening and a shift will happen, and thus reliable results may not be obtained with this technique.

In addition, when there is no high-resolution spectrometer since a finite slit width is used, the radiation is not monochromatic under these conditions; thus, the band determined experimentally comprises no true physical constants of the absorbing molecule but depends on the instrumental conditions employed. As reported by (Ramsay, 1951), for slit width equal to one half of the true width of the absorption band, the true and apparent molecular extinction coefficient differs by about 20% on infrared spectrometer. The difference in integrated intensity measurement, however, is only 2 - 3%; the decrease in peak intensity produced by finite slit width is roughly compensated by an increase in the band width. Furthermore, it is also reported that the finite spectral line width of the radiation source in absorption spectroscopy leads to a marked deviation from Beer-Lambert's relationship when quantitative determination is carried out on spectrally narrow transitions. In addition measuring the intensity of absorption by the integrated method provides additional information about the nature of the absorbing molecules and establishes accurate evaluation of UV-Vis absorption band intensity.

Therefore, in this research, the integrated absorption coefficient technique was proposed to determine integrated absorption cross-section, oscillator strength and number densities of caffeine in different coffee beans. Absorption cross-section and oscillator strength are the intrinsic ability of molecules to absorb light, and they are proportional to the intensity of the transition. Experimental determination of molecular transition probabilities is important both for direct application to emission, absorption and dispersion radiation. It is also useful for providing stringent tests of atomic structure calculations.

The integrated absorption coefficient technique and peak height measurements (Beer-Lambert's law) were compared for calibration and real sample analysis, and the results obtained by the two methods were comparable.

MATERIALS AND METHODS

Chemicals and samples

Dichloromethane bought from (Aldrich, Germany), distilled water, caffeine from Evan Company (England) and Arabic coffee samples were provided by Ethiopia coffee quality inspection center. The coffee samples were collected from south west of Ethiopia randomly with out considering their varieties.

Instrumentation

For UV-Vis absorption measurement of caffeine double beam, double monochromator, and ratio recording UV-Vis-NIR spectrometer, Perkin Elmer Lambda 19 (Perkin Elmer, D-7770 Ueberlingen, and Germany) operated in the wave length regions of 170 - 3200 nm were used. The instrument was operated by 4.3 UVCSS software and its resolution 0.05 - 5 nm. Scanning speed 240 nm per min and slit width 2 nm was used. Data acquisition performed by computer interfaced with spectrometer.

Methods for measuring integrated absorption cross-section and oscillator strength of caffeine in water and dichloromethane

Caffeine concentration of $(1.25 - 14.20) \times 10^{-5}$ and $(1.78 - 9.48) \times 10^{-5} \text{ mol L}^{-1}$ were prepared in distilled and dichloromethane respectively for calibration curves as shown in Tables 1 and 2. Validation parameters, Integrated absorption cross-section and oscillator strength were calculated from these solutions. Deconvolution or resolution at peak height processed on the basis of a least square procedure, where minimal values of the difference between the real spectrum and the stimulated (fitted) one were considered..

Methods for measuring number density of caffeine in coffee beans

In order to measure number density of caffeine in coffee beans by integrated absorption technique, caffeine should be extracted and purified from coffee solution. For coffee sample preparation and extraction of caffeine previously developed procedures and reported by (Belay et al., 2008) were used. The number density of caffeine in coffee beans were calculated from the area of Gaussian function fitted to the spectra in the wave number regions of $(20,000 - 50,000) \text{ cm}^{-1}$.

RESULTS AND DISCUSSION

Validation of the method

The UV-Vis absorption of caffeine was validated in terms of linearity, sensitivity, precision (repeatability) and limit of detection (LOD). The calibration curve correlating the integrated absorption coefficient with the corresponding number density was constructed. From calibration curve, it was observed that the area of absorption coefficient of caffeine increased from the lowest number density to the highest both in water and dichloromethane as shown in Tables 1 and 2 respectively. The calibration equations are $(Y = 32.73 + 223.72x, R = 0.9999, S.D = 59.42, p < 0.0001, N = 14)$ for caffeine dissolved in water, and $(Y =$

Table 1. Shows number density of caffeine dissolved in distilled water and the corresponding peak absorbance, integrated absorption coefficients, and integrated molar decadic absorption coefficient.

Number density of caffeine in mol L ⁻¹	Peak of absorbance	$\int \text{adv}$ (integrated absorption coefficients) in cm ⁻²	$\int \epsilon \text{d}\nu$ (integrated molar decadic absorption coefficients) in L mol ⁻¹ cm ⁻²
14.20 x 10 ⁻⁵	1.65	6202.39	4.36 x 10 ⁷
6.90 x 10 ⁻⁵	0.78	2952.96	4.28 x 10 ⁷
5.36 x 10 ⁻⁵	0.59	2331.66	4.35 x 10 ⁷
4.46 x 10 ⁻⁵	0.50	2019.00	4.53 x 10 ⁷
3.80 x 10 ⁻⁵	0.43	1777.98	4.67 x 10 ⁷
3.38 x 10 ⁻⁵	0.38	1454.85	4.30 x 10 ⁷
3.34 x 10 ⁻⁵	0.37	1553.26	4.32 x 10 ⁷
2.96 x 10 ⁻⁵	0.33	1424.66	4.60 x 10 ⁷
12.74 x 10 ⁻⁵	0.31	1189.82	4.20 x 10 ⁷
2.27 x 10 ⁻⁵	0.26	1026.46	4.50 x 10 ⁷
1.96 x 10 ⁻⁵	0.22	848.74	4.33 x 10 ⁷
1.71 x 10 ⁻⁵	0.19	762.82	4.46 x 10 ⁷
1.37 x 10 ⁻⁵	0.15	599.25	4.37 x 10 ⁷
1.25 x 10 ⁻⁵	0.14	522.75	4.18 x 10 ⁷

Table 2. Shows number density of caffeine dissolved in dichloromethane and the corresponding peak absorbance, integrated absorption coefficients, and integrated molar decadic absorption coefficient.

Number density of caffeine in mol L ⁻¹	Peak of absorbance	$\int \text{adv}$ (integrated absorption coefficients) in cm ⁻²	$\int \epsilon \text{d}\nu$ (integrated molar decadic absorption coefficients) in L mol ⁻¹ cm ⁻²
9.48 x 10 ⁻⁵	0.98	3976.31	4.19 x 10 ⁷
6.32 x 10 ⁻⁵	0.64	2649.17	4.19 x 10 ⁷
4.74 x 10 ⁻⁵	0.48	2020.82	4.26 x 10 ⁷
4.45 x 10 ⁻⁵	0.45	1916.05	4.23 x 10 ⁷
3.79 x 10 ⁻⁵	0.38	1611.29	4.25 x 10 ⁷
3.37 x 10 ⁻⁵	0.34	1430.00	4.18 x 10 ⁷
3.16 x 10 ⁻⁵	0.32	1412.00	4.50 x 10 ⁷
2.97 x 10 ⁻⁵	0.30	1256.00	4.24 x 10 ⁷
2.67 x 10 ⁻⁵	0.27	1200.30	4.36 x 10 ⁷
2.37 x 10 ⁻⁵	0.24	1044.08	4.40 x 10 ⁷
2.10 x 10 ⁻⁵	0.21	926.00	4.41 x 10 ⁷
1.78 x 10 ⁻⁵	0.18	781.76	4.39 x 10 ⁷

64.58 + 212.21*x, R = 0.9999, S.D = 24.86, p < 0.0001, N = 12) for caffeine dissolved in dichloromethane where Y represents the area of absorption coefficient of caffeine in cm⁻¹ and x the concentration in mg L⁻¹. From the analysis of calibrations, a linear dataset was obtained. Similarly, with peak height measurements, a linear fit with (R = 0.9999) was obtained. Therefore, the methods are valid in terms of sensitivity. Over all method of repeatability was also determined by calculating the coefficient of variance (C.V) and a value ranged from (0.99 - 5.56) % were obtained. These results suggested that the proposed method is valid in terms of precision.

The methods are also validating in terms of limits of

detection. The limits of detection defined by the analytic concentration giving a signal equal to the blank signal plus three times the standard deviation of the blank were 1.65 mg L⁻¹ for caffeine dissolved in water and 1.05 mg L⁻¹ for caffeine dissolved in dichloromethane respectively. These results are in a good agreement with one reported by (Huck et al., 2005; Bouhsain et al., 1999).

Integrated absorption cross-section and oscillator strength of caffeine in water and dichloromethane

For intensity of incident wave propagating a distance l into

the absorbing medium, the Beer-Lamberts Law was expressed by (Rao, 1975).

$$I = I_0 10^{-a l} \quad (1)$$

Where P_λ and P_λ^0 are the transmitted and incident light intensities, respectively, and a_λ is the absorption coefficient. From equation (1), absorption coefficient was expressed as

$$a = \frac{1}{l} \log\left(\frac{I_0}{I}\right) \quad (2)$$

The absorption cross-section σ_λ related to the absorption coefficient a_λ at a single frequency for N number of molecules per unit volume was expressed by the following relation (Rao, 1975; Thorne, 1988).

$$a = N\sigma \quad (3)$$

However, in a UV-Vis spectrometer, the absorption of molecules in a liquid occurs over a certain range of frequencies rather than at a single frequency. Therefore, absorption coefficient measured at any single frequency may not express the true intensity of the molecular transition. Integrated absorption coefficient which is the sum of absorption coefficients for all frequencies in the band is preferable in such cases; the techniques are useful for different applications, since it is independent of the line function which may vary by parameters like pressure, temperature, concentration of the solute and solute-solvent interaction. In addition, the techniques are very important in the absence of a high-resolution spectrometer. Therefore, in liquids and solutions where the above effects are observed, the true integrated absorption intensity of a band should be defined by the following equation (Rao, 1975; Thorne, 1988; Milonni and Eberly, 1988)

$$a_t = \int a dv \quad (4)$$

Substituting equation (2) into (4), the integrated absorption coefficient was expressed as

$$a_t = \frac{1}{l} \int \log\left(\frac{I_0}{I}\right) dv \quad (5)$$

The integral being measured over the limit of the absorption band. From the integrated absorption coefficient having no line shape dependence, the integrated absorption cross-section was calculated using the following equation (Milonni and Eberly, 1988).

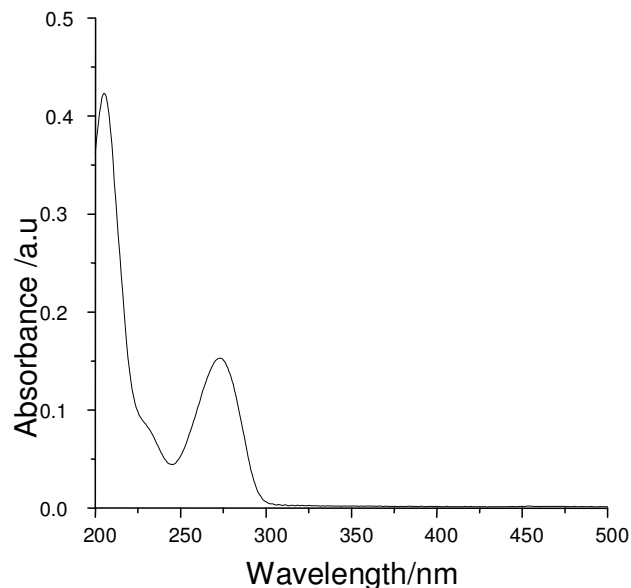


Figure 1. Shows absorbance versus wavelength of caffeine in distilled water.

$$\sigma_t = \frac{1}{NI} \int \log \frac{I_0}{I} dv \quad (6)$$

Where; σ_t are the integrated absorption cross-section, a_t the integrated absorption coefficient, and N is the number density.

For calculating the integrated absorption cross-section of caffeine, the absorbances versus wavelength of caffeine in water and dichloromethane (Figures 1 and 2) are recalculated in to absorption coefficient versus wave number (Figure 3) using Origin 6.1 software. For deconvolution the overlapped spectra of caffeine and determine the area under peak, Gaussian function were fitted to the spectra of absorption coefficient versus wave number (Figure 4). From area of Gaussian function fitted to the spectra, integrated absorption cross-section of caffeine was calculated in distilled water and dichloromethane in the wave number regions of 20,000- 39,06220 cm^{-1} .

The calculated integrated absorption cross-section for independent measurements of caffeine in water and dichloromethane were $(4.44 \pm 0.18) \times 10^7$ and $(4.32 \pm 0.11) \times 10^7 \text{ L mol}^{-1} \text{ cm}^{-2}$, respectively as mentioned in table 1 and 2. On the other hand corresponding, the peak absorption cross-section of caffeine at the center was calculated in both the solvents using equation (3). The calculated absorption cross-section of caffeine at peak was $(1.12 \pm 0.02) \times 10^4$ and $(1.01 \pm 0.01) \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$. The integrated absorption cross-section and peak cross-section obtained in this research agree with one reported by (Canosa-Marttin et al., 1987).

The oscillator strength was considered the other useful

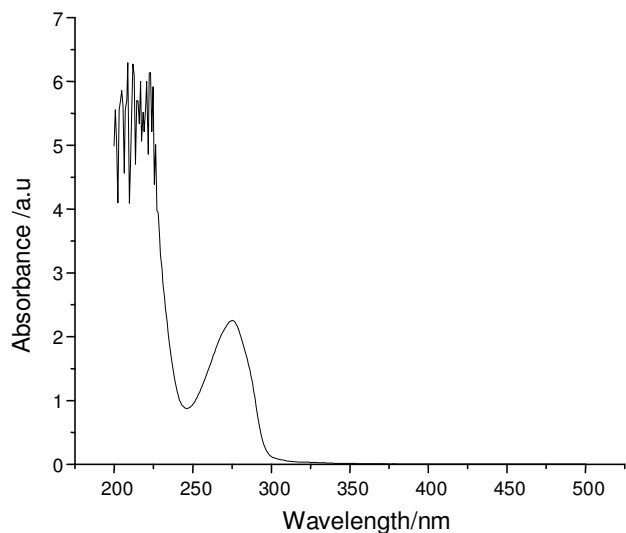


Figure 2. Shows absorbance versus wavelength of caffeine in dichloromethane.

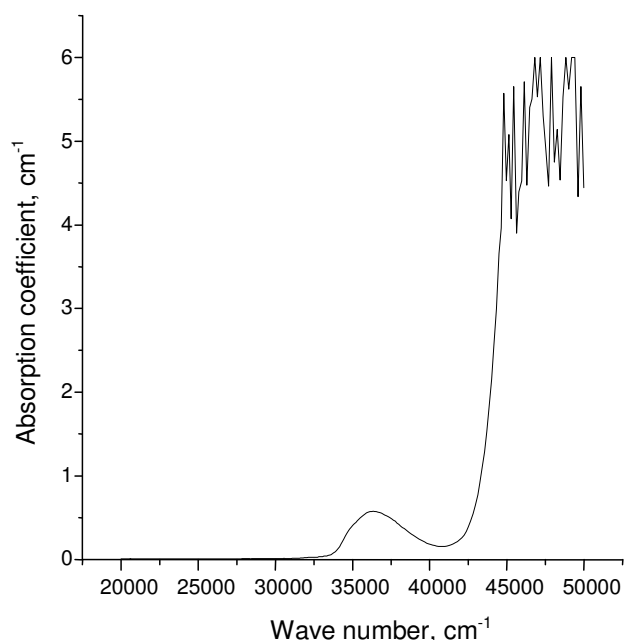


Figure 3. Shows the absorption coefficient versus wave number of caffeine in dichloromethane.

parameter providing the intensity of transition; it expresses the relative strength of electron transition. It is one of the most 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 needs to relate the magnitude of the absorption signal to its concentration. Oscillator strength

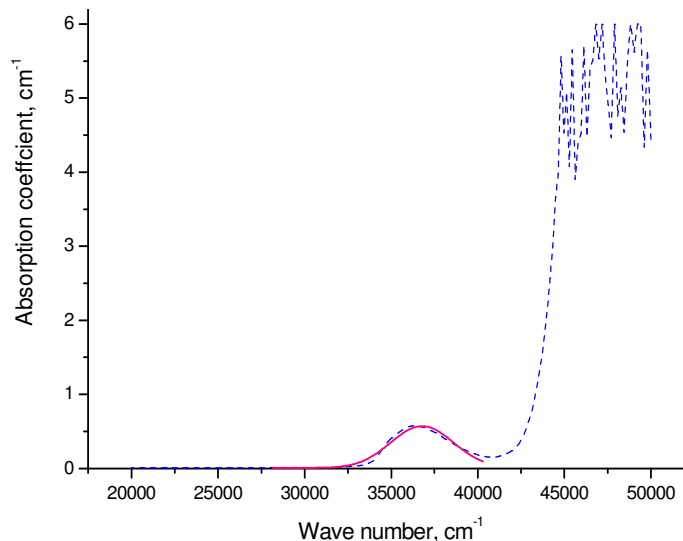


Figure 4. Shows the Gaussian function fitted to the absorption coefficient versus wave number of caffeine dissolved in dichloromethane. The line shows (—) Gaussian function and the dot shows (.....) absorption coefficient versus wave number.

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. Oscillator strength was related to the molar decadic absorption coefficient by the following equation (Forsman and Clark, 1973; Georgakopoulous et al., 2004; Radwan, 2007).

$$f = 4.32 \times 10^{-9} \frac{\text{mol cm}^2}{\text{L}} \int \epsilon \, d\nu \quad (7)$$

$$\epsilon = \frac{1}{lc} \log\left(\frac{I_0}{I}\right) \quad (8)$$

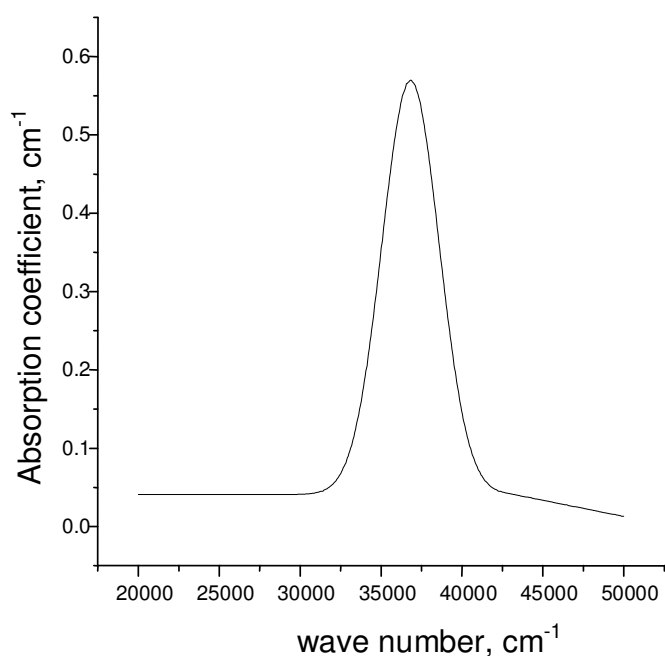
Where; ϵ is molar decadic absorption coefficient carrying the unit $\text{L mol}^{-1} \text{cm}^{-1}$ and wave number ν in cm^{-1} . By equation (7), the oscillator strength of caffeine was calculated in distilled water and dichloromethane solution for different concentrations or number densities mentioned in Tables 1 and 2. The Gaussian function was fitted to the spectra of molar decadic absorption coefficient versus wave number and; from area of Gaussian function oscillator strength was calculated in wave number regions $20,000 - 39,062 \text{ cm}^{-1}$.

The calculated oscillator strength of caffeine in water and dichloromethane are 0.19 ± 0.01 and 0.18 ± 0.01 respectively.

The other basic parameters describing any individual absorption band is a band width at half maximal intensity, defined as follow (Antonov and Nedlcheva, 2000)

Table 3. Shows the percentage of caffeine in coffee beans calculated by integrated absorption coefficient and Beer-Lambert's law.

Types of coffee sample	Number density calculated by integrated absorption coefficient in mol L ⁻¹	Number density calculated by Beer-Lambert's law in mol L ⁻¹	Percentage of caffeine calculated by integrated absorption coefficients (w/w %)	Percentage of caffeine calculated by Beer-Lambert's law (w/w %)
Sample- 1	(3.53) × 10 ⁻⁵	(3.66) × 10 ⁻⁵	1.22	1.26
Sample -2	(3.55) × 10 ⁻⁵	(3.86) × 10 ⁻⁵	0.90	0.95
Sample -3	(3.89) × 10 ⁻⁵	(4.16) × 10 ⁻⁵	1.05	1.12
Sample -4	(4.33) × 10 ⁻⁵	(4.65) × 10 ⁻⁵	1.12	1.20
Sample -5	(4.43) × 10 ⁻⁵	(4.85) × 10 ⁻⁵	1.15	1.26
Sample -6	(4.62) × 10 ⁻⁵	(5.05) × 10 ⁻⁵	1.05	1.14
Sample -7	(4.76) × 10 ⁻⁵	(4.95) × 10 ⁻⁵	1.22	1.27

**Figure 5.** Shows the area of Gaussian function fitted to the spectra of caffeine in the wave number regions of 20,000 - 50,000 cm⁻¹.

$$\Delta v_{1/2} = v_1 - v_2 \quad (9)$$

It measures the anti-bonding character of the excited state. The anti-bonding character is a function of internuclear distance and in some cases the change of the band width can indicate the change of this distance. In this research, the band width at half maximum of caffeine calculated in water and dichloromethane were 4243 and 4532 cm⁻¹ respectively.

Number density of caffeine in coffee beans by integrated absorption coefficient

Integrated absorption techniques were developed to

determine the number density of caffeine in coffee beans, instead of Beer-Lambert's law. An equation relating integrated absorption coefficient with number density and oscillator strength for the Gaussian shaped spectrum will be given by Thorne (1988).

$$\int a \, dv = 2.65 \times 10^6 N f \quad (10)$$

Where N is number density in molecules cm⁻³, a_λ in m⁻¹ and v in Hz; f is oscillator strength of the transition molecule. Since direct application of UV-Vis spectrometer cannot be used for the determination of caffeine in coffee beans owing to the matrix effects of UV absorbing substances, caffeine was extracted from coffee solution by dichloromethane before applying the technique. Chlorinated solvents are good at extracting caffeine, especially dichloromethane having an extraction efficiency of 98 - 99% (Clarke and Macarae, 1985; Belay et al., 2008).

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. From area of Gaussian function fitted to the spectra (Figure 5), the number density of caffeine was calculated in the frequency region of 20,000 - 50,000 cm⁻¹ using equation (9).

By these methods, the number density of caffeine was calculated for seven different coffee samples (samples 1 through 7) collected from different regions of Ethiopia. The calculated number density ranged from (3.53 - 4.76) × 10⁻⁵ mol L⁻¹ as shown in Table 3. On the other hand, for the purpose of comparison with other techniques same samples were analyzed by our previously reported method (Belay et al., 2008) or Beer-Lambert's law and the results are in the range of (3.66 - 4.94) × 10⁻⁵ mol L⁻¹. The corresponding percentages of caffeine were 0.90 - 1.22% and 0.95 - 1.27% obtained by integrated absorption coefficient and Beer-Lambert's law, respectively. The coefficients of variation between the two methods were about 3.17 - 8.73%. This discrepancy was expected due to the confidence of Gaussian fit to the experimental

data. Thus, from the comparison of the two methods, the new analytical methods are considered valid in terms of accuracy.

Moreover, the caffeine levels reported in this research were within the range of previously reported by HPLC methods. The higher and lower amounts of caffeine reported by (Dessalegn et al., 2007) for Ethiopian coffee samples are 0.91 - 1.32% with an average 1.10% by analysis 42 coffee samples. Similarly, (Ky et al., 2000) also reported a minimum of 0.96% and a maximum of 1.22% caffeine level in coffee Arabica (native to Ethiopia) using HPLC after analyzing 38 coffee samples.

Conclusion

Integrated absorption techniques were developed to determine the number density and optical transition properties of caffeine. The integrated absorption coefficient has more advantages since independent of line function. The effects of line broadening and shift in peak intensity due to, temperature and pressure variation, solute-solvent interaction and a finite slit width of UV-Vis spectrometer do not affect the intensity of the absorbing molecules. Therefore, the techniques are the best for evaluating the UV-Vis absorption intensity; moreover, it is also sensitive, precise, and accurate for determining caffeine in coffee beans.

ACKNOWLEDGMENT

The authors would like to thank polymer physics (physics department), inorganic and analytical chemistry (chemistry department) of AAU for allowing us the laboratory facilities. In addition we like to thank the Ethiopia Coffee Quality Inspection Center for providing the coffee samples.

REFERENCES

- Alpdogan G, Karabina K, Sungur S (2002). Derivative spectrophotometer determination of caffeine in some beverages. *Turk. J. Chem.* 26: 295-302.
- Antonov L, Nedeltcheva D (2000). Resolution of overlapping UV-Vis absorption bands and quantitative analysis. *Chem.Soc.Rev.* 29: 217-227.
- Belay A, Ture K, Redi M, Asfaw A (2008). Measurement of caffeine in coffee beans by UV-Vis spectrometer. *Food chemistry*, 108: 310-315.
- Bouhsain Z, Garrigues JM, Garrigues S, Guardia M (1999). Flow injection Fourier transform infrared determination of caffeine in coffee. *Vib. Spectros.* 21: 143-150.
- Canosa-Martin CE, Fowles M, Houghton PJ, Wayne RP (1987). Absolute absorption cross-section measurement on NO₃. *J. Chem. Soc. Faraday Trans.* 83: 1465-1474.
- Casal S, Oliveira MB, Ferreira MA (2000). HPLC/diode applied to the thermal degradation of trigonelline, nicotinic acid and caffeine in coffee. *Food Chem.* 68: 481-485.
- Clarke RJ, Macarae R (1985). *Coffee*. Elsevier, Amsterdam, Chemistry 1: 1-50.
- De Aragao NM, Veloso MCC, Bispo MS, Ferreira SLC, de Andrade JB (2005). Multivariate optimization of the experimental conditions for determination of three methylxanthines by reversed phase high performance liquid chromatography. *Talanta* 67: 1007-1013.
- Dessalegn Y, Labuschagne MT, Osthoff G, Herselman L (2007). Variation of green bean caffeine, chlorogenic acid, sucrose and trigonelline contents among Ethiopian Arabica coffee accessions. *Ethiopian J. Sci.* 30: 77-82.
- Forsman EN, Clark KC (1973). Measurement of the oscillator strength of the O (¹S-¹P⁰) transition at 1217.6 Å. *Phys. Rev. A* 7: 1203-1208.
- Georgakopoulos S, Grondelle RV, Zwan GVD (2004). Circular dichroism of carotenoides in bacterial light-harvesting complexes: experimental and modeling. *Biophys. J.* 87: 3010-3022.
- Huck CW, Uggebachler WG, Bonn GK (2005). Analysis of caffeine theobromine and theophylline in coffee by NIR spectroscopy compared to HPLC coupled to mass spectrometry. *Anal. Chim. Acta* 538: 195-203.
- Ky CL, Louarn J, Guyot B, Dussert S, Hamon S, Noirot M (2000). Caffeine, trigonelline chlorogenic acids and sucrose diversity in wild coffee arabica and canephore. *Food Chem.* 75: 223-230.
- L-Martinez L, Lopez-de-Alba PL, G-Campos R, L-Redriquez L (2003). Simultaneous determination of methylxanthines in coffee and tea by UV-Vis spectrophotometry and partial least squares. *Anal. Chim. Acta* 493: 83-94.
- Milonni PW, Eberly JH (1988). *Lasers*. Wiley, New York, pp 83-85.
- Minamisawa M, Yoshida S, Takai N (2004). Determination of biologically active substances in roasted coffee using a diode-HPLC system. *Anal. Sci.* 20: 325-328.
- Najafi NM, Hamid AS, Afshin RK (2003). Determination of caffeine in black tea by Fourier transforms IR spectrometry using multiple linear regressions. *Microchem. J.* 75: 151-158.
- Paradkar MM, Irudayaraj J (2002). Rapid estimation of caffeine content in tea, coffee and soft drinks by FTIR-ATR spectroscopic method. *Food Chem.* 67: 2507-2511.
- Radwan RM (2007). Electron induced modifications in the optical properties of polypropylene. *J. Phys D: Appl. Phys.* 40: 374-379.
- Ramsay DA (1951). Intensity and shapes of infrared absorption bands of substances in the liquid phase. Contribution from the division of chemistry, Publication of National research council of Canada 74: 72-80.
- Rao CNR (1975). *Ultra-violet and visible spectroscopy chemical applications*. Butterworth and Co Ltd, England pp 1-12.
- Thorne AP (1988). *Spectrophysics*. Chapman and Hall Ltd, London pp.318-320.
- Yukawa GS, Mune M, Otani H, Tone Y, Liang X-M, Iwahashi H, Sakamoto W (2004) Effects of coffee consumption on oxidatives susceptibility of low-density lipo proteins and serum lipid level on humans. *J. Biochem. (Moscow)* pp.170-174.