

Full Length Research Paper

Determination of oscillator strength of caffeine and caffeine in tea leaves by the integrated absorption coefficient technique at two different temperatures

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In this paper, oscillator strength of caffeine in dichloromethane, water, chloroform, and ethyl acetate were calculated in the wave number range of 32, 000 to 41,000cm⁻¹. It was found that oscillator strength of caffeine in dichloromethane, water, chloroform, and ethyl acetate were 0.230 ± 0.001 , 0.170 ± 0.0003 , 0.114 ± 0.001 , 0.150 ± 0.001 respectively. Caffeine content of real tea leaves using integrated absorption coefficient method were also calculated for different samples at two different temperatures. The result agrees with that obtained using Beer's law with a little less in the case of integrated absorption coefficient technique with higher value at the boiling temperature and smaller extraction time.

Key words: Tea leaves, integrated absorption coefficient, caffeine, oscillator strength, UV-visible spectroscopy.

INTRODUCTION

Processed tea, which is one of the most popular beverages, is manufactured from the young tender leaves of the plant *Camellia sinensis* (Cabrera et al., 2003). Tea is not only used as fresh drink but also as traditional herb which has many benefits for human health. Recently, several studies led to the acceptance of tea as a useful cancer preventive agent (Jankun et al., 1997; Borrelli et al., 2004; Scalbert et al., 2005; Yang et al., 2004) which increased public awareness of the health protective characteristics of tea. There are two major kinds of tea; black tea and green tea, and they both contain caffeine (1 to 5 % of its dry weight) (Amra et al., 2006). Tea composition varies with climate, season, tea variety, and age of the leaf.

Due to the physiological and psychological effects of caffeine, decaffeination is currently a popular method to minimize the caffeine content in tea leaves. Decaffeination is done by the use of solvent, which can extract caffeine. For this purpose, the currently available solvents are chloroform, dichloromethane, ethyl acetate, super critical carbon dioxide, etc. Dichloromethane is used to decaffeinate a high proportion of conventional teas. It is also a relatively non-toxic solvent and is often

used in place of chloroform. Ethyl acetate also removes caffeine from tea leaves effectively; it can also extract other chemical components as well (Mumin et al., 2006). Studies on green tea decaffeinated with ethyl acetate have shown the potential for up to 30% of Epigallocatechin Gallate (EGCG, considered to be the beneficial component in green tea) and other beneficial antioxidant compounds to be extracted along with caffeine (Kirmer, 1998; Johnson, 1988; Jefferson, 1998).

Due to the widespread consumption of caffeine, it is important to collect precise information on their content in foods. Most research activities have been focused on chromatographic methods; however, spectrophotometric determination is preferred because of its rapidity, high accuracy and reproducibility (Komes et al., 2009; Guzin et al., 2002). Furthermore, UV-visible spectrophotometer is cheap and it is found in many laboratories. But caffeine content in tea leaves cannot be determined directly using UV-visible spectrometer due to the matrix effect of UV absorbing substances (Guzin, 2002; Zhang et al., 2005).

Caffeine content in tea leaves can be obtained using UV-visible spectrometer by Beer-Lambert's law. 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

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broadening and a shift will happen, and thus, reliable results may not be obtained with this technique. However, the integrated absorption coefficient is independent of line function which varies due to pressure, temperature, and solute solvent interaction. 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-visible absorption band intensity. Therefore, in this paper we have used UV-visible spectrometer for the determination of caffeine content in tea leaves using integrated absorption coefficient technique at 30°C and boiling temperature of 94°C. The results obtained using integrated absorption coefficient technique and Beer-Lambert's law was also compared. Finally, oscillator strengths of caffeine in different solvents were also calculated.

MATERIALS AND METHODS

Samples

Four different Ethiopian commercial tea samples named Addis, black lion, cinnamon black teas and Ethiopian green tea. All the tea leaves were purchased from supermarkets in Addis Ababa. All the samples were from tea bags except black lion. Cinnamon tea is black tea leaves blended with cinnamon.

Instrumentation

The absorption spectra were recorded on a Perkin-Elmer Lambda 19 spectrometer with wavelength range of 170 to 3200 nm and double monochromator which offers the advantage of low levels of stray light that is significant to measure a high value of absorbance using 1 cm cuvette. The investigated compound was measured in the UV-visible spectrum range of 200 to 500 nm with a spectral band width of 2 nm and scan speed of 480 nm min⁻¹.

Lab apparatus

Microbalance (Mettler At 250, 0.1/0.01 mg) for measuring mass of caffeine, balance (BP 210S, d = 0.1 mg, Max 210 g) for measuring mass of tea leaves, measuring cylinder, magnetic stirrer, glass filter (2 'Pyrex', England), beakers, thermometer, 1 cm quartz cuvette, separating funnel, funnel, ice bath, and distilled water was used.

Chemicals

Dichloromethane (assay: 99.6%, Aldrich Germany), chloroform (assay: 99.8%, European Union), ethyl acetate (assay: 99%, Indian), caffeine (M.W. 194.19 g/mol, Aldrich Germany).

Methods for measuring oscillator strength of caffeine in water, dichloromethane, chloroform, and ethyl acetate

Caffeine concentration of (14.419 to 2.670)10⁻⁵M, (9.475 to 1.1560)10⁻⁵M, (14.240 to 4.747)10⁻⁵M, (10.299 to 2.943)10⁻⁵M were prepared in distilled water, dichloromethane, chloroform, and ethyl acetate respectively. Absorbance (optical density); which is a

dimensionless quantity versus wavelength of the solution was measured using UV-visible spectrometer. Then, integrated absorption coefficient and oscillator strength were calculated from the data of each spectra using origin 6.1 soft ware along with Equations 1 and 2.

Methods for determination of caffeine content in tea leaves

50 mg of tea leaves was first dissolved in distilled water at two different temperatures (30°C and boiling temperature of 94°C) and then, caffeine was extracted using dichloromethane. Caffeine was repeatedly extracted four times from tea infusion. To ensure the reliability of the experiment, three independent measurements (n=3) were taken for each sample. For 94°C, we used smaller extraction time than 30°C. Then, the absorption spectrum of the solution were taken, from which, contents of caffeine for four different samples of tea leaves were calculated using integrated absorption coefficient technique. The absorption coefficients of caffeine were calculated in the wave number regions of 32, 000 to 41,000 cm⁻¹.

RESULTS AND DISCUSSION

In this section, the results of the experiments were presented based on the experimental procedures of the previous section. Each data in these experimental activities was found from computerized recorder interfaced with the spectrometer. It was then analyzed using origin 6.1 soft ware.

Validation of the method

From the UV-visible absorption spectrum of caffeine in dichloromethane, water, chloroform, and ethyl acetate, the calibration graph correlating the integrated absorption coefficient versus concentration was constructed. With this, the linear regression coefficients of 0.99978, 0.99998, 0.99986, 0.9994 and standard deviations of 31.18222, 9.16031, 17.58995, and 39.14415 were obtained for caffeine in dichloromethane, water, chloroform, and ethyl acetate respectively. Thus, for the analysis of the calibration, a linear data set was obtained. The absorption spectra of pure caffeine and caffeine extracted by dichloromethane exactly overlaps (Figure 1). This indicates that dichloromethane was a good solvent used to extract caffeine from tea leaves.

Integrated absorption coefficient and oscillator strength of caffeine in different solvents

From UV-visible absorption spectra, the integrated absorption coefficient and oscillator strength of caffeine were calculated in dichloromethane, water, chloroform, and ethyl acetate. The absorption coefficient determines how far into a material, light of a particular wavelength can penetrate before it is absorbed. The integrated absorption coefficient a_t which is the sum of absorption

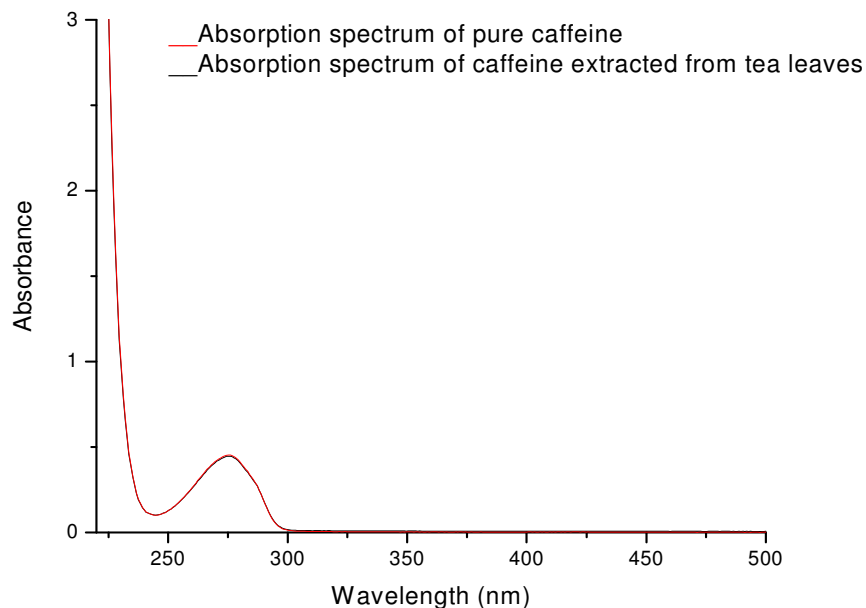


Figure 1. Absorption spectra of pure caffeine and caffeine extracted from tea leaves.

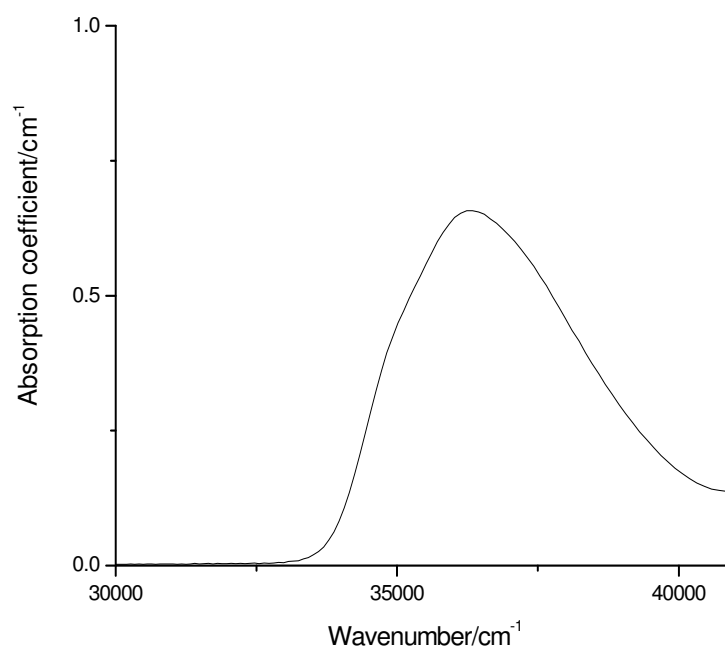


Figure 2. a_ν versus ν of caffeine in dichloromethane.

coefficient a_λ for all frequencies in the band is expressed as

$$a_i = \int a_\lambda d\nu, \quad (1)$$

where ν is the frequency. Figure 2 shows the absorption

coefficient versus wave number of caffeine in dichloromethane. The integrated absorption coefficient is independent of line function, which may vary with parameters like pressure, temperature, and solute solvent interaction. It thus provides a measure of the inherent absorbing strength of atoms.

In this experimental activity, the integrated absorption coefficient was found by recalculating the absorbance

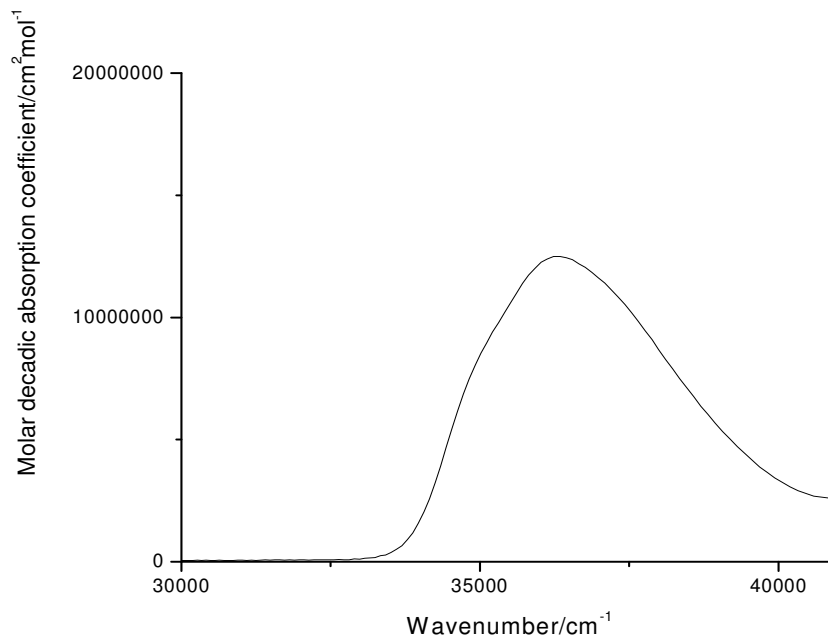


Figure 3. $\epsilon(\nu)$ versus ν for caffeine in dichloromethane.

versus wave length into absorption coefficient versus wave number in the wave number region of 32, 000 to 41,000 cm^{-1} . The other important parameter, which provides the relative strength of electron transition, is the oscillator strength (f). Oscillator strength relates to molar decadic absorption coefficient (ϵ) by the following equation (Georgakopoulos et al., 2004; Radwan, 2007).

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

The molar decadic absorption coefficient measures the intensity of optical absorption at a given wavelength.

Thus in this experimental activity, oscillator strength of caffeine in different solvents was found by recalculating absorbance versus wavelength into molar decadic absorption coefficient versus wave number along with Equation 2 using origin 6.1 software. Figure 3 shows the molar decadic absorption coefficient versus wave number of caffeine in dichloromethane. Furthermore, absorption coefficient is related to the number density and oscillator strength by Equation 3 (Throne, 1988).

$$\int a_{\lambda} d\nu = 2.65 \times 10^{-6} Nf \quad \dots(3)$$

Where N is number density in molecules m^{-3} , f oscillator strength of the transition molecule a_{λ} absorption coefficient in m^{-1} , and ν is the frequency in Hz. From N, concentration (c) ($N = cN_a$, where N_a is Avogadro's number) of caffeine was determined, which in turn is

used to calculate mass (m) of caffeine ($c = m/MV$, where M molecular mass of caffeine, and V volume of solution). Finally, percentage of caffeine was calculated using this result.

The integrated absorption coefficient, integrated molar decadic absorption coefficient, and oscillator strength of caffeine in dichloromethane, water, chloroform, and ethyl acetate are indicated in Table 1. As indicated in Table 1, the oscillator strength of caffeine in dichloromethane is greater than in the other solvents.

Caffeine content in tea leaves using integrated absorption coefficient technique

From Equation (3), number density of caffeine in tea leaves was calculated for caffeine in real tea leaves where f is the oscillator strength of pure caffeine in dichloromethane (Table 1). From N, concentration, c, of caffeine was determined, which was in turn used to calculate mass of caffeine. Finally, percentage of caffeine was calculated. Using this method, caffeine content in four Ethiopian tea leaves were calculated at 30°C and boiling temperature. This result was compared with the result obtained using Beer's law (Table 2). From Table 2, for both temperatures, the percentage of caffeine, using both methods are similar except a little smaller in the case of integrated absorption coefficient methods. This small difference will be due to the fact that, in the case of Beer's law it was calculated only at maximum peak of the absorption spectrum. When we compare the content of caffeine at 30°C and boiling temperature, the percentage

Table 1. Integrated absorption coefficient and oscillator strength of caffeine in dichloromethane, water, chloromethane, and ethyl acetate.

Solvent	Integrated absorption coefficient (cm ⁻²)	Integrated molar decadic absorption coefficient (L mol ⁻¹ cm ⁻²)	Oscillator strength
Dichloromethane	1626.50 ± 36.16	(5.22±0.03)10 ⁷	0.230±0.001
Water	2519.43±33.66	(3.92±0.01)10 ⁷	0.170±0.0003
Chloroform	2162.92±29.30	(2.64±0.01)10 ⁷	0.114±0.001
Ethyl acetate	1890.59±31.62	(3.39±0.02)10 ⁷	0.150±0.001

Table 2. Comparison of caffeine content in tea leaves using absorption coefficient technique and Beer's law at 30 °C and boiling temperature ,94 °C (n = 3).

Sample	30 °C			94 °C		
	Concentration of caffeine using integrated absorption coefficient(mol/m ⁻³)	Percentage of caffeine using integrated absorption coefficient	Percentage of caffeine using Beer's law	Concentration of caffeine using integrated absorption coefficient(mol/m ⁻³)	Percentage of caffeine using integrated absorption coefficient	Percentage of caffeine using Beer's law
Addis black tea	(3.55±0.11)10 ⁻⁵	1.86±0.05	2.01±0.04	(3.77±0.06)10 ⁻⁵	2.00±0.02	2.11±0.04
Ethiopian green tea	(3.51±0.06)10 ⁻⁵	1.88±0.02	2.08±0.01	(3.78±0.12)10 ⁻⁵	2.01±0.07	2.15±0.12
Black lion tea	(2.76±0.02)10 ⁻⁵	1.47±0.004	1.50±0.00	(2.95±0.03)10 ⁻⁵	1.57±0.02	1.60±0.01
Cinnamon black tea	(3.49±0.22)10 ⁻⁵	1.82±0.11	1.98±0.09	(3.41±0.15)10 ⁻⁵	1.82±0.09	1.87±0.08

of caffeine at the boiling temperature is greater with smaller extraction time. At both temperatures, Ethiopian green tea leaves has greater caffeine content than the other tea leaves studied.

Caffeine content in tea leaves reported in this research is in the range of values 1 to 5% reported by Amra et al. (2006). As it can be seen in this research, the maximum content of caffeine is almost similar to the results obtained for the caffeine contents of marketed tea by Misra et al. (2009) using HPLC in which the maximum concentration of caffeine in tea samples of marketed tea was 2.145%. Mumin et al. (2006) also reported using HPLC that, caffeine content for black tea was 3.34% and green tea was 2.44%.

Furthermore, Guzin et al. (2002) reported that caffeine content in tea, using derivative spectrometric and HPLC methods were 1.53% and 1.51% respectively. Precision of the method was determined by three replications of each sample. The precision (%RSD) of the replications was found to be less than two which is indicative of a precise method.

Conclusion

A new technique for measuring the quantity of caffeine in tea leaves has been described using integrated absorption coefficient technique. Using

this technique, contents of caffeine in four different tea samples were calculated at 30 °C and boiling temperature (94 °C). The result agrees with the range of values in literature using other analytical methods. The results at the two temperatures reveal that, caffeine is better extracted at the boiling temperature than at 30 °C and more extraction time. Therefore, it can be said that, by taking more extraction time and at higher temperatures, more caffeine can be extracted than at boiling temperature and less extraction time.

This technique is more advantageous for evaluating the UV-Visible absorption intensity since the effect of line broadening due to temperature, pressure, and other variation do not affect the

intensity of the absorbing molecule.

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