SPECTROPHOTOMETRIC INVESTIGATION OF MAJOR BIOACTIVE COMPOUNDS OF COFFEE BEANS

A Dissertation Submitted to the Department of Physics Addis Ababa University

In Partial Fulfilment of the Requirements of the Degree of Doctor of Philosophy in Physics

By: Abebe Belay Gemta

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Approved by the Examination Committee

Dr. Lemi Demeyu, Chairman ———————

Prof. A. V. Gholap, Advisor ———————

Prof. Maria L. Calvo, External Examiner ———————

Dr. Gizaw Mengistu, Internal Examiner ———————

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Dedication

This Work is dedicated to :

My Father: **Belay Gemta Ruda**

My mother: **Weyitu Kebebew Kelume**

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Abstract

In this research the methods of measuring major bioactive compounds in coffee beans, their optical transition properties, self-association, hetero-association and thermodynamic properties were investigated by experimental and computational methods. The implemented methods for determination the contents of biocactive compounds in coffee beans are the extraction of caffeine from water solution or liquid-liquid extraction and fitting Gaussian function to the spectrum of the compound by non-linear curve fitting based on the Lavenberg-Marquardt algorithm to eliminate the possible interferences. The contents of caffeine and chlorogenic acids determined by these methods for green coffee beans are in the ranges of 0.90-1.27 $\%$, 6.05-6.25 $\%$ respectively. The optical transition properties of caffeine, caffeic and 5-caffeoylquinic acids were determined in different solvents by integrating the absorption coefficients in the wave number regions. The calculated values of transition dipole moment of caffeine, caffeic and 5-caffeoylquinic acids in water in their wave number regions are 10.40×10^{-30}, 19×10^{-30}, $20\times10^{-30}Cm$ respectively. The corresponding oscillator strength are 0.19, 0.49 and 0.56. The concentration dependent self-association of caffeic, 5-caffeoylquinic acids and their hetero-association were analyzed by dimer model and Benesi-Hildebrand approaches. The thermodynamic parameters, enthalpy, Gibbs free energy and entropy of dimerzation reactions were calculated from Van't Hoff equation. The calculated molar enthalpy change for caffeic and 5-caffeoylquinic acids of self-association are -63.20, -12.89 $kJmol^{-1}$ respectively.In addition, the time dependent Schrodinger equations were solved by semi-classical approach to relate the theoretical expressions with the experimentally measurable quantities.

Introduction

Most of our knowledge about atoms and molecules are based on spectroscopic methods of investigation (Demtroder, 1995). The structure and their interaction with the surrounding is derived from absorption or emission spectra generated, when electromagnetic radiation interacts with matter (Demtroder, 1995; Savanberg, 1991). The three important parameters which characterize an electronic transition and necessary to interpret the absorption spectra are wavelength, oscillator strength and electronic transition dipole moment. The wavelength is inversely proportional to the transition energy, the oscillator strength is proportional to the intensity of transition and the electric field dipole transition moment is a vector that depends on both the ground and excited state wave function and that couples the transition to the electric field of light. The transition probabilities determined by spectroscopy for atoms and molecules are directly important in several experimental and theoretical studies (Thorne, 1988; Rao, 1975). On the other hand, the information obtained from applied spectroscopy can be useful for various kinds of analysis. For instance, the atomic or molecular absorption or emission are useful for qualitative and quantitative chemical analysis (Thorne, 1988; Rao, 1975). Thus, it is possible to say spectroscopy has an outstanding contribution for the present state of atomic and molecular physics, chemistry and molecular biology (Demtroder, 1995).

There are several spectroscopic techniques which are distinguished on the basis of the frequency ranges in which measurements are made as well as the type of information sought (Rao, 1975; Bauman, 1962). The molecular spectroscopy which works in the wavelength regions of UV-Vis have various applications. For quantitative determination of one or more species in mixtures by Beer-Lambert's law and qualitative analysis for identification of pure compound, for determination of the presence or absence of a particular species in a mixture or the identification of certain functional groups in a compound un-

der structural investigation (Thorne, 1988; Rao, 1975; Bauman, 1962). Moreover it is also useful for studies the rate of chemical reaction, and the thermodynamic properties of the molecules. In this research, it is intended to investigate the qualitative and quantitative characterization, compositional analysis of some biochemical compounds of coffee beans (caffeine and chlorogenic acids) using computational and experimental methods.

Previously many workers have reported that coffee beans have many biologically active compounds that are of particular interests (Minamisawa et al., 2004; Yukawa, 2004; Wen et al., 2004). The most biologically active substances in coffee beans are caffeine, chlorogenic acid, and others (Fujioka et al., 2008; Minamisawa, et al. 2004; Ky et al., 2001; De Maria et al., 1995). The amounts of these compounds depend on type of coffee bean, degree of maturation, roasting, and to some extent on environmental condition and agricultural practice (Clarke and Macarae, 1985; Minamisawa et al., 2004; Farah et al., 2006). Consuming high concentration of caffeine can cause various physiological and psychological effects on human being (Clarke and Macarae, 1985; Bolton and Null, 1981), on the other hand this compound is used as additive in soft drinks and preparation of drugs in different pharmaceutical companies. Similarly, the chlorogenic acids of coffee beans possess potential antioxidant activities which play an important role in protecting cells and organs from oxidative degeneration (Ramalakshmi et al., 2009; Manch et al., 2004; Yukawa, 2004; Svillas et al., 2004). The quality of coffee beverages is also strictly related to the chemical compositions of these compounds (Farah et al., 2006; Farah et al. 2005b; Franca et al., 2005; Mazzafera, 1999). Chlorogenic acid and caffeine increase the bitterness, the former after degradation in to phenolic derivative and later without any degradation (Farah et al. 2005a).

Today intensive researches have been conducted on the quantitative and qualitative characterization and analysis of these compounds in coffee beans by chemical and physical methods to use for various applications in biological system. The molecular interaction of these compounds with toxic metal ions (Cornard et al., 2008; Cornard and Lapouge, 2004) and with other aromatic molecules like, protein (Suryaprakash, 1995), Caffeine (D'Amelio et al., 2009) and Beta-Cyclodextrin (Gronas et al., 2009; Irwin et al., 1999) have been also reported by theoretical as well as spectroscopic techniques to design more advanced and controllable carries of drugs and food components. In view of the beneficial properties

of bioactive compounds of coffee beans, it is of interest to investigate the quantitative and qualitative characterization and analysis of these compounds in coffee beans, and study the self-association, the complexation with sodium and thermodynamic properties of these compounds by simple methods. The most economical, time saving and simple method for characterization, analysis of these compounds in coffee beans, study their self-association and complexation with sodium are computational and UV-Vis spectrophotometric methods. The techniques are non-destructive and of interest due to the recent development of powerful optical instruments like CCD detector, which permits the detection of numerous substances at low concentration.

Therefore, the objective of the thesis work is spectrophotometric characterization of these bioactive compounds in different solvents and based on this, develop reliable, fast, simple and inexpensive methods for determination of caffeine, chlorogenic acids in coffee beans by computational and spectroscopic methods. In addition, we investigate the concentration dependent self-association, the complexation with sodium ions, and study of thermodynamic properties of these compounds. The specific objectives are 1. Determine the optical transition properties (transition dipole moment, oscillator strength, integrated absorption cross-section and molar extinction coefficients) of caffeine, 5-caffeoylquinic acid and caffeic acid, 2. Develop simple and fast method to analyze the contents of caffeine, chlorogenic acids in coffee beans by UV-Vis spectrophotometry, 3. Investigate the concentration dependent self-association of 5-caffeoylquinic and caffeic acids and their complexation with sodium ions. 4. Calculate the thermodynamic properties of the self-association of 5-caffeoylquinic and caffeic acids using Van't Hoff's equation.

The thesis is organized as follows. In Chapter 1, literature review related to bioactive compounds are presented. The chemical and physical properties these compounds, their contents in coffee beans, the physiological and psychological effects in biological systems, and their roles in determining the quality of coffee beans are discussed. Moreover, the different physical and chemical methods to analyze these compounds in coffee beans and their interaction with aromatic compounds and metal ions are reviewed.

In Chapter 2, the derivation of electromagnetic radiation from Maxwell's equations and the quantum mechanical derivation that is often used for qualitative and quantitative understanding how transitions are induced in molecular system when it interacts with elec-

tromagnetic radiation are presented. The Schrodinger's equation is introduced and simple problems to illustrate its relation to quantities that are important in UV-Vis Spectroscopy are solved.

In Chapter 3, the materials and methods used in this work are presented. This chapter has two sections, the first section of this chapter deals with the description of various chemicals, samples and instruments used to carry out this research and the second section of this chapter deals with the methods of measuring the optical transition properties of caffeine, 5-caffeoylquinic and caffeic acids as well as the mathematical and experimental procedures used to analyze these compounds in coffee beans are presented. In addition, methods of studying the concentration dependent self-association, the complexation of these compounds with sodium and the thermodynamic properties of caffeic acid and 5-caffeoylquincic acid are presented.

In Chapter 4, the results and discussion of the thesis are presented. The calculated values of molar extinction coefficients, transitional dipole moment, oscillator strength, and integrated absorption cross-section of caffeine are reported. In addition, the caffeine contents measured in coffee beans both by Beer-Lambert's law and integrated absorption coefficients techniques are presented. Similarly, the optical transition properties, the molar extinction coefficient, oscillator strength, transition dipole moment, integrated absorption cross-section and solvent effects of 5-caffeoylquincic and caffeic acids are presented. In addition, the contents of CGA measured for different coffee beans of Ethiopia by UV-Vis spectrophotometry are presented. At the end of this chapter, the parameters calculated for concentration dependent self-association, and complexation with sodium ions and thermodynamic of properties of caffeic acid and 5-caffeoylquincic acid are presented. The parameters are the extinction coefficients, dimerzation constants, and the change in enthalpy, Gibbs free energy and entropy of the system calculated using dimer model and Benesi-Hildebrand approach.

Finally, conclusion is given in Chapter 5.

1

Literature Review

In this chapter, the literature review related to the chemical and physical properties of major bioactive compounds in coffee beans, their contents in coffee beans, the various physiological and psychological effects in biological system, along with their relation with coffee quality are presented. In addition, the various chemical and physical methods used to analysis the contents of these compounds in coffee beans and their interaction with aromatic compounds, and metal ions are reviewed.

1.1 Properties and Analysis of Caffeine and Chlorogenic Acids of Coffee

1.1.1 Caffeine

1.1.1.1 Properties and Composition of Caffeine

Caffeine (1,3,7-trimetylxanthine) is one of the main alkaloid found in various kinds of foods and drinks that we consume in daily life (Mumin et al., 2006; Singh and Sahu, 2006; Najafi et al., 2003). It is naturally found in leaves, seeds or fruits of 63 plant species (Mumin et al., 2006). The most common sources of caffeine are coffee, coca beans, cola nuts and tea leaves (Mumin et al., 2006). The chemical formula for caffeine is $C_8H_{10}N_4O_2$ and chemical structure is shown in Fig 1.1. Pure caffeine occurs as odorless, white powder. It has molecular weight of 194.19 g, melting point of 236 ^0C, sublimation point of 178 ^0C and pH values in the range of 6 to 9 (Mumin et al., 2006; Clarke and Macare, 1985).

The caffeine contents of green coffee varies widely between the different Arabica and Robusta species and even within each species there is a very wide range of values of caf-

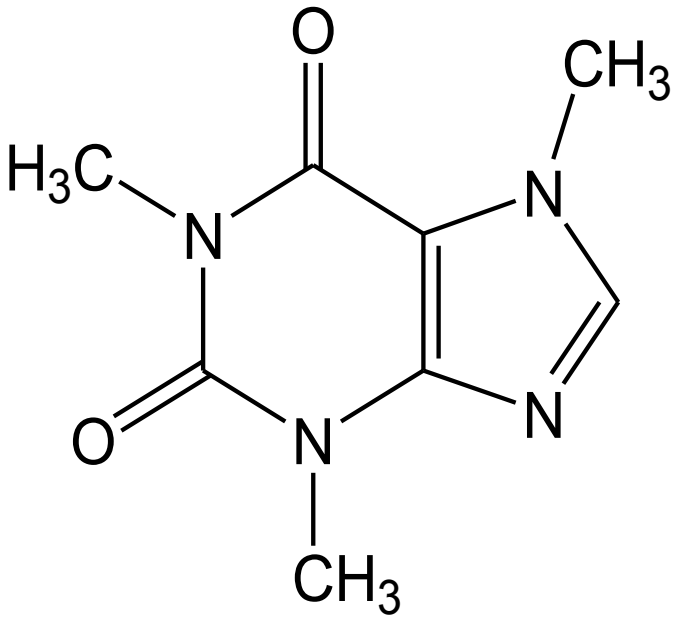

Figure 1.1: The Chemical Structure of Caffeine.

feine contents(Silvarolla et al., 2004; Ky et al., 2001). Robusta coffee in general has higher caffeine content with an over all mean value 2.2 %, whereas that of Arabica is about 1.2 % with a range of 0.6-1.9 % (Belay , 2010; Belay et al., 2008; Clarke and Macare, 1985; Franca et al., 2005). Intermediate value have also been reported for commercially less important species such as Liberica with mean value 1.35 and Arabusta about 1.72 % respectively (Clarke and Macarae, 1985). The coffee paracoffea genus in Africa and Asia are available with very low caffeine contents of about 0.2 % (Clarke and Macarae, 1985). The effects of environmental and agricultural factors are less important than genetic variation in controlling the caffeine contents of green beans and it is also reported that fertilizer in particular potassium, phosphate, magnesium and calcium do not have a significant effect on caffeine (Clarke and Macarae, 1985). Further more, roasting does not have applicable effect on caffeine, other than causing a slight relative increase in its concentration due to the loss of other compounds (Farah et al., 2006; Clarke and Macarae, 1985).

1.1.1.2 **Physiological and Psychological Effects of Caffeine**

The effects of caffeine on humans depends on concentrations consumed. Consumption of high concentration of this compound causes various physiological and psychological

effects such as relaxation of bronchial muscle, stimulation of the central nervous system, gastric acid secretion and dieresis (Zhang et al., 2005; Minamisawa, 2004; Yukawa et al., 2004; Bolton and Null, 1981). Increases in concentration of caffeine in vivo are also a key marker for various disorders, including heart disease, kidney malfunction and asthma. Moreover our sleeping habit, performance, and concentration are modified by caffeine (Zhang et al., 2005; Najafi et al., 2003; Singh and Sahu, 2000; Bolton and Null, 1981). Caffeine is rapidly and completely absorbed from gastrointestinal tract with in a short period of time from consumption and then distributed through out the body(Clarke and Macarae, 1985). However, it is not removed from the circulation until metabolized initially into paraxanthine, thephylline and thyobromine then into derivative of uric acid and diaminourcil. Thus, the plasma half life of caffeine in man that is, the time required for its level to be diminished by 50 $\%$ as a result of biotransformation and excretion is 5-6 hours (Clarke and Macarae, 1985). When caffeine concentration are 15-30 M, effects such as, mild anxiety, respiratory stimulation, cardiovascular effects, diuresis and increase in gastric secretion can be observed. At levels between 150-200 M, a symptom of acute toxicity may appear and these includes severe restlessness, excitement, muscular tension, twitching and cardiovascular disturbance such as tachycardia (Barone and Roberts, 1996; Clarke and Macarae, 1985). The International Olympic Committee classified caffeine as a drug of abuse when it is present in human urine with a concentration higher than 12 $\frac{\mu g}{ml}$ (Aragao et al., 2005; L-Martinez et al., 2003).

On the other hand, besides the physiological and psychological effects of caffeine, the chemical analysis of caffeine in coffee beans have been used as an additional tool for evaluating coffee quality. It has been reported that higher caffeine contents is associated with less quality samples compared to other Arabic samples (Farah et al., 2006; Farah et al., 2005b). Also because of adverse effects mentioned above, there is a demand for decaffeinated of coffee and about 10 $\%$ of the world production of green coffee, and 20 $\%$ of that imported in to some European countries goes for decaffeination in coffee industry (Clarke and Macarae, 1985). However, decaffeination of coffee by chemicals in industry is expensive and it also adversely changes the taste and aroma, thus reducing the quality of coffee. Recently the Nara Institute of Science and Technology in Japan has created transgenic plant with 70 $\%$ lower caffeine contents. The researchers design to repress a

gene, which activated one of the three enzymes, involved in caffeine biosynthesis (Ogita et al., 2003). However, the genetic engineering process takes longer time and is not yet cost effective so there is also activity in searching for coffee beans naturally low in caffeine. Recently, after screening 300 Ethiopian coffee trees Brazilian researchers discovered three naturally decaffeinated varieties, which they named AC1, AC2 and AC3. Analysis of these new varieties showed they contain 0.07 % caffeine compared to the caffeine found in natural coffee beans (Sivarolla et al., 2004). It is hoped that this new coffee will provide an alternative to the artificial decaffeinated coffee that currently supplies the market for this beverage . Thus, because of the importance of caffeine level in coffee simple analytical methods are required in order to characterize and identify the origin of low caffeine coffee beans obtained in Ethiopia.

1.1.1.3 **Methods Developed for Analysis of Caffeine**

Due to above mentioned facts many chemical and physical methods have been developed for the determination of caffeine in coffee and other beverages. The most widely used methods for the determination of caffeine in beverages are based on UV-Vis Spectrophotometry and partial least square (L-Martinez, 2003; Oztem et al., 2003), UV-Vis Spectrophotometry (Singh and Sahu, 2006; Belay et al., 2008; Belay, 2010), derivative spectrophotometry (Alpdogan et al., 2002; Lau et al., 1992), HPLC (Mumin et al., 2006; Aragao et al., 2005; Minamisawa, 2004; Qi et al., 2002; Ortega-Burrales et al., 2002; Casal et al., 2000), Fourier Transform Infrared (FTIR) Spectroscopy (NajafI et al., 2003; Paradkar and Irudayaraj, 2002; Bousain et al., 1999), NIR Reflectance Spectrometry (Chen et al., 2006; Huck et al., 2005; Rodriquez-Saona et al., 2005), Raman Spectroscopy (Edawards et al., 2005) and Capillary Electrophoresis (Zhang et al., 2005) are very commonly used techniques.

The spectrophotometric method is fast, simple, accurate, reproducible and inexpensive procedure as compared to other methods, but it is not possible to determine caffeine directly in coffee beans by conventional UV-Vis absorption measurement due to the spectral overlap of UV absorbing substances in the sample (Belay, 2010; Belay et al., 2008; Zhang et al., 2005). Derivative spectrophotometry is relatively easy, but; it is not reliable for the small concentration of caffeine in samples. By HPLC methods many caffeine contents were determined in various foods using different procedures since it provides the most re-

liable method. However, the relatively high cost of equipments and solvents, combined with the requirement for specialist operator attention, prohibit its use in small industrial laboratories where only a few analysis are performed each day (Zhang et al., 2005; Alpdogan et al., 2002). Other methods such as FTIR, Raman and NIR Reflectance Spectrometry are equally versatile and powerful analytical tools for analyzing contents of caffeine in coffee (Huck et al,. 2005; Rodriquez-Saona et al., 2005), and do not require use of expensive chemicals. However, such instruments are not available in most laboratories in coffee producing countries (especially in Africa and Asia), because of the large initial capital cost involved in their purchase. Although, there are many reports on analysis of caffeine in coffee beans and other beverages by different techniques, however, the qualitative and quantitative characterization of caffeine in coffee beans by a UV-Vis spectrophotometry is not yet reported. The characterization and analysis include the determination of the optical transition properties of caffeine and determines its contents in coffee beans using Beer-Lambert's and integrated absorption techniques.Detail methods of studies and the results obtained by UV-Vis spectrophotometry available in Chapter 3 and 4 of this thesis.

1.1.2 **Chlorogenic Acids**

The other major bioactive compounds in coffee beans are chlorogenic acids. In the next section, a review related to properties, and physical and chemical methods developed for analysis of chlorogenic acids in coffee beans are presented.

1.1.2.1 **Chemical Structure and Composition of Chlorogenic Acids**

Chlorogenic acids (CGA) are the main phenolic compound found in green coffee beans and have been studied for more than century (Clifford, 1979). They are esters of quinic acid and various class of hydroxycinnamic acids, chiefly caffeic acid (3,4-dihydroxycinnamic acid), ferulic acid (3-methoxy-4-hydroxycinnamic acid), p-coumaric (4-hydroxycinnamic acid), and sinapic acids acid (3,5-dimethoxy-4-hydroxycinnamic acid) (Zhu et al., 2006; Manach et al., 2004). However, the major hydroxycinnamic acid in CGAs in food is caffeic acid (Manach et al., 2004; Clifford, 1999). The CGAs are subdivided according to the nature and number of cinnamic substituents, and their esterification position in the cyclohexane ring of quinic acid. The IUPAC number system for quinic acid (1L-1(OH), 3,

4, 5-terahydroxy-cyclohexane carboxyl acid) has axial hydroxyl group on carbon 1 and 3 and equatorial hydroxyl on carbon 4 and 5. Esters of this acid are usually formed on 5 but also on carbon 3, 4 and less commonly on carbon 1 (Clifford, 2000; Clifford, 1979). The main groups of CGA compounds found in green coffee beans are shown in Fig 1.2. These groups of compounds are, caffeoylquinic acids with 3 isomers (3-, 4-, and 5-CQA); dicaffeoylquinic acids (diCQA) with 3 isomers (3-,4-diCQA; 3,5-diCQA; 4,5-diCQA); feruloquinic acids (FQA), with 3 isomers (3-, 4-, and 5-FQA); p-coumaroylquinic acids (pCoQA) with 3 isomers (3-, 4-, and 5-pCoQA) and six mixed diesters of caffeoylferuloy-quinic acids (CFQA) (Clifford, 2003; Trugo and Macrae, 1984; Clifford and Wight, 1976). Caffeoylquinic acids (CQA), dicaffeoylquinic acids (diCQA) and feruloylquinic acids (FQA) represent 98 % of CGA (Farah and Donangelo, 2006; Clarke and Macarae, 1985) and minor class, such as diferuloylquinic acids ((diFQA), di-p-coumaroylquinic acid (di-p-CoQA), dimethoxycinnamoylquinic acids and others, which together constitute less than 1 % of the total CGA content as recently identified (Perrone et al., 2008). The major representative of hydrox-

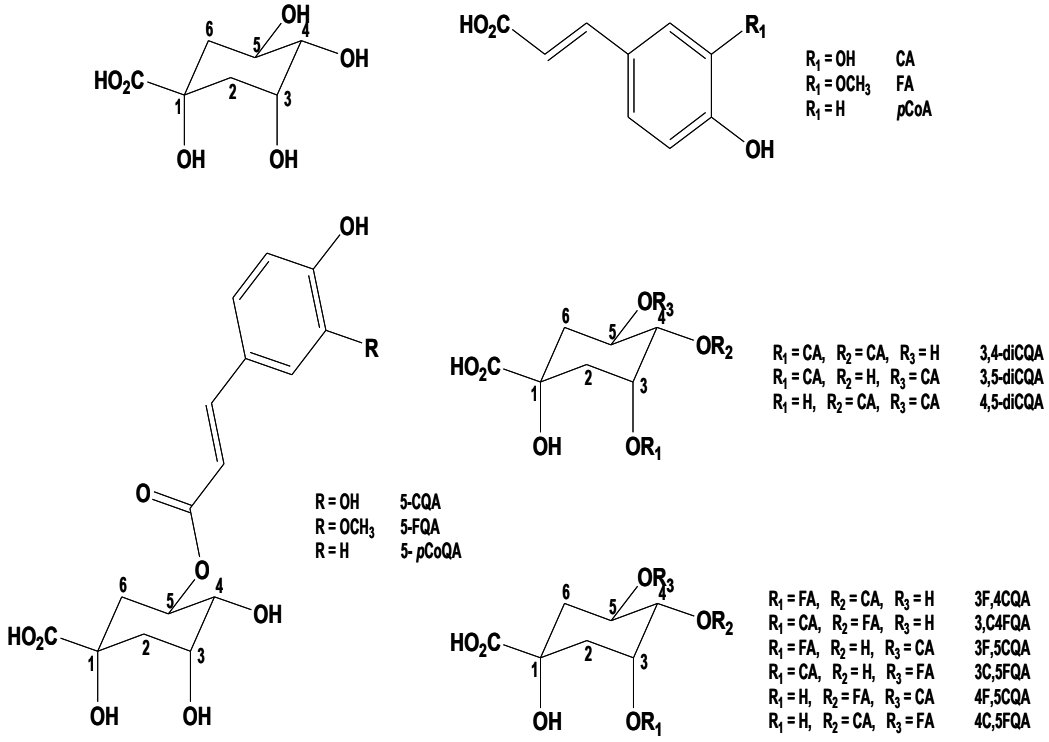

Figure 1.2: The Chemical Structure of Hydroxycinnamic Family (Caffeic, Coumaric Ferrulic, and Sinapic Acids).

ycinnamic acid is caffelic acid, which occurs in food mainly as an ester with quinic acid called chlorogenic acids (Manach et al., 2004; Kono et al., 1995; Clifford, 1999).

The total CGA content of green coffee beans is varying according to genetic species, degree of maturation and less importantly agricultural practices, climate and soil (Farah et al., 2005b; Clifford, 1985). In general, the percentage of CGA for regular green coffee beans on the dry matter varies from 4-8.4 % for Arabic and 7-14.4 % for Canephora with some hybrids presenting intermediate levels (Farah et al., 2005a, 2005b; Trugo and Macrae, 1984b; Clifford and Wight, 1976). On the other hand, a low CGA content 1.2 % has reported in beans of coffea pseudozanquebariae, caffeine free species native of East Africa. Similar low CGA contents have been also observed in some other low caffeine species from Africa (Clifford, 1985).

1.1.2.2 **Antioxidant Properties of Chlorogenic Acids**

Chlorogenic acids are the most abundant poly phenols in coffee which are responsible for substantial part of coffee antioxidants (Svilaas et al., 2004; Wen et al., 2004; Pellegrini et al., 2003; Richelle et al., 2001). Epidemiological study indicates that due to chlorogenic acids and its derivatives coffee shows tea catechin analog biological effect like antioxidation, antimutation, anticarcinogenic, antibiotic, antihypercholesterolemia, antihypertensive, metal chelation and inflammatory action (Svilaas et al. 2004; Wen et al. 2004; Yukawa 2004). Moreover, there are also reports that hydroxycinnamic acid derivative have a promising role in protection from radiation and photooxidation (Zhu et al. 2006). In addition to these properties of the coffee beverage, commercial antioxidants are also manufactured from coffee that consists of 55 % chlorogenic acids which regulates the blood sugar and perhaps help for the management of obesity (Abidoff, 1999). Clinical studies indicate that CGAS can lower blood glucose level by 15-20 % (Abidoff, 1999). Furthermore, the risks of type-2 diabetes also decreased with increasing coffee consumption. It is expected that the chlorogenic acids are the main active component to antidiabetic effect according to pharmacology studies (Salazar-martinez et al., 2003; Vandam and Feskens, 2002; Abidoff, 1999).

CGAs also have physiological function in coffee plant and contributes to control seed germination and cell growth through regulation of the level of indolacetic acids (Clifford,

1985). Moreover, it has been reported that due to antioxidants and antibiotic properties hydroxycinnamoylqunic acids are involved in numerous biological plant functions such as pest and disease resistance (Matsuda et al., 2003; Takahama, 1998). The mono (Szalma et al., 2005) and dicaffeoylqunic acids (Cole 1984) are involved in the insect resistance in different cultivated species.

In addition to their the antioxidant properties and physiological function, CGAs play important role in the formation of pigments, taste and flavor of coffee beans, which ultimately determine the quality and acceptance of the beverages. They also contribute to the final acidity of the beverages, as result of maillard and strecker's reaction bitterness and the formation of lactones and other phenol derivatives responsible for flavor and aroma (Variyar et al., 2003; Trugo and Macrae, 1984a; Clifford and Wight, 1976). According to literature report a total CGA level in coffee has an inverse association with coffee quality with higher content observed in lower quality samples. Farah et al. (2006) observed a strong association between the level of CQA and FQA and low cup quality. The Lower CGA level of coffee Arabica in beverage quality when compared with coffee Canephora also appear to explain this situation . The large difference in CGAs content of these species was considered one of the factors responsible for flavor difference between the two species (Ky et al., 2001; Trugo and Macrae, 1984b).

1.1.2.3 **Methods Developed for Analysis Chlorogenic Acids**

Several methods have been developed for determination of chlorogenic acids and its derivatives in coffee beans and other plants. The most widely used methods are HPLC (Farah et al., 2006; Farah et al., 2005b; Negishi et al., 2004; Ky et al., 2001; Pedrosa et al., 2000), Capillary Electrophoretic (Jiang et al., 2004). Although these methods are powerful for quantification of the content of chlorogenic acids and its derivatives, however, they have been criticized as being tedious, time consuming and some are limited to large sample volume extraction procedures and requiring long time for reaction. Moreover, most of these instruments are very expensive which are not available in moderate laboratories in many coffee producing countries.

There are also some less expensive physical methods developed for qualitative and quantitative characterization of chlorogenic acids. For example UV-Vis and Fluorescence

Spectroscopy used to study the suppression of N-nitrosating reaction by CGAs (Kono et al., 1995). Other examples include measurements of the rates of oxidation of hydroxycinnamic by HRP/H2O2 or tyrosinase /O2 (Moridani et al., 2001), the application of laser flash photolysis technique to investigate the antioxidant properties of CGA (Zhu et al., 2006) and determination of CGA contents in fresh and processed potatoes (Dao and Friedman, 1992) and coffee beans (Belay et al., 2009) using UV-Vis spectrophotometry.

Despite the characterization of the compound using the above mentioned techniques, it is worthwhile to mention that simple and systematic method for measuring the CGA in green and roasted coffee beans using UV-Vis Spectroscopy applied by Belay et al. (2009). Moreover, the investigation of the optical transition properties (molar decadic absorption coefficient, integrated absorption cross-section, oscillator strength and transition dipole moment) of CGA, the effects of solvents, and the self-association and hetero-association were not explored by above mentioned instrument in the past. The detail methodologies and results obtained in thesis are presented in Chapter 3 and 4 respectively.

1.2 Complexation of Caffeic and 5-caffeoylqunic Acids

Caffeic and 5-caffeoylqunic acids are one of the most common phenolic acids frequently occuring in fruits, vegetables, cereals legumes and in beverage of plant origin such as wine, tea and coffee (Iglesias et al., 2009; Jiang et al., 2005; Cornard et al., 2008; Scalbert et al., 2005; Dillard and German, 2000; Prior, 2003). The compounds have attracted the attention of researchers due to health promoting attributes, which include lowering the risks of cardiovascular disease, cancer, diabetes, and other conditions associated with aging. The biological mechanisms behind these effects are protection against free radicals, free radical mediated, inflammation and viral infection (Rice-Evans et al., 1996; Kono et al., 1997; Robinson and Chew, 1999; Iglesias et al., 2009; Jiang et al., 2005; Giacomelli et al, 2002). On the other hand, caffeic and 5-caffeoylqunic acids are multifunctional natural organic acid substances that play significant role in binding toxic metals in the natural environment (Cornard et al., 2008; Cornard and Lapouge, 2004). The complexation of caffeic and 5-caffeoylqunic acids with series of di and trivalent metal ions have been studied and reported by different workers using theoretical and spectroscopic techniques: the complexation with Al (III) (Cornard et al., 2006; Cornard et al., 2004; Khvan et al., 2001), copper

(II), Ni(II), Zn (II), Co (II), lead (II) (Khvan et al., 2001; Cornard et al., 2008), and iron (III) (Hynes et al., 2004). Moreover the molecular complexation of these compounds with Beta-Cyclodextrin (Gornas et al., 2009; Irwin et al., 1999), caffeine (Sondheiemer et al., 1961; D'Amelio et al., 2009), and protein (Suryaprakash and Prakash, 1995) have been reported to design more advanced and controllable carriers of drugs and food components.

Although from pervious research reports, it is possible to find the complexation of these compounds with other metal ions, polyphenols, and aromatic-hydroxy acid molecules, there are lack of information on the concentration dependent self-association, their thermodynamic properties and the complexation with sodium ions. Usually, investigation of self-associated molecules using UV-Vis spectroscopy is limited due to difficulty in obtaining the spectra of highly concentrated solutions and need of careful examination of changes in the apparent molar extinction coefficients over a wide range of concentrations (Dearden, 1963). It is known that, the formation of dimer complicates the use of Beer-Lambrt's law. Extinction coefficients and shape of the absorption band of the fraction of dimerized molecules are usually unknown that often leads to difficulty in the interpretation of experiment (Marme et al., 2005). Therefore, study of the self-association and hetero-association of the solute is an important phenomenon. This is accounted in analyzing and interpreting the spectroscopy, photophysics and photochemistry of the system (Dearden, 1963; Marme et al., 2005). Moreover, in order to design a more advanced and controllable carries of drugs or food components it is necessary to know the association mechanisms to control the processes. .

On the other hand the solvent effects and the parameters measuring the transition probability of the compound (oscillator strength, transition dipole moment and integrated absorption cross-section) which are also useful for providing stringent tests of atomic structure calculations (Radwan, 2007; Liptay, 1969), were not calculated for caffeic and 5-caffeyloquinic acids in different solvents in the past. The details study for the complexations, the thermodynamic and optical transition properties of caffeic and 5-caffeoylquinic acids using UV-Vis spectrophotometric are presented in thesis in Chapter 3 and 4 respectively.

2

Interaction of Light with Matter

One of the most important and interesting aspects of spectroscopy are study the propagation of light through matter. In this chapter the interaction of light with matter by classical and semi-classical theory are discussed. In the first two sections of this chapter the base for electromagnetic theory is reviewed. Starting with Maxwell's equations, the wave equations in material media are described by classical electromagnetic theory to show the various optical phenomena exhibited by medium like absorption and dispersion of the light. The last two sections of this chapter deal with the mathematical derivation that can often used for qualitative and quantitative understanding of how transitions induced in molecular system are presented by semi-classical approach. The time dependent Schrodinger equation has been solved to relate, the theoretical expressions, (the transition diploe moment) with the experimental quantities, (the molar extinction coefficient, extinction coefficient) that are important in UV-Vis spectroscopy.

2.1 Maxwell's Equations and the Wave Equations

All the macroscopic aspects of the static and dynamics of the electromagnetic field in the presence of the material media are described by four Maxwell's Equations (Atikins and Friedman, 1997). These equations are the most fundamental description of electric and magnetic field. The differential form of Maxwell's equations are given by

$$\vec{\nabla} \times \vec{E} = -\frac{\partial \vec{B}}{\partial t} \tag{2.1}$$

$$\vec{\nabla} \times \vec{H} = \frac{\partial \vec{D}}{\partial t} + \vec{J} \tag{2.2}$$

$$\vec{\nabla}.\vec{D} = \rho \tag{2.3}$$

$$\vec{\nabla}.\vec{B} = 0 \tag{2.4}$$

The four quantities which describe the electromagnetic nature of matter at a given point are the electric charge per unit volume (ρ) , the electric dipole moment per unit volume or polarization (P) , the magnetic dipole moment per unit volume or magnetization (M) , and the electric current per unit area or current density (J) (Fowler, 1975). The material media responses are expressed by the material relation $\vec{D} = \epsilon_0 \vec{E} + \vec{P}$, and $\vec{B} = \mu_0 \vec{H} + \mu_0 \vec{M}$, where $\vec{D}$ is the electrical displacement, $\vec{E}$ is the electric field strength, $\vec{H}$ is the magnetic field strength, $\vec{B}$ is magnetic induction and ϵ_0, μ_0 are permittivity and permeability in vacuum respectively.

For non-magnetic and non-conducting media, the volume density of electric charge and magnetization are zero, thus the Maxwell's equations expressed in Eqs (2.1) to (2.4) take the form of the following (Attwood, 1999; Millonni and Eberly, 1988; Fowler, 1975).

$$\vec{\nabla} \times \vec{E} = -\mu_0 \frac{\partial \vec{H}}{\partial t} \tag{2.5}$$

$$\vec{\nabla} \times \vec{H} = \varepsilon_0 \frac{\partial \vec{E}}{\partial t} + \frac{\partial \vec{P}}{\partial t} \tag{2.6}$$

$$\vec{\nabla}.\vec{E} = 0 \tag{2.7}$$

$$\vec{\nabla}.\vec{H} = 0 \tag{2.8}$$

From the above Maxwell's equations the general wave equation for electric field can be obtained by taking the curl of Eq (2.5) and time derivative of Eq (2.6) and eliminating the magnetic field. Thus,

$$\vec{\nabla} \times (\vec{\nabla} \times \vec{E}) + \frac{1\partial^2 \vec{E}}{c^2 \partial t^2} = -\mu \frac{\partial^2 \vec{P}}{\partial t^2} \tag{2.9}$$

By general identity of vector calculus the first left hand side of Eq (2.9) can be expressed as follows (Attwood, 1999; Millonni and Eberly, 1988)

$$\vec{\nabla} \times (\vec{\nabla} \times \vec{E}) = \vec{\nabla}(\vec{\nabla}.\vec{E}) - \vec{\nabla}^2\vec{E} \tag{2.10}$$

Substituting Eq (2.10) in Eq (2.9) the general wave equation for electric field becomes

$$\vec{\nabla}^2\vec{E} - \vec{\nabla}(\vec{\nabla}.\vec{E}) - \frac{1}{c^2}\frac{\partial^2\vec{E}}{\partial t^2} = \frac{1}{\varepsilon_0 c^2}\frac{\partial^2\vec{P}}{at^2} \tag{2.11}$$

where,

$$\epsilon_0\mu_0 = \frac{1}{c^2} \tag{2.12}$$

Eq (2.12) represents the speed of light in vacuum, on the other hand Eq (2.11) is a partial differential equation with the term on the right side a source term shows the presence of polarization within the medium. The way in which the propagation of light is affected by the source is revealed by the solution of the wave equation when the source term is included. For non-conducting (a neutral isotropic dielectric media) the polarization term is important. This term leads to an explanation of many optical effects including dispersion and absorption (Attwood, 1999; Millonni and Eberly, 1988; Fowler, 1975). For solenoidal field Eq (2.11) becomes

$$\vec{\nabla}^2\vec{E} - \frac{1}{c^2}\frac{\partial^2\vec{E}}{\partial t^2} = \frac{1}{\epsilon_0 c^2}\frac{\partial^2\vec{P}}{\partial t^2} \tag{2.13}$$

Thus, Eq (2.13) is the fundamental electromagnetic wave equation. In order to make any use of it, it is necessary to specify the polarization. However this can not be done solely with in the frame work of Maxwell's equations, since polarization is the property of the material media. We need to know how a dipole moment density is produced in the medium. For this purpose in the next section, a theory of dielectric media will be introduced. First however, this section would be completed with a discussion of the solution of the Maxwell's equation in vacuum, where a wave equation (2.13), does not contain the polarization term (Attwood, 1999; Millonni and Eberly, 1988; Fowler, 1975);

$$\vec{\nabla}^2\vec{E} - \frac{1}{c^2}\frac{\partial^2\vec{E}}{\partial t^2} = 0 \tag{2.14}$$

For a wave propagating in the z-direction in free space the solution of wave equation becomes

$$\vec{E}(\vec{z},t) = \vec{\epsilon}E_0 cos(\omega t - \kappa z) \tag{2.15}$$

where the wave vector and phase velocity are defined as follows

$$\kappa_0^2 = \frac{\omega_0^2}{c^2} \tag{2.16}$$

$$v_{p=\frac{\omega}{\kappa_0}} = c \tag{2.17}$$

2.2 Propagation of Light in Isotropic Dielectric

To express the polarization in dielectric due to propagation of light, a simple Lorentz model is considered. The classical theory satisfactorily treats many important features of the interaction of light with matter by adopting the hypothesis that an electron exert a binding force on nucleus (Millonni and Eberly, 1988; Fowler, 1975). This hypothesis states that an electron in an atom responds to light, as if, it was bound to its atom or molecule by simple spring. As consequence, the electron can be imagined to oscillate about the nucleus and this leads to the following equation of motion (Millonni and Eberly, 1988; Fowler, 1975):

$$m\frac{d^2\vec{x}}{dt^2} = -e\vec{E}(R,\vec{t}) - K\vec{x} \tag{2.18}$$

where K is the spring constant.

On the other hand, when field applied to dielectric substances, each electron is displaced by some distance x from its original position, thus each atom has a dipole moment . If the density of atoms is denoted by N, then the density of dipole moment is N times the individual dipole moment of each atom (Millonni and Eberly, 1988). Therefore, the polarization density induced in the medium by the field is given by

$$\vec{P} = N\vec{p} = Ne\vec{x} \tag{2.19}$$

Eq (2.13) tells us how the electric field depends upon the dipole moment density of the medium and Newton's equation (2.18) shows how the electron displacement depends up on the electric field while Eq (2.19) connects these two basic equation by relating polarization to displacement (Fowler, 975). The electron oscillator model thus ties the Maxwell's equation with Newton's law of motion. Solution of these coupled equation will provide mutual interaction of light and matter. Further, if atoms or molecules of the medium absorb radiation energy while it transits through the materials, the strength of EMR would be

reduced. This absorption can be further explained by the assumption of Lorentz electron oscillator of Eq (2.18). The classical damped harmonic oscillators due to bound electron amend Newton's force of equation (2.18) as follows:

$$m\frac{d^2\vec{x}}{dt^2} = -e\vec{E}(\vec{R},t) - K\vec{x} + \vec{F}_{fric} \tag{2.20}$$

Since the assumption is compatible with the idea of frictional drag, and a frictional damping force is proportional to the velocity of the electron,and some proportionality constant designated by γ. Therefore the differential equation of the motion becomes

$$m\frac{d^2\vec{x}}{dt^2} + m\gamma\frac{d\vec{x}}{dt} + K\vec{x} = -e\vec{E} \tag{2.21}$$

Now to find the trial solution of Eq (2.21), let us consider the assumption that the electric field varies harmonically with the time and the motion of electron has also similar harmonic time dependence $\vec{x} = e^{i\omega t}$, thus on substitution of this field into Eq (2.21) results in

$$(-m\omega^2 - i\omega m\gamma + K)\vec{x} = e\vec{E} \tag{2.22}$$

Consequently, the polarization of Eq (2.19) would be given by

$$\vec{P} = \frac{Ne^2}{-m\omega^2 - i\omega m\gamma + K}\vec{E} \tag{2.23}$$

A more significant way of writing Eq (2.23) is in which the equation of resonance frequency of bound electrons have been written. Thus Eq (2.23) can be written as

$$\vec{P} = \frac{Ne^2/m}{\omega_0^2 - \omega^2 - i\omega\gamma}\vec{E} \tag{2.24}$$

Apart from Lorentz model polarization can also be expressed using dielectric constant or susceptibility (Demtroder, 1995)

$$\vec{P} = \epsilon_0(\epsilon_r - 1)\vec{E} = \epsilon_0\chi\vec{E} \tag{2.25}$$

The relative dielectric constant is related to refractive index n by

$$n = \sqrt{\epsilon_r} \tag{2.26}$$

Substituting Eq (2.26) into Eq (2.25) and comparing it with Eq (2.24) yields

$$n^2 - 1 = N\frac{e^2}{\epsilon_0 m(\omega_0^2 - \omega^2 + i\gamma\omega)} \tag{2.27}$$

Using the approximation of $n^2 - 1 \approx 2(n-1)$ which is sufficiently accurate for the most purpose and the above equation can be reduced to

$$n = 1 + N\frac{e^2}{2\epsilon_0 m(\omega_0^2 - \omega^2 + i\gamma\omega)} \tag{2.28}$$

In order to make clear the physical implication of Eq (2.28) or the complex index of refraction, let us separate the real and the imaginary part as follows:

$$n = n^{'} - i\kappa \tag{2.29}$$

For electromagnetic wave

$$\vec{E} = E_0 exp[i(\vec{w}t - kz)] \tag{2.30}$$

passing through a medium in the z direction, with the refractive index n has same frequency as in vacuum or $\omega_n = \omega_0$, but the wave vector becomes $k_n = k_0 n$, and inserting this into Eq (2.30), it becomes

$$E = E_0 e^{-k_0\kappa z} e^{i(\omega t - k_0 n^{'} z)} \tag{2.31}$$

In Eq (2.31), the imaginary part $\kappa(\omega)$ of the complex refractive index n describes the absorption of electromagnetic wave. At penetration depth the amplitude of $E_0 exp(-k_0 kz)$ has decreased to $\frac{1}{e}$ of its value at z=0. On the other hand, the real part $n^{'}(\omega)$ represents the dispersion of the wave, that means the dependence of the phase velocity $v(\omega) = \frac{c}{n^{'}(\omega)}$ on the frequency. The intensity which is $I \propto E^* E$ then decrease as

$$I = I_0 e^{-2\kappa k_0 z} = I_0 e^{-\alpha z} \tag{2.32}$$

where the absorption coefficient, $\alpha = 2k_0\kappa = \frac{4\pi\kappa}{\lambda_0}$ is proportional to the imaginary part of the complex refractive index. The frequency dependent α and $n^{'}$ using the above equation as the real and imaginary parts becomes

$$\alpha = \frac{Ne^2\omega_0\gamma\omega}{c\epsilon_0 m[(\omega_0^2 - \omega^2)^2 + \gamma^2\omega^2]} \tag{2.33}$$

$$n^{'} = 1 + \frac{Ne^2(\omega_0^2 - \omega^2)}{2\epsilon_0 m[(\omega_0^2 - \omega^2)^2 + \gamma^2\omega^2]} \tag{2.34}$$

The above two equations are the Kramers-Kronig dispersion relation and they are the absorption and dispersion of the complex refractive index (Demtroder, 1995). In addition,

from the above equations the real and imaginary part of dielectric constant, susceptibility can also be obtained, however since classical way of interaction of light with matter are not our interest we moved to the next section where the interaction of light with matter is treated by semiclassical approach.

Therefore, in the next sections the absorption and emission of light by molecular system would be presented by semi-classical approach in which molecular system is treated quantum mechanically and the radiation field is treated classically. For molecular system instead of absorption coefficient the absorption cross-section and molar extinction coefficient were used to express the phenomena of absorption and these will be discussed in detail in the next two sections.

2.3 Absorption and Emission of Radiation

Absorption and emission are one of the phenomena in which the electromagnetic radiation interact with matter and any process taking place in an atom or molecule associated with absorption or emission of radiation is transition (Piepho and Schatz, 1983; Levine, 1975; Bauman, 1962; Barrow, 1962) . To discuss transition in molecular states the time-dependent Schrodinger equation is derived (Levine, 1975). It allows calculating the atomic scale phenomena as a function of time as well as space. Thus, in the next section the general solution for time dependent Schrodinger equation will be derived

2.3.1 The Time Dependent Schrodinger Equation

In this section the transition dipole moment of the molecule are derived from time dependent Schrodinger equation by semiclassical approach in which the radiation of incident upon an atom is described by classical electromagnetic plane wave and the atom on the other hand treated quantum mechanically (Demtroder, 1995). In order to simplify the equation, it is restricted to two level system with the state $|K\rangle$, $|m\rangle$ and energy E_k and E_m.

In spectroscopy, if a system that is initially in some stationary state of definite energy, exposed to electromagnetic radiation for a limited time, then it undergoes transition to some other stationary states . If $\hat{H}^0$ be the Hamiltonian of the molecular system in the absence of radiation field, the time independent equation becomes (Piepho and Schatz,

1983; Levine, 1975; Bauman, 1962; Barrow, 1962)

$$\hat{H}^0\psi_k^{(0)}(x) = E_k^{(0)}\psi_k^{(0)}(x) \tag{2.35}$$

where the energy and the wave function are, $\psi_k^{(0)}(x)$ and $E_k^{(0)}$ for unperturbed system. On the other hand if $\hat{H}'(t)$ be the Hamiltonian due to interaction between the system and radiation, the time dependent Schrodinger equation can be written as follows to indicate the state change with time (Demortoder, 1995; Svanberg, 1991):

$$i\hbar\frac{\partial\psi}{\partial t} = \hat{H}'\psi \tag{2.36}$$

During the time interval the perturbation becomes, the sum of unperturbed Hamiltonian of the free atom plus the perturbation operator (Demortoder, 1995; Svanberg, 1991):

$$i\hbar\frac{\partial\psi}{\partial t} = (\hat{H}^0 + \hat{H}')\psi \tag{2.37}$$

Where the general state function expressed as a linear superpositions of

$$\psi(x,t) = \Sigma c_k exp(-iE_k^{(0)}t/\hbar)\psi_k^{(0)}(x) \tag{2.38}$$

For two level systems Eq (2.38) reduced to a sum of two terms, according to the following

$$\psi(x,t) = c_k(t)\psi_k^{(0)}e^{-\frac{iE_kt}{\hbar}} + c_m(t)\psi_m^{(0)}e^{-\frac{iE_mt}{\hbar}} \tag{2.39}$$

The coefficient $c_k(t)$ and $c_m(t)$ are the time dependent probability amplitudes of the two atomic states of k and m (Demortoder, 1995). This means that the value $|c_k(t)|^2$gives the probability of finding the system in level $|k\rangle$ at time t. In addition the relation of $|c_k(t)|^2 + |c_m(t)|^2 = 1$ holds at all times t, if the decay into other level is neglected.

If further substituting Eq (2.38) in to Eq (2.37) yields

$$i\hbar\frac{d}{dt}(\Sigma c_k(t)exp(-\frac{iE_k^0t}{\hbar})\Psi_k^0) = (\hat{H}^0 + \hat{H}')\Sigma c_k exp(-iE_k^{(0)}t/\hbar)\psi_k^{(0)} \tag{2.40}$$

where, the relation in Eq (2.35) has been used to cancel equal terms on both sides. Further multiplication by conjugate with $\psi_n^*(n = k,m)$ and spatial integration result in the following two equations:

$$\frac{d(c_k(t))}{dt} = -\frac{i}{\hbar}\{c_k(t)\hat{H}'_{kk} + c_m(t)\hat{H}'_{km}e^{\frac{i(E_k-E_m)t}{\hbar}}\} \tag{2.41}$$

$$\frac{d(c_m(t))}{dt} = -\frac{i}{\hbar}\{c_m(t)\hat{H}'_{mm} + c_k(t)\hat{H}'_{mk}e^{\frac{-i(E_k-E_m)t}{\hbar}}\} \tag{2.42}$$

with the spatial integral

$$\hat{H}'_{km} = \int \psi_k^* \hat{H}' \psi_m d\tau = -e\vec{E}\int \psi_k^* \vec{r}\psi_m d\tau = -\vec{E}.\vec{\mu}_{km} \tag{2.43}$$

$$\vec{\mu}_{km} = \vec{\mu}_{mk} = -e\int \psi_k^* \vec{r}\psi_m d\tau \tag{2.44}$$

Eq (2.44) is the atomic transition dipole moment. It depends on the wave functions ψ_k and ψ_m of the two states and are determined by charge distribution in these states (Barrow, 1962; Demtroder, 1995). On the other hand, Eq (2.41) and (2.42) show the rate at which a system can be changed from one stationary to another under the influence of perturbing effect. They are the basic equations, which must be solved to obtain the probability amplitudes of $c_k(t)$ and $c_m(t)$ (Demtroder, 1995; Barrow, 1962). To further proceed with Eq (2.41) and (2.42), it is necessary to be more specific about the perturbation . Thus, in the next section some features of the interaction between the electric field and molecular system would be treated.

2.3.2 **Interaction of EMR with Molecular System**

When electromagnetic radiation falls on a molecule the oscillating electric field of the radiation can disturb the energy of the molecule and allow it to escape from its initial stationary state, which is characterized by quantum number k (Demtroder, 1995; Levine, 1975; Barrow, 1962). Eq (2.45) which describes the x-component of the radiation at the position occupied by the molecule becomes

$$\vec{E}_x = \frac{\vec{E}_x^{(0)}(e^{i\omega t} + e^{-iwt})}{2} \tag{2.45}$$

This field can act on a dipole moment component to produce a change in energy and is responsible for the change in Hamiltonian that occurs when radiation falls on the system. The change in the Hamiltonian therefore, is given by

$$\hat{H'} = -\vec{E}_x\vec{\mu_x} \tag{2.46}$$

Substituting Eq (2.45) into (2.46), the perturbed Hamiltonian becomes

$$\hat{H}' = -\frac{\vec{E}_x^{(0)}(e^{i\omega t} + e^{-iwt})}{2}\mu_x \tag{2.47}$$

Before substituting this Eq in (2.41) and (2.42) a weak-field approximation was considered (Demtroder, 1995), in which at time $t = 0$, the atoms are in the lower state which implies $c_k(t) = 1$ and $c_m(t) = 0$. By assumption that the field amplitude to be sufficiently small for the time $t < T$ and the population of E_m remains small compared with that of E_k. Therefore with this assumption the first approximation of Eq (2.41) and (2.42) become

$$\frac{dc_k(t)}{dt} = 0 \tag{2.48}$$

$$\frac{dc_m}{dt} = \frac{i}{2\hbar}E_x^{(0)}\langle\psi_m^{(0)}|\mu_x|\psi_k^{(0)}\rangle[e^{it(\omega+\omega_{mk})} + e^{it(\omega_{mk}-\omega)}] \tag{2.49}$$

where,

$$|\mu_{xkm}| = \int < \psi_m^{(0)} \mid \mu_x \mid \psi_k^{(0)} > dx \tag{2.50}$$

With the initial condition of $c_k(0) = 1$ and $c_m(0) = 0$ the integration over the time interval 0 to t will give

$$c_k(t) = 0 \tag{2.51}$$

$$c_m(t) = \frac{E_x^0|\mu xkm|}{2}[\frac{e^{it(E_m-E_k-h\nu)} - 1}{E_m - E_K - h\nu} + \frac{e^{it(E_m-E_k+h\nu)} - 1}{E_m - E_K + h\nu}] \tag{2.52}$$

The process of interest here is the process in which the system goes from lower energy level k to higher level m. For such arrangement of energies, the denominator of the first term of Eq (2.52) will go to zero when the radiation frequency is such that

$$h\nu = E_m - E_k \tag{2.53}$$

On the other hand for emission case the second term is important since the energy in sate m is greater than that of state K. Thus, for assumed energy level pattern Eq (2.52) reduces to

$$c_m(t) = \frac{E_x^0|\mu\_xkm|}{2}[\frac{e^{it(E_m-E_k-h\nu)} - 1}{E_m - E_K - h\nu}] \tag{2.54}$$

The transition probability of absorption from k to m state becomes

$$c_m^*(t)c_m(t) = E_0^2|\mu_{xkm}|^2(\frac{sin^2(\frac{E_m-E_k-h\nu}{2\hbar})t}{(E_m - E_k - h\nu)^2}) \tag{2.55}$$

The total probability of a transition to state m is found by summing the infinitesimal probabilities over the various frequencies. Since the energy density in an electromagnetic wave is given by ,$u = \frac{E_0^2 \epsilon_0}{2}$ the transition probabilities calculated proportional to the energy density of the incoming radiation. If we consider a situation in which the in coming radiation is electromagnetic waves of many frequencies, it is the best to replace u with energy density in the range $d\omega$(that means $u \longrightarrow d\omega\rho(\omega)$ and integrate over the frequency spectrum. Moreover since the term in the bracket is sharply peaked about $\omega \approx \omega_{mk}$ while $\rho(\omega)$ is slowly varying we can move ρ out side of the integral.

$$c_m^*(t)c_m(t) = \frac{2\rho(\omega)}{\epsilon_0\hbar^2}|\mu_{xkm}|^2 \int sin^2 \frac{(\omega_{mk} - \omega/2)t}{(\omega_{mk} - \omega)^2} d\omega \tag{2.56}$$

After integration, Eq (2.56) becomes

$$c_m^*(t)c_m(t) = \frac{\pi\rho(\omega)}{\epsilon_0\hbar^2}|\mu_{xkm}|^2 t \tag{2.57}$$

Eq (2.57) gives the probability that any molecule will make a transition to sate m after having been illuminated for a time t. The integration of Eq (2.56) carried using the following integration technique

$$\int \frac{sin^2 ax}{x^2} dx = \pi a \tag{2.58}$$

The number of transition to m per second or rate of transition probability becomes

$$\frac{d(c_m^* c_m)}{dt} = \frac{\pi\rho(\omega)}{\epsilon_0\hbar^2}|\mu_{xkm}|^2 \tag{2.59}$$

For isotropic radiation the three components of the radiation dipole interaction are equal and one can write

$$\frac{d(c_m^* c_m)}{dt} = \frac{\pi\rho(\omega)}{3\epsilon_0\hbar^2}|\mu_{km}|^2 \tag{2.60}$$

This is the rate of change of the system as a result of absorption under the perturbing effect of the electric field of the radiation and it is usually written with Einstein's coefficient of induced absorption so that Eq (2.60) becomes (Demtroder, 1995; Levine, 1975; Barrow, 1962)

$$\frac{d(c_m^* c_m)}{dt} = B_{km}\rho \tag{2.61}$$

From the Eq (2.60) it is obvious that the rate of transition depends on transition moment and energy density of the radiation (Demtroder, 1995; Levine, 1975; Barrow, 1962).

To complete the general study of the process by which radiation is absorbed, it remains only to compare this derived expression with the quantities usually encountered in the experimental determination of the absorption of radiation. Thus in the next sections the experimentally observable quantities, the molar extinction coefficient, are related to the parameter which reflects the detail molecular properties of the system, namely the transition integral.

2.3.3 Beer-Lambert's Law and Integrated Absorption Technique

In optics, the Beer-Lambert's law relates the absorption of light to the properties of the materials through which the light travels. The law states that there is a logarithmic dependence between the transmission, T, of light through substance and the absorption coefficient of substance, α, and the distance the light travel through the material (the path length), l. The absorption coefficient can, in turn be written as a product of either a molar absorption coefficient of the absorber, ϵ, and the concentration c, of the absorbing species in material or the absorption cross-section, σ, and number density N of the absorbers.

For liquids, these relations are usually written written as

$$T = \frac{I}{I_0} = 10^{-\alpha l} = 10^{-\epsilon c l} \tag{2.62}$$

where, I_0 and I are the intensities of the incident and transmitted light respectively. For gases and in particular among physicist and for some spectroscopic techniques, they are normally written as Eq of (2.33) (Bauman, 1962; Thorone; 1988; Millonni and Eberly, 1988). The transmission for liquid substances expressed in terms of absorbance as follow,

$$A = log(\frac{I_0}{I}) = \epsilon c l \tag{2.63}$$

The absorption cross-section σ, related to the absorption coefficient α at a single frequency for N number of molecules per unit volume can be expressed by the following relation,(Thorone; 1988; Millonni and Eberly, 1988)

$$\alpha(\nu) = \sigma(\nu) N \tag{2.64}$$

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 (Thorone, 1988; Rao, 1975). 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 technique is 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 (Thorone, 1988; Rao, 1975; Liptay, 1969). In addition, the technique is very important in the absence of a high-resolution spectrometer (Ramasy, 1951). 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:

$$\alpha_t = \int \alpha d\nu \tag{2.65}$$

Using Eq (2.63) into (2.65) yields

$$\alpha_t = \frac{1}{l} \int log(\frac{I_0}{I}) d\nu \tag{2.66}$$

From the integrated absorption coefficient, the integrated absorption cross-section can be calculated using the following equation (Milonni and Eberly, 1988).

$$\sigma_t = \frac{1}{Nl} \int log(\frac{I_0}{I}) d\nu \tag{2.67}$$

where, σ_t is the integrated absorption cross-section, and α_t the integrated absorption coefficient, and N the number density.

2.3.4 Comparison the Theoretical Results with Experimental Quantities

Now, let us relate the measurable quantities, the molar decadic absorption coefficient ε to quantum mechanical expression that means the theoretical expression for the rate of transfer of molecules from state k to m of Eq (2.60).

The molar decadic absorption coefficient, Eq (2.63) related to the absorption coefficient of equation (2.33) by the following (Barrow, 1962; Banwell, 1972):

$$\alpha(\nu) = ln(10)\varepsilon(\nu)c \tag{2.68}$$

Substituting this equation to Eq (2.33), for diluted and short path length it becomes

$$I = I_0(1 - ln(10)\varepsilon cl) \tag{2.69}$$

Taking differential of the both sides of Eq (2.69) gives

$$-dI = I_0 ln(10)\varepsilon c dl \tag{2.70}$$

Where the concentration c,

$$c = \frac{N}{N_a} \tag{2.71}$$

Substituting Eq (2.71) into (2.70), leads to

$$-dI = \varepsilon \frac{N}{N_a} I_0 ln(10) dl \tag{2.72}$$

From theoretical expression, if $\frac{d(c_m c_m^*)}{dt}$ is the rate of probability for a single molecule changes as a result of absorption of radiation under perturbing effect of electric field radiation then $\frac{d(c_m c_m^*)}{dt} N dl$ is the number of molecules excited in a layer dl with energy absorption $h\nu_{mk}$, so the loss in intensity becomes (Barrow, 1962; Banwell, 1972).

$$-dI = \frac{d(c_m c_m^*)}{dt} N h\nu_{mk} dl \tag{2.73}$$

Comparing Eq (2.72), containing an experimental quantities with Eq (2.73) the theoretical expression, the rate of probability can be expressed as follows,

$$\frac{d(c_m c_m^*)}{dt} = \frac{\varepsilon(\nu) c\rho ln(10)}{N_a h\nu} \tag{2.74}$$

where, $I_0 = \rho c$

If the energy density is assumed to be constant through out the bands the total rate of probability for the entire absorption band is obtained by integrating over the entire frequency range. Therefore, the total rate of transition probability (Michale, 1999; Liptay, 1969; Barrow, 1962) is given by

$$\frac{c\rho ln(10)}{N_a h} \int \frac{\varepsilon(\nu) d\nu}{\nu} = \frac{1|\mu_{km}|^2 \rho}{6\epsilon_0 \hbar^2} \tag{2.75}$$

The total intensity of the band is obtained by measuring $\varepsilon(\nu)$ in the region of absorption and usually determined by integrating the area under the graph. So the integrated absorption coefficient due to transition can be expressed as (Michale, 1999; Liptay, 1969; Barrow, 1962)

$$I_A = \int \frac{\varepsilon(\nu) d\nu}{\nu} = \frac{2\pi^2 N_a |\mu_{mk}|^2}{3 ln(10) c\epsilon_0 h} = S\frac{|\mu_{mk}|^2}{3} \tag{2.76}$$

where, $S = 2.9352 \times 10^{60} C^{-2} mol^{-1}$. Eq (2.76) relates the experimentally measured molar decadic absorption coefficient with the quantum mechanical expression the transition dipole moment. The transition dipole moment is a vector that depends on both ground state and excited state wave function and couples the transition to the electric field of light.

On the other hand, oscillator strength was considered the other useful parameter providing the intensity of transition; it expresses the relative strength of electron transition (Radwan, 2007; Throne, 1988; Rao, 1975) . 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 can be determined directly through absolute emission, absorption or dispersion measurement. Oscillator strength is related to the molar decadic absorption coefficient by the following equation (Radwan, 2007; Georgakopoulous et al., 2004; Forsman and Clark, 1973):

$$f = 4.32 \times 10^{-9} \frac{molcm}{L} \int \varepsilon d\nu \tag{2.77}$$

Measurements of emission, absorption and dispersion intensities of the molecules give their number density and oscillator strength. Absorption and dispersion measurement involves the number density of the lower level of the transition, and emission measurement involves that of the upper level. An equation relating integrated absorption coefficient with number density and oscillator strength for the Gaussian shape is given by (Radwan, 2007; Thorne, 1988):

$$\int \alpha(\nu) d\nu = 2.65 \times 10^{-6} N f \tag{2.78}$$

where N is number density in molecules cm^{-3}, α in m^{-1} and ν in Hz; f is oscillator strength of the transition molecule.

3

Materials and Methods

In this chapter the materials and methods of the thesis are presented. The first section of this chapter describes the various chemicals, samples and instruments used to carry out this research. Moreover, the basic components of UV-Vis Spectrophotometry and working principles of the optical systems are discussed in details. In the second section of this chapter the different methods applied to determine the contents of caffeine and chlorogenic acid in coffee beans by UV-Vis spectroscopy are presented. The computational and experimental procedures used to analyze these compounds in coffee beans have been also reported. At the last section of this chapter methods of studying the optical transition properties, solvent effects, the self-association, and the thermodynamic properties of caffeic acid and 5-caffeyloquinic acids are presented. Moreover, method of study the complexation of sodium with caffeic acid and 5-caffeyloquinic acids are also presented.

3.1 Materials

In this section the the different chemicals, laboratory apparatus, and the components of Lambda 19 UV-Vis Spectrophotometry with its working principles will be discussed.

3.1.1 Chemicals and Samples

Ethanol, Methanol, Acetonitrile, Dichloromethane, Chloroform, bought from (Aldrich-Sigma, Germany) and distilled water were used as solvents with out further purification. For standard solution preparation a commercially bought caffeine (Evan, England), 5-caffeoylquinic and caffeic acids (Aldrich-Sigma, Germany) were used. On the other hand,

to study the hetero-association and the thermodynamic properties of caffeic acid and chlorogenic acid solutions of sodium hydroxide dissolved in water were used. For analysis of caffeine and chlorogenic acid in green and roasted Arabic coffee beans, the coffee samples were provided by Ethiopia Coffee Quality Inspection Center. The coffee samples were washed at the export standard of the center. The samples were collected from different regions of Ethiopia. The specific areas are Benchi maji, Gediyo Yirgachefe, Tepi, Godere, Goma, Limu, Bebeka, West wellega and Besema with out considering their varieties.

3.1.2 Apparatus and Instrument

The laboratory apparatus and instrument used for the experiment are the following. Beakers, measuring cylinders, pipettes, volumetric flasks, spatula, magnetic stirrer with hot plate, funnel, separatory funnel, glass filter, 1 cm quartz cuvete and 250-450 μm sieve are some of the apparatus used . For measuring the mass of caffeine, 5-caffeoylquinic acid, caffeic acid and coffee samples micro balance and digital balance with accuracy of 0.0001g were used. For electronic absorption measurement of standard solutions and coffee samples a double monochromator UV-Vis-NIR spectrometer, Perkin Elmer Lambda 19 (Perkin Elmer, D-7770 Ueberlingen, Germany) with wavelength ranges of 170-3200 nm is used. The instrument operated by a powerful soft ware package termed UVCSS. It provides a wide range of operating mode for the instrument and it also includes comprehensive data handling and file management capabilities. Scanning speed of 240 nm per min and slit width 2 nm were used during spectral data acquisition. The instrument is a PC-driven spectrometer.

3.1.3 Basic Components of UV-Vis Spectrophotometry and Working Principles

The basic components of double beam spectrophotometry are light sources, monochromator, focusing devices, sample cell compartment and detector (Mann et al., 1997; Ewing, 1975; Bauman, 1962). The sources are a deuterium lamp which covers ultraviolet (UV) range and tungsten-halogen lamp for visible (VIS) and near infrared (NIR) ranges (Elmer, 1993). The monochromator isolate radiant energy of desired wavelength by dispersing the

beam in to its components and placing the light path in a slit that lets pass only a narrow wavelength band. The focusing devices are a combination of lenses, slits, and mirrors inserted in to light path to render the light rays parallel or to isolate narrow portion of the light beam or its spectrum. The detectors are a photo sensetive materials, mostly a photomultiplier tube for UV and VIS ranges and PbS only for NIR range. When light strikes the PMT, it generates electron which passes through a series of dynode that amplify the signal several times (Mann et al., 1997; Ewing, 1975; Bauman, 1962).

Fig 3.1 shows the schematic optical components of Lambda 19 spectrometry and its working principles. For operation in the near infrared and visible ranges, source mirror (M_1) reflects the radiation from tungsten-halogen lamp onto mirror M_2, at the same time it blocks the radiation from deuterium lamp. Similarly for operation in the ultraviolet range, M_1 is raised to permit radiation from deuterium lamp to strike mirror M_2. The radiation from the respective source lamp is reflected from mirror M_2 via mirror M_3 through an optical filter wheel assembly (FW) to mirror M_4. The filter wheel is driven by a steeping motor to be in synchronization with monochromators. Depending on the wavelength being produced, the appropriate optical filter is located in the beam path to prefilter the radiation before it enters the first monochromator. From mirror M_4 the radiation is reflected through the entrance slit of monochromator 1. This radiation is collimated at mirror M_5 and reflected to grating table G_1. Depending on the current wavelength range, the collimated radiation beam strikes either the UV/VIS grating or the NIR grating. The radiation is dispersed at the grating to produce the spectrum. The rotational position of the grating effectively selects a segment of the spectrum, reflecting this segment to mirror M_5 and hence through the exit slit. The exit slit restricts the spectrum segment to a near-monochromatic radiation beam.

The exit slit of monochromator 1 serves as the entrance slit of monochromator 2. The radiation is reflected via mirror M_6 to appropriate grating table G_2 and hence back via mirror M_6 through the exit slit mirror M_7. The rational position of grating table G_2 is synchronized to that of G_1. The radiation emerging from the exit slit exhibits high spectral purity with an extremely low stray radiation content.

From mirror M_7 the radiation beam is reflected via torroid mirror M_8 to the chopper assembly (C). As the chopper rotates, a mirror segment, a window segment, and dark seg-

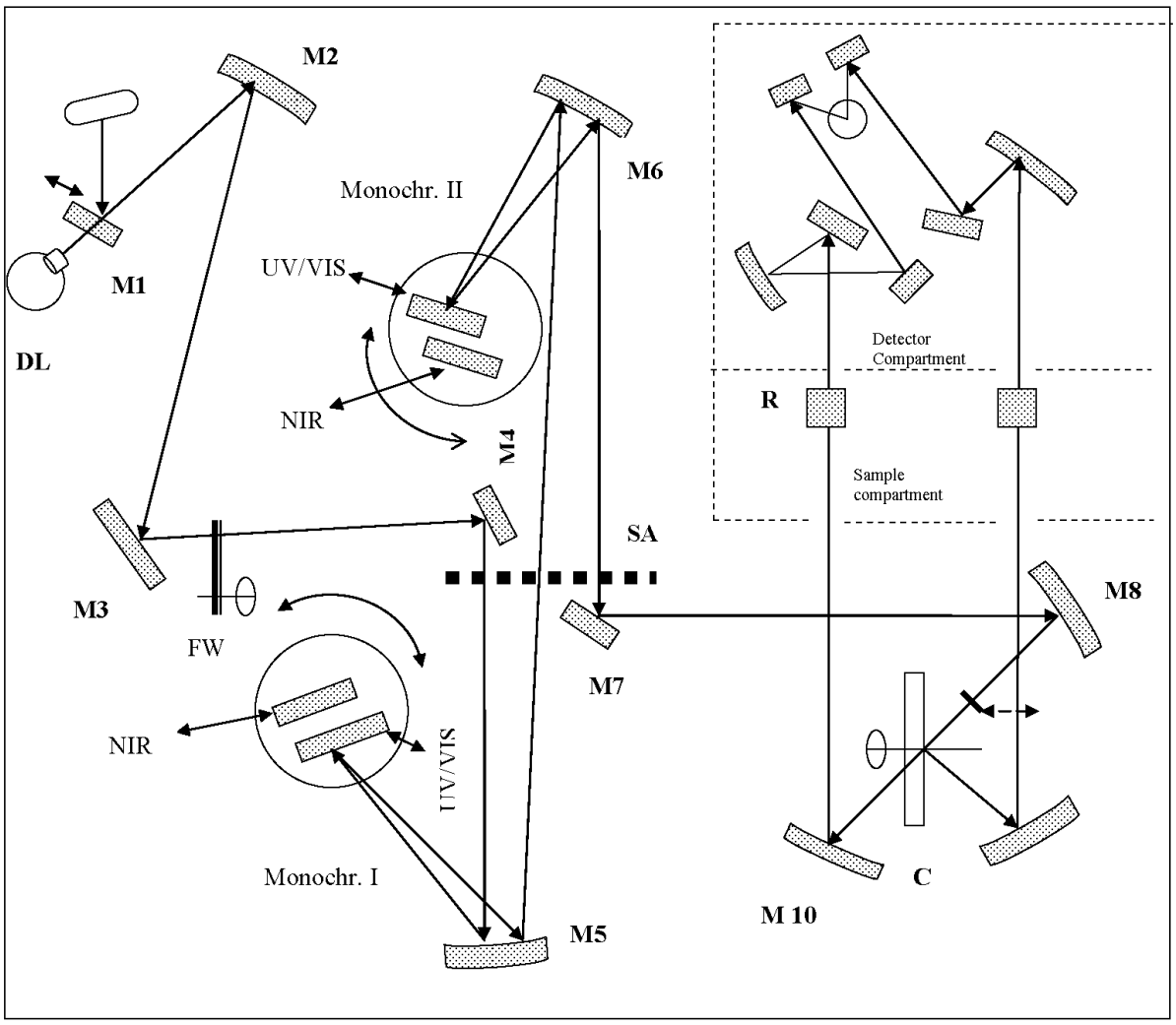

Figure 3.1: The Schematic Optical Components of Lambda 19 Spectrophotometry.

ment are brought alternatively into radiation beam. When a mirror segment enters the beam, radiation is reflected via mirror M_9 to create the sample beam (S). When a window segment enters the beam the radiation passes through to mirror M_{10} and is then reflected to form the reference beam (R). When a dark segment is in the beam path, no radiation reaches the detector, permitting the detector to create the dark signal. The radiation passing alternately through the sample and reference beam is reflected by the optics of the detector assembly onto the appropriate detector.

3.2 Methods

3.2.1 Methods of Measuring Caffeine by Beer-Lambert's Law

3.2.1.1 Standard Solution Preparation

For standard solution preparation a commercially bought caffeine was dissolved in water and dichloromethane solutions. The solutions were stirred and heated gently for an hour using magnetic stirrer. The absorbance of the solutions were measured by UV-Vis spetrometer in the wavelength regions of 200-500 nm. From absorbance of solutions molar extinction coefficient and transition dipole moment of caffeine were calculated in water and dichloromethane using Eqs (2.63) and (2.76) respectively.

3.2.1.2 Coffee Sample Preparation

For coffee sample preparation, green and roasted (light, medium and dark) coffee ground and sieved through $250\mu m$ sieve to get a uniform texture. An accurately weighed amount of sieved coffee ($50mg$) was dissolved in 25 ml of distilled water. The solution was stirred for half an hour using magnetic stirrer and heated gently to remove caffeine easily from the solution. In addition the solution was filtered through glass filter to get rid of particles from solution. After filtration extraction of caffeine by liquid-liquid method follows (Belay et al., 2008).

3.2.1.3 Liquid-Liquid Extraction of Caffeine

Extraction of caffeine from water solution or liquid-liquid extraction is a separation process in which a solute is distributed between two immiscible solvents and these process were implemented by chloroform and dichloromethane solution. Extraction of caffeine by chloroform has many interfering substances as compared to dichloromethane. Hence dichloromethane solution was selected for extraction of caffeine from coffee beans. It has also been reported in literature that both solvents are useful for decaffeinating caffeine from coffee beans (Belay et al., 2009a; Belay et al., 2008; Rofti, 1971; Clarke and Macrea, 1985; Mumin, et al., 2006). However, the most widely used solvent currently for decaffeinating in coffee beans is dichloromethane. The efficiency of dichloromethane to extract

caffeine from coffee beans is 98-99 $\%$ as reported by (Clarke and Macrea, 1985).

Extraction has been performed according to the following procedures. The coffee solution prepared above was mixed with dichloromethane by volume ratio of $(25ml : 25ml)$. The mixtures solution was stirred for 10 minutes and caffeine was extracted from coffee solution by dichloromethane. The extraction proceeds for 3 times by the same method. The caffeine extracted at each round stored in volumetric flasks. Finally the absorbance of the solutions were measured by UV-Vis spectrophotometry in the spectral range of 200 -500 nm against the corresponding reagent blank (Belay et al., 2008; Belay et al., 2009). All glasswares were thoroughly cleaned, rinsed with distilled water and dried before being used. After extraction, there is still interfering substance which was eliminated by Gaussian function based on non-linear curve fitting.

3.2.1.4 **Non-linear Curve Fitting of Gaussian Function**

In addition to caffeine spectrum there are interfering band from other coffee components extracted by dichloromethane and the peak of this band was observed at the wavelength regions of 305, 324 nm for green and roasted coffee beans respectively (Belay et al., 2008). The compound attributes to this is known to be chlorogenic acid related compound. It is clear that this interfering band has an effect on the maximum peak of caffeine. Therefore, in this research the matrix was eliminated by fitting the the following equation of Gaussian function to the experimental data.

$$y = y_0 + Ae^{-(\frac{x-x_c}{w})^2} \tag{3.1}$$

where y_0 represents the minimum point, A its amplitude, x_c is the central wavelength and w is full width at half maxima of the equation. It was fitted by Non-linear curve fitting based on the Lavenberg-Marquardt algorithm. The four quantities of Gaussian function are serving as searching parameters in order to achieve minimum discrepancy between the experimental data and Gaussian function. Thus, the peak absorbance for calculating the concentration of caffeine obtained after subtracting the fitted Gaussian function from the total caffeine spectrum.

3.2.2 Method of Measuring Caffeine by Integrated Absorption Coefficient

3.2.2.1 Integrated Absorption Cross-Section and Oscillator Strength

For measuring integrated absorption cross-section and oscillator strength of caffeine solutions ranged $(1.25 - 14.20) \times 10^{-5} molL^{-1}$ and $(1.78 - 9.48) \times 10^{-5} molL^{-1}$ were prepared in water and dichloromethane respectively . The absorbance versus wavelength measured by spectrophotometry re-calculated into absorption coefficient versus wave number using Origin 6.1 software. For deconvolution the overlapped spectra of caffeine and determine the area under the peak, Gaussian function was fitted to the spectrum of absorption coefficient versus wave number of caffeine. From the area of Gaussian function fitted to the spectrum the integrated absorption cross-section of caffeine was calculated in the wave number regions of 20,000-39,062 cm^{-1} or using Eq (2.67). In order to deconvolute the overlapped spectra at peak height a least square procedures were implemented, where the minimal values of the difference between the real spectrum and simulated (fitted) one were considered.

Similarly the corresponding oscillator strengths of caffeine was measured from the same solution. The Gaussian function fitted to the spectrum of molar decadic absorption coefficient versus wave number. From area under Gaussian function fitted to the spectrum the oscillator strength was calculated in the regions of 20,000-39,062 cm^{-1} or using Eq (2.77).

3.2.2.2 Number Density of Caffeine in Coffee Beans

After integrated absorption cross-section and oscillator strength were calculated in various solvents analysis of caffeine in coffee beans were carried out by integrated absorption technique. The coffee samples were prepared according to the procedures mentioned in section 3.2.1.2 and 3.2.1.3 respectively. By above mentioned method the number density of caffeine in coffee beans were calculated in the ranges of 20,000-50,000 cm^{-1} from Gaussian function fitted to the spectrum of absorption coefficient versus wave number or by Eq (2.78).

In this case the Origin 6.1 software was used for data analysis such as changing the ASCII file to the spectra; for integrating the absorption coefficient, cross-section and molar

decadic absorption coefficient across the entire absorption band. Moreover, it has also useful for measurement of height and line width of the spectrum; and to draw graphs.

3.2.3 Methods of Measuring CGA in Coffee Beans

3.2.3.1 Standard Solution Preparation

For the standard solutions preparation a commercially bought 5-caffeoylquinic acid was dissolved in polar-solvents (ethanol, methanol, acetonitrile and water). 5-caffeoylquinic acid is one of the highest percentage of chlorogenic acids groups found in coffee beans. The solution of the compound was stirred in dark room using magnetic stirrer, and absorbance was measured immediately after stirring. From UV-Vis absorption spectrum, the optical transition properties (molar decadic absorption coefficient, oscillator strength, integrated absorption cross-section and transitional dipole moment) and validation parameters were calculated. For integrating the absorption coefficient and molar decadic absorption coefficient in the frequency regions origin6.1 software was used.

3.2.3.2 Coffee Sample Preparation

Green and roasted (light, medium and dark) coffee beans were ground and screened through 250-450 μm sieve to get a uniform texture. An accurately weighed amount of sieved coffee (6 mg) was dissolved in water 25 ml. The solutions were stirred for an hour using magnetic stirrer and heated gently to increase the solubility of caffeine in water solution. In addition, solution was filtered by glass filter to get rid of the suspended particles from the solutions (Belay et al., 2009b).

3.2.3.3 Liquid-liquid Extraction and Absorption Measurement Procedures

In coffee sample chlorogenic acids and caffeine spectra make interference in the wavelength regions of 200-500 nm. To resolve (deconvolute) the two overlapped spectra; caffeine was extracted from water solution by dichloromethane. For caffeine extraction previously developed procedures by (Belay et al., 2008) were used. After caffeine was extracted from the solution, the chlorogenic acids remain as residue in coffee beans. From the residual solution, concentration of chlorogenic acid was measured in the wavelength region

of 200-500 nm against the corresponding blank (distilled water) by Beer-Lambert's Law at $\lambda_{max} = 324nm$ (Belay et al., 2009b). All glass wares, cuvette were thoroughly cleaned, rinsed with distilled water and dried before use.

3.2.4 Methods of Measuring Solvent Effects and Optical Transition Properties of 5-Caffeoylquinic and Caffeic Acids

The solvent effects and optical transition properties of CGA and caffeic acid were measured in polar solvents. For calculating the optical transition properties of 5-caffeoylquinic and caffeic acids in different polar-solvents the absorption measured as absorbance versus wavelength was re-calculated into the spectra of absorption coefficient (cm^{-1}) versus wave number (cm^{-1}). By integrating absorption coefficients in the wave number regions oscillator strength, transition dipole moment and integrated absorption cross-section were calculated in ethanol, methanol, acetonitrile and water respectively.

3.2.5 Methods of Study the Self-Association, Complexation of Sodium Ions and Thermodynamic Properties of Caffeic and 5-Caffeoylquinic Acids

The experimental method applied to study the self-association of caffeic is based on change in the shape of the spectrum, shift of maximum absorbance and isobestic points at three wavelengths indicating at least the existence of two different species (Belay, 2009c). The self-association of the compound was studied over the concentration range of (1.49-23.9) $\times 10^{-5}$ M in water solutions, since self-association can be detected over a wide range of concentration and need a careful examination of the changes in the apparent molar extinction coefficients. The absorbance as a function of concentration has been measured at the wavelength which showed maximum absorption to obtain the greatest accuracy of detection. For numerical analysis of the extinction coefficients and equilibrium constants, of self-association of caffeic acid, dimer model were used. Numerical procedure of fitting the experimental data was carried out by non-linear curve fitting based on Levenberg-Marquardt algorithm. The extinction coefficients and equilibrium constants are used as search parameters, in order to achieve minimum discrepancy between the experimental

data and theoretical values .

On the other hand for studying the complexation of sodium with caffeic acid , a solution of sodium hydroxide, concentration (0.97-7.46)$\times 10^{-3}$ M and caffeic acid (9.16$\times 10^{-5}$)M solution were prepared in distilled water. The electronic absoption spectra of the complex solution recorded at various concentrations of sodium hydroxide solutions. The association constant and molar extinction coefficient of the complexes calculated from equation of Benesi-Hildebrand at 343 nm (Benesi and Hildebrand, 1949) The thermodynamic parameters of caffeic acid self-association have been studied in the temperature range of 328-348 K.

The self-association of the 5-caffeoylquinic was studied over the concentration range of $(3.02 - 17.50)\ \times 10^{-5}$ M in water solutions, since self-association can be detected over a wide range of concentration and need a careful examination of the changes in the apparent molar extinction coefficients. The absorbance as a function of concentration has been measured at absorption maxima 324 nm to obtain the greatest accuracy of detection. For numerical analysis the (molar extinction coefficients and dimerzation constant), dimer model equation fitted to the experimental data. Numerical procedure of fitting the experimental data was carried out by non-linear curve fitting based on Levenberg- Marquardt algorithm. The molar extinction coefficients and equilibrium constants were used as searching parameters, in order to achieve minimum discrepancy between the experimental data and equations. The thermodynamic parameters (enthalpy, Gibb's free energy and entropy) have been determined for the temperature range of 290-328 K using Van't Hoff's equation.

The complexations of sodium ions with 5-caffeoylquinic acid have been studied by Benesi-Hildebrand method (Benesi and Hildebrand, 1949). For this purpose the solution of sodium hydroxide, whose concentration ranges are (1.13-22.46)$\times 10^{-3}$ M and 5-caffeoylquinic acid 2.20$\times 10^{-5}$ M solution were prepared in distilled water. The association constant and molar extinction coefficient of the complexes calculated from equation of Benesi-Hildebrand at 370 nm (Benesi and Hildebrand, 1949). The UV-Vis spectra were recorded half an hour after the solution preparation in order to ensure that the equilibrium was reached. The solutions were stored in the dark to avoid photo degradation of the compounds.

4

Results and Discussion

In this chapter the results and discussion of the thesis are presented. In the first two sections of this chapter the molar extinction coefficients, transitional dipole moment, oscillator strength, and integrated absorption cross-section of caffeine in water, dichloromethane and the contents of caffeine in coffee beans calculated by both Beer-Lambert's law and integrated absorption coefficients techniques were presented. Similarly in section 3 of this Chapter the results obtained in relation to the analysis of chlorogenic acids in coffee beans were presented. In section 4 of this chapter the molar extinction coefficient, oscillator strength, transition dipole moment, integrated absorption cross-section calculated in different solvents and the effects of solvents on 5-caffeoylquinic and caffeic acids were presented. At the last section of this chapter the concentration dependent self-association of the compounds, their complexation with sodium ions and thermodynamic properties were presented. The calculated values of molar extinction coefficients and dimerzation constants of the self-association were determined by dimer model and their complexation with sodium by Benesi-Hildebrand approach were presented. In addition, the thermodynamic properties such as the change in enthalpy, the Gibb's free energy and entropy calculated for self-association of the compounds are also presented in this section.

4.1 Measuring Caffeine by Beer-Lambert's Law

4.1.1 UV-Vis Absorption and Transition Dipole Moment of Caffeine

Fig 4.1 shows the UV-Vis absorption spectrum of caffeine in water in the wavelength regions of 200 -500 nm at room temperature. In this region caffeine has two isolated peaks

at 206, 272, and shoulder around 229 nm respectively. The intensity of caffeine in water drop to zero for wavelength greater than 300 nm and start to rise below this wavelength. The peak absorbance of caffeine observed in water at these wavelengths are quite similar with one reported by Clarke and Macrae (1985). The molar decadic absorption coefficient measures the intensity of optical absorption was calculated at λ_{max} =272 nm using Equation (2.63). The molar decadic absorption coefficient of caffeine in water was computed and a value of ε_{max} =1115 $m^2 mole^{-1}$ was obtained .

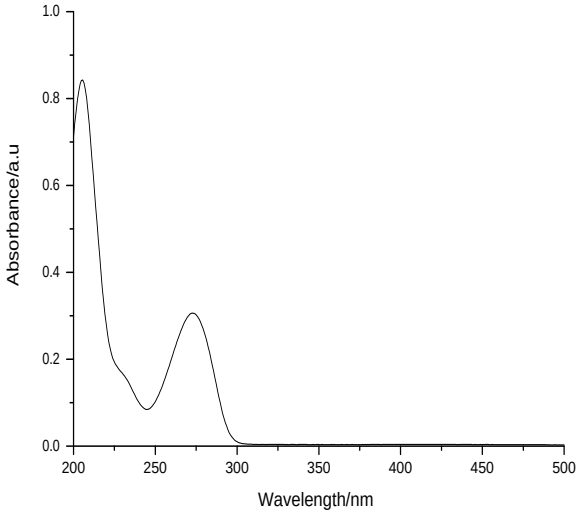

Figure 4.1: UV-Vis Absorption Spectrum of Caffeine in Water.

The transitional dipole moment of caffeine, which is related to the molar decadic absorption coefficient by the integral absorption coefficient, was calculated using Eq (2.76). Fig 4.2 shows the spectra of caffeine in water given as a function of molar decadic absorption coefficient over wave number versus wave number ($\frac{\varepsilon(\nu)}{\nu}$ versus ν). The integrated area under this curve, I =105.92 $m^2 mole^{-1}$ was obtained by integrating the molar absorption coefficient over wave number from 33,000 -41,000 cm^{-1} for $c = 1.09 \times 10^{-4} M$. Thus, the calcuated transitional dipole moment of caffeine in water is μ = $10.40 \times 10^{-30}\ Cm$.

Similarly, Fig 4.3 shows the UV-Vis absorption spectra of caffeine in dichloromethane in the wavelength region of 200-500 nm. The molar decadic absorption coefficient of caffeine in dichloromethane, ε_{max} = 1010 $m^2 mole^{-1}$ was obtained. The area under graph of

$\frac{\varepsilon(\nu)}{\nu}$ versus ν of caffeine in dichloromethane for $c = 1.03 \times 10^{-4} M$ is I =114.16 $m^2 mole^{-1}$ in the wave number regions of 33,000-41,000 cm^{-1}. From the integral absorption coefficient the transitional dipole moment of caffeine in dichloromethane was, μ = $10.80 \times 10^{-30}\ Cm$. From the above discussion the molar absorption coefficient of caffeine at peaks are higher

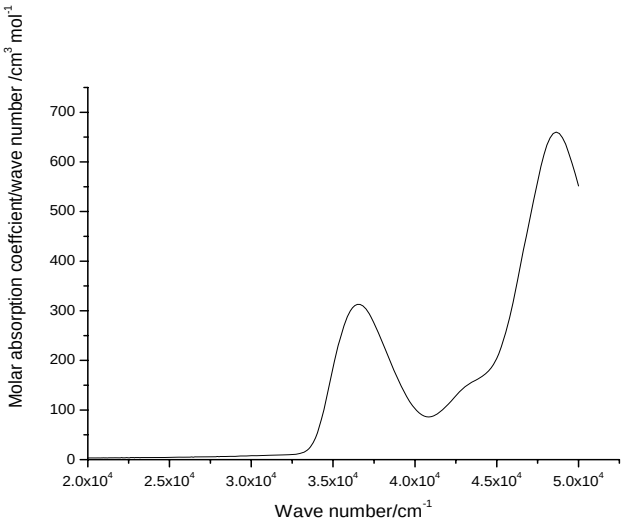

Figure 4.2: Molar Decadic Absorption Coefficient Over Wave Number Versus Wave Number of Caffeine in Water.

in water as compared to dichloromethane, however, the total integrated absorption coefficients of this compound in the wave number regions of 33,000-41,000 cm^{-1} is higher in dichloromethane than water. After optical transition of caffeine determined in solvents its contents in coffee beans were determined using the above calculated parameters.

4.1.2 Determination of Caffeine in Green Coffee Beans

By UV/Vis spectrophotometer a direct measurement of caffeine in coffee beans are impossible owing to the matrix effect of UV absorbing substances (Zhang et al., 2005; Ortega-Barrales et al., 2002). This effect is clearly seen in Fig. 4.4 for band of coffee beans dissolved in water. To resolved this interfering substances caffeine was extracted from coffee solution by dichloromethane . Previously many workers,(Mumin et al., 2006; Clarke et al., 1985) reported that dichloromethane is the most commonly employed method for ex-

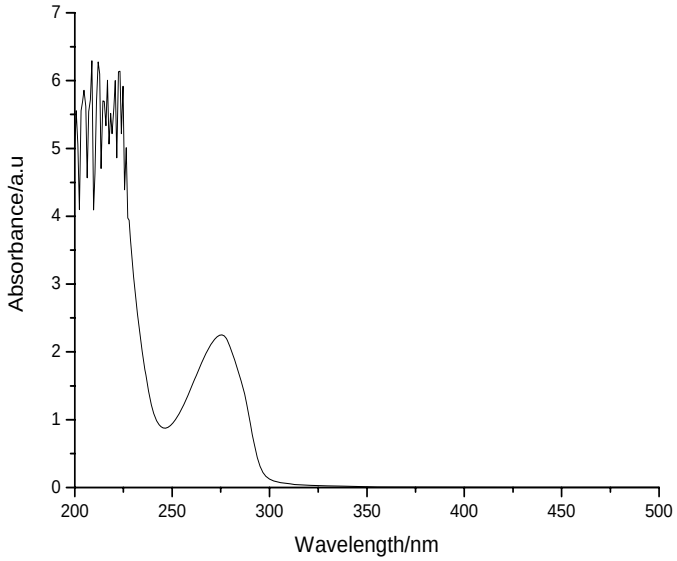

Figure 4.3: UV-Vis Absorption Spectrum of Caffeine in Dichloromethane.

traction of caffeine from green coffee beans. Moreover, many commercial products also uses dichloromethane for decaffeinating coffee beans since its efficiency is about 98-99 $\%$. In this research the extraction of caffeine from coffee solution performed for three rounds with equal amounts of dichloromethane solutions. However, the amount of caffeine extracted at each round of extraction is not equal. Fig 4.5 shows absorbance versus wavelength of caffeine extracted by dichloromethane solution from water at three rounds of extraction. From total amounts of caffeine about 83.57 $\%$ of caffeine extracted from coffee in the first round and only about 12.14 and 4.29 $\%$ of caffeine extracted in the second and third rounds, respectively. The extraction technique still could not completely remove the possible interference of caffeine spectra. Hence, it is impossible to obtain the qualitative and quantitative information from the spectrum of unresolved band. The peak of this interference band was observed at the wavelength of 305-308 nm as shown in Fig 4.6. This peak is due to the complexation of (caffeine-chlorogenic acid) compounds. Previously many workers reported the complexation of caffeine-chlorogenic acid in coffee beans (D'Amelio et al., 2009; Campa et al., 2005; Belay et al., 2008; Clarke et al., 1985; Martin, 1970). To eliminate this interference band Gaussian function given in Equation (3.1) was fitted to the spectrum of caffeine using non-linear curve fitting. It is assumed that the Gaussian function fit experimental data more than other functions. Fig 4.6 shows the

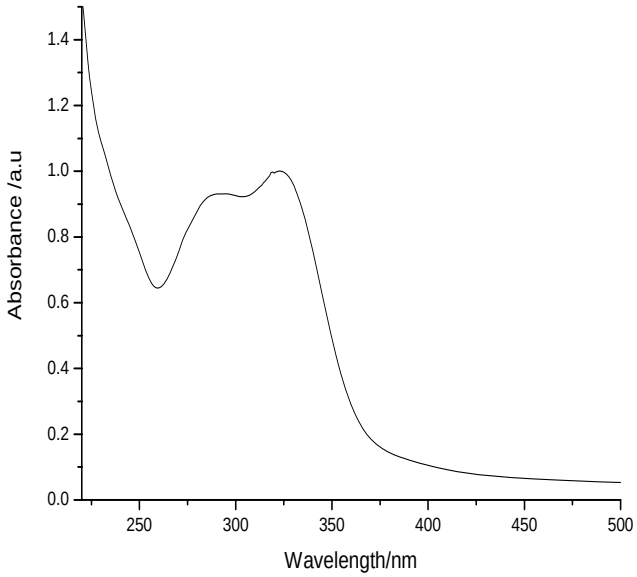

Figure 4.4: UV-Vis Spectrum of Coffee Dissolved in Water.

spectrum of caffeine extracted by dichloromethane solution and Gaussian function fitted to this spectrum to eliminate the peak due to caffeine-chlorogenic acid complexation. After the inferences were eliminated the shape and peak of the two spectra are exactly similar and this is justified by overlapping the two spectra. Therefore, it can be concluded that the applications of experimental and computational methods enable to determine the contents of caffeine in coffee beans using UV-Vis spectrophotometry.

By these methods the contents of caffeine in coffee beans were determined for various samples collected from different regions of Ethiopia. The mean percentage of caffeine in coffee beans calculated for five independent measurements ranged from 1.1± 0.01, to 1.19± 0.02 $\%$ (Belay et al., 2008) respectively and shown in Table 4.1. Further more, the caffeine contents determined by this method is in a good agreement with the caffeine contents of Arabica coffee beans reported by various analytical techniques. The caffeine contents of Arabica coffee beans reported by Farah et al. (2006) using HPLC are 1.23 ± 0.06, 0.96 ± 0.01 $\%$ for highest and lowest respectively. The derivative spectrophotometry techniques applied for analysis of the contents of caffeine in coffee beans as determined by Alpdogan et al. (2002) was 1.36 ± 0.03 $\%$.

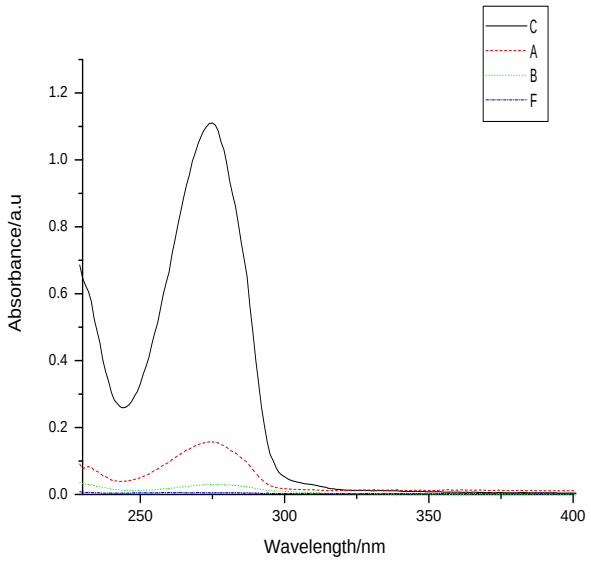

Figure 4.5: The Over Lapped Spectra of Caffeine Extracted for various Round of Extraction (A) for First Round, (B) for Second Round and (C) for Third Round .

4.1.3 **Determination of Caffeine in Roasted Coffee Beans**

The caffeine contents in roasted coffee beans are also determined by the methods mentioned in Section (4.1.2). The only differences unlike the green coffee beans the peak of inferences in roasted coffee were observed at higher wavelength of 324 nm. Table 4.2 shows the percentage of caffeine calculated for coffee roasted at light, medium and dark temperature respectively. Generally the caffeine contents of roasted coffee is higher than green cof-

Table 4.1: The Percentage of Caffeine in Green Coffee Beans Measured by UV-Vis Spectrophotometry for Samples Collected from Different Regions of Ethiopia.

Type of Coffee Samples	UV-Vis spectroscopy (w/w) %
Sample-A	0.9 ± 0.01
Sample-B	1.01 ± 0.04
Sample-C	1.07 ± 0.02
Sample-D	1.27 ± 0.02

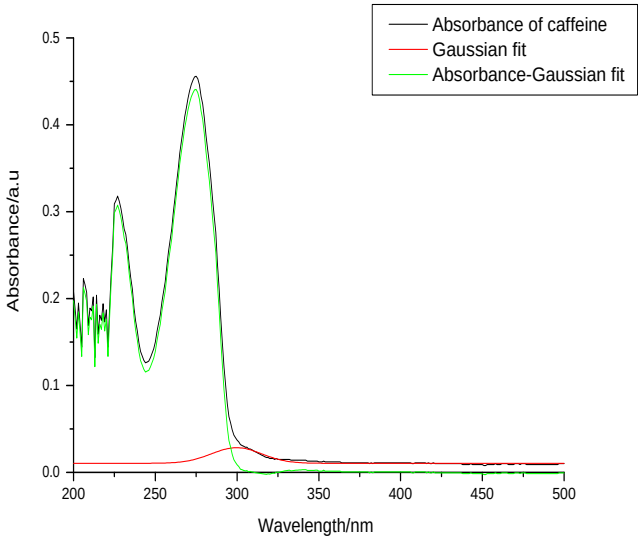

Figure 4.6: The UV-Vis Absorption Spectrum of Caffeine Extracted from Coffee Solution, Gaussian Function Fitted to the Spectrum and Spectrum of Caffeine after Gaussian Function Subtracted.

fee beans. The difference in caffeine contents among same species of coffee beans which are roasted at different temperatures are due to removal of some compounds from coffee beans. The caffeine content for coffee roasted at medium temperate greater than light, this is because water and carbon dioxide which account about 20 % of the total coffee beans were removed at this temperature. On the other hand a decrease in the percentage of caffeine was observed at the dark , where it is expected that the caffeine contents might melt and removed by evaporation.

Table 4.2: Percentage of Caffeine in Roasted Coffee Beans Measured by UV-Vis Spectrophotometry.

Coffee Samples	Light Roasted %	Medium Roasted %	Dark Roasted %
Sample-1	1.33	1.48	1.33
Sample-2	1.58	1.62	1.58
Sample-3	1.18	1.25	1.27

4.2 Determination the Contents of Caffeine by Integrated Absorption Coefficient

4.2.1 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 4.3 and 4.4 respectively. The calibration equations are (Y=32.73+223.72X, R=0.9999, S.D=59.42, P$<$0.0001, N=14) for caffeine dissolved in water, and (Y = 64.58+ 212.21 * X, R=0.9999, S.D=24.86, P$<$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 mgL^{-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.

The method of repeatability was also determined by calculating the coefficient of variance (C.V) and its value ranged from (0.99-5.56) $\%$. These results suggested that the proposed method is valid in terms of precision. The methods are also validated 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 mgL^{-1} for caffeine dissolved in water and 1.05 mgL^{-1} for caffeine dissolved in dichloromethane respectively. These results are in a good agreement with one reported by (Huck et al., 2005; Ohasmann et al., 2002; Pardakar et al., 2002; Bouhsain et al., 1999) using Near Infra Red and Fourier Transform Infra Red spectrometry .

Table 4.3: Number Density of Caffeine in Water and its Corresponding Peak Absorbance, Integrated Absorption Coefficients and Integrated Molar Decadic Absorption Coefficients.

N of caffeine in $molL^{-1}$	A in $a.u$	$\int a d\nu$ in cm^{-2}	$\int \epsilon d\nu$ in $Lmol^{-1}cm^{-2}$
14.20×10^{-5}	1.65	6202.39	4.36×10^{7}
6.90×10^{-5}	0.78	2952.96	4.28×10^{7}
5.36×10^{-5}	0.59	2331.66	4.35×10^{7}
4.46×10^{-5}	0.50	2019.00	4.53×10^{7}
3.80×10^{-5}	0.43	1777.98	4.67×10^{7}
3.38×10^{-5}	0.38	1454.85	4.30×10^{7}
3.34×10^{-5}	0.37	1553.26	4.32×10^{7}
2.96×10^{-5}	0.33	1424.66	4.60×10^{7}
2.74×10^{-5}	0.31	1189.82	4.20×10^{7}
2.27×10^{-5}	0.26	1026.46	4.50×10^{7}
1.96×10^{-5}	0.22	848.74	4.33×10^{7}
1.71×10^{-5}	0.19	762.82	4.46×10^{7}
1.37×10^{-5}	0.15	599.25	4.37×10^{7}
1.25×10^{-5}	0.14	522.75	4.18×10^{7}

4.2.2 Integrated Absorption Cross-Section and Oscillator Strength of Caffeine

For calculating the integrated absorption cross-section of caffeine, the absorbance versus wavelength of caffeine in water and dichloromethane Fig 4.1 and 4.2 are recalculated in to absorption coefficient versus wave number Fig 4.7 (dichloromethane) 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 Fig 4.8. 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 20,000-39,062 cm^{-1} using Eq (2.67).

The calculated integrated absorption cross-section obtained for independent measurements of caffeine in water and dichloromethane were $(4.44 \pm 0.18) \times 10^7$ and $(4.32 \pm 0.11) \times 10^7$ $Lmol^{-1}cm^{-2}$ as indicated in Table (4.3) and (4.4) respectively. On the other hand the corresponding peak absorption cross-section of caffeine at the center was calculated in both solvents using Eq (2.64). The calculated absorption cross-sections of caffeine at peaks were $(1.12 \pm 0.02) \times 10^4$ and $(1.01 \pm 0.01) \times 10^4$ $Lmol^{-1}cm^{-1}$ respectively. The integrated absorption cross-section and peak cross-section obtained in this work are within the range of values reported by other workers (Belay, 2010; Canosa-Martin et al., 1987).

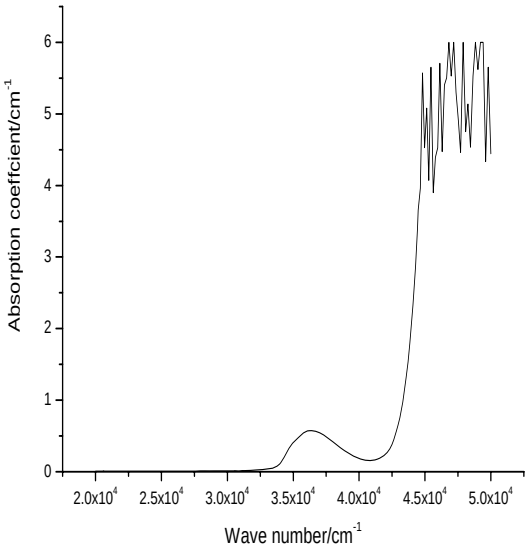

Figure 4.7: The Absorption Coefficient Versus Wave Number of Caffeine in Dichloromethane in the Wave Number Regions of 20,000-50,0000 / cm^{-1}.

The oscillator strength was considered the other useful parameter providing the intensity of transition; it expresses the relative strength of electron transition. In this work, the oscillator strength of caffeine in water and dichloromethane were calculated by absorption measurements. Oscillator strength related to the molar decadic absorption coefficient by Eq (2.77). The oscillator strength of caffeine was calculated for different concentrations or number densities mentioned in Tables 4.3 and 4.4. The mean values of oscillator strength of caffeine in water and dichloromethane are 0.19 ± 0.01 and 0.18 ± 0.01, respectively (Belay, 2010).

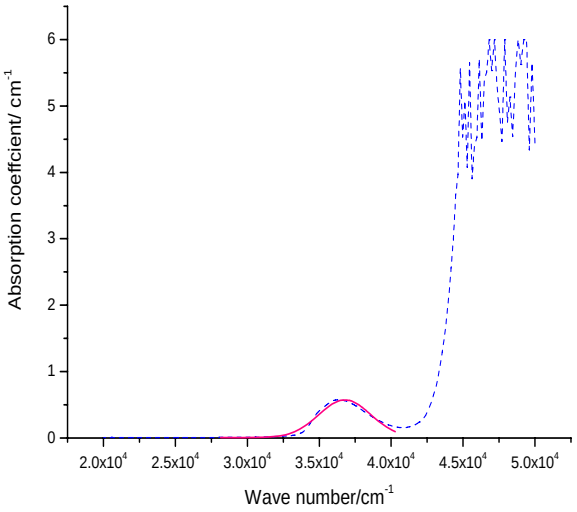

Figure 4.8: The Gaussian Function Fitted to the Absorption Coefficient Versus Wave Number of Caffeine Dissolved in Dichloromethane. The Line Shows (-) Gaussian Function and the Dot Shows (..) Absorption Coefficient Versus Wave Number of caffeine.

The other basic parameters describing any individual absorption band is a band width at half maximal intensity (Antonov and Nedltcheva, 2000). 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 and the values of 4243 and 4532 cm^{-1} are obtained respectively.

4.2.3 Number Density of Caffeine in Coffee Beans by Integrated Absorption Coefficient

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 the area of Gaussian function fitted to the spectra in Fig 4.9, the number density of caffeine was calculated in the frequency region of 20,000-50,000 cm^{-1} using Eq (2.78). By this method, the number density of caffeine was calculated for seven different coffee samples (samples 1 through 7) collected from different regions of

Table 4.4: Number Density of Caffeine in Dichloromethane and its Corresponding Peak Absorbance, Integrated Absorption Coefficients and Integrated Molar Decadic Absorption Coefficients.

N of caffeine in $molL^{-1}$	A in $a.u$	$\int a d\nu$ in cm^{-2}	$\int \epsilon d\nu$ in $Lmol^{-1}cm^{-2}$
9.48×10^{-5}	0.98	3976.31	4.19×10^{7}
6.32×10^{-5}	0.64	2649.17	4.19×10^{7}
4.74×10^{-5}	0.48	2020.82	4.26×10^{7}
4.45×10^{-5}	0.45	1916.05	4.23×10^{7}
3.79×10^{-5}	0.38	1611.29	4.25×10^{7}
3.37×10^{-5}	0.34	1430.00	4.18×10^{7}
3.16×10^{-5}	0.32	1412.00	4.50×10^{7}
2.97×10^{-5}	0.30	1256.00	4.24×10^{7}
2.67×10^{-5}	0.27	1200.30	4.36×10^{7}
2.37×10^{-5}	0.24	1044.08	4.40×10^{7}
2.10×10^{-5}	0.21	926.00	4.41×10^{7}
1.78×10^{-5}	0.18	781.76	4.39×10^{7}

Table 4.5: Number Density of Caffeine in Green Coffee Beans Calculated by Integrated Absorption Coefficient and Beer-Lambert's Law.

Coffee Samples	N by I.A.C/$molL^{-3}$	N by Beer-Lambert's Law/$molL^{-3}$
Sample-1	3.53$\times 10^{-5}$	3.66$\times 10^{-5}$
Sample-2	3.55$\times 10^{-5}$	3.86$\times 10^{-5}$
Sample-3	3.89$\times 10^{-5}$	4.16$\times 10^{-5}$
Sample-4	4.33$\times 10^{-5}$	4.65$\times 10^{-5}$
Sample-5	4.43$\times 10^{-5}$	4.85$\times 10^{-5}$
Sample-6	4.62$\times 10^{-5}$	5.05$\times 10^{-5}$
Sample-7	4.76$\times 10^{-5}$	4.95$\times 10^{-5}$

Ethiopia. The calculated number density ranged from $(3.53-4.76)\times 10^{-5}\, molL^{-1}$ as shown in Table 4.5. On the other hand, for the purpose of comparison with other technique same samples were analyzed by the previously reported method (Belay et al., 2008) or Beer-Lambert's law and the results are in the range of $(3.66-4.95)\times 10^{-5}\, molL^{-1}$. 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 to be 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 were 0.91-1.32 $\%$ with an average of 1.10 $\%$ from analysis of 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 from analysis 38 coffee samples.

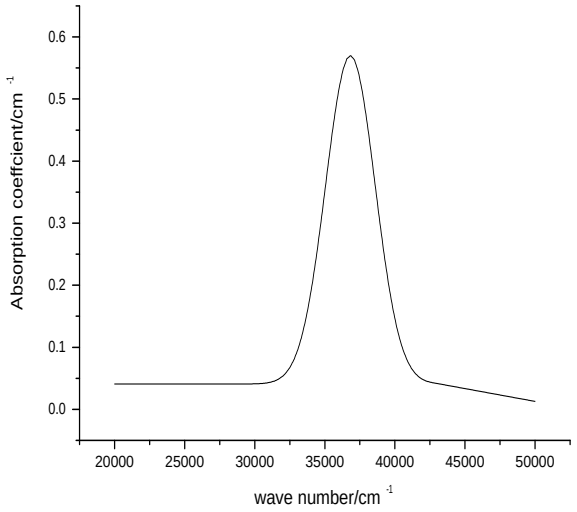

Figure 4.9: The Gaussian Function Fitted to the Spectrum of Caffeine in the Wave Number Regions of 20,000-50,000 cm^{-1}.

4.3 Measuring the Contents of Chlorogenic Acids (CGA) in Coffee Beans

4.3.1 UV-Vis Absorption Spectra of 5-caffeoylquinic in Water

Fig 4.10 shows the UV-Vis absorption spectra of 5-caffeoylquinic measured in distilled water in the wavelength regions of 200-500 nm at room temperature. In these regions 5-caffeoylquinic has two maximum points; the first maximum being at 217 nm with shoulder at 240 nm and the second peak was at 324 nm with shoulder at 296 nm and minimum point was at 262 nm respectively. The peak point at 324 nm was the highest peak corresponding to the HOMO→LUMO transition which presents mainly a $\pi \rightarrow \pi^*$ character with electron density localization on the benzene ring and carbon chain Cornard et al. (2008). This large absorbance seem to be promising to improve the sensitivity for CGA determination in coffee beans. The other peak and shoulders are also presents the same $\pi \rightarrow \pi^*$ transitions Cornard et al. (2008).

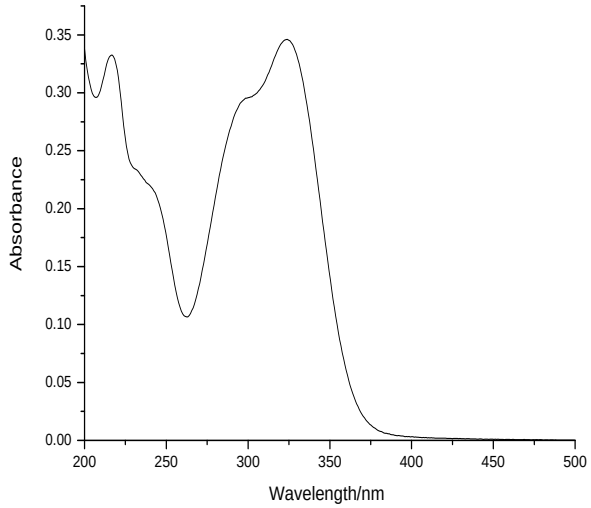

Figure 4.10: The UV-Vis Absorption Spectrum of 5-Caffeoylquinic Acid Dissolved in Water.

4.3.2 **Validation of the Method**

The UV-Vis absorption was validated in terms of limit of detection (LOD), limit of quantification (LOQ), linearity, and standard deviation (SD). The calibration graph correlating the absorption intensity with the corresponding concentration was constructed for 5-caffeoylquinic acid at the highest peak for concentration range of (3.02-11.00)$\times 10^{-5} M$. The calibration equation is (Y= -0.00921+0.19252X, R=0.99, S.D= 0.045, N=7, P<0.0001) where Y, represents the peak height at λ_{max}=324 nm and X concentration in mgL^{-1} .

The limit of detection (LOD) and quantification (LOQ) decide the sensitivity of the method and calculated from the peak-to-noise ratios. In the present study the LOD and LOQ values of CGA were 16, 54 mgL^{-1} respectively. These values were in a good agreement with the result reported by Urakova et al. (2008) using column liquid chromatographic method.

4.3.3 Determination the Contents of CGA in Green and Roasted Coffee Beans

By UV-Vis spectroscopy a direct determination of CGA content in coffee beans are impossible. It was observed that, there is spectral interference from caffeine and CGA in the wavelength regions of 200-500 nm. Fig 4.4 shows the spectrum of coffee beans dissolved in water. Two peaks were observed in these wavelength regions, the first peak was observed in the regions of 250-300 nm which belongs to the peak of caffeine and the other peak was observed in the regions of 300-350 nm and this peak corresponds to the peak of CGA (Belay et al., 2009, ; Belay et al., 2008).

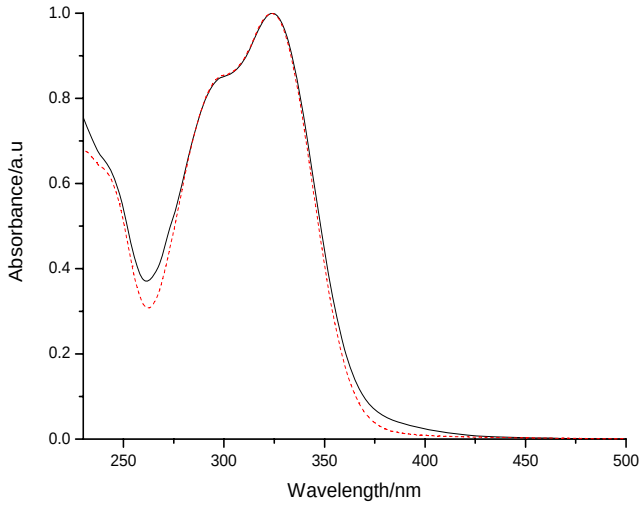

Figure 4.11: The Overlapped Spectra of Pure CGA and CGA after Caffeine Extracted from Coffee Beans by Dichloromethane. The Dot Shows 5-Caffeoylquinic Dissolved in Water and Line Shows CGA after Caffeine Extracted from Coffee Solution.

For quantitative determination of CGA in coffee beans using UV-Vis spectrometry the overlapped spectral band should be resolved or deconvoluted. In this research, the two overlapped spectra were deconvoluted by extracting caffeine from water solution using dichloromethane (Belay et al., 2008). After caffeine extracted from coffee solution the remaining residue is the CGA of coffee beans (Belay et al., 2009). Fig 4.11 shows the over-

Table 4.6: Percentage of CGA in Green Coffee Beans Calculated by UV-Vis Spectrophotometry

Coffee Samples	CGA by UV-Vis Spectroscopy (w/w $\%$
Sample-1	6.25±0.32
Sample-2	6.06±0.17
Sample-3	6.05±0.33
Sample-4	6.19±0.23
Sample-5	6.15±0.25

lapped spectra of pure CGA dissolved in water and CGA after caffeine extracted from coffee solution. The two spectra are exactly similar to each other both in peaks and shapes. The similarity in peak and shape of the two shows there is no overlap band from other components of coffee in these regions, and this shows the specificity of the method.

The accuracy and matrix effects of the method were evaluated using the recovery tests. The recovery of the method was performed by adding a known amount of pure 5-caffeoylquinic acid concentration to the coffee solution. The percent of recovery obtained in different coffee varieties were 91-98.43 $\%$. From the result of recovery test the matrix effects in the sample studies were minor. Thus, the method is valid statistically for determination of CGA in coffee beans. The recovery test obtained in this research is similar with the one reported by Fujioka et al. (2008), for 3-CGA, caffeic acid, ferulic in brewed coffee. The precision (repeatability) of the method was observed by measuring the absorbance of the solution (containing 6 mg of coffee samples in 25 ml of water) and a coefficient of variation 2.80 - 5.45 $\%$ were obtained for different coffee varieties. From the results of coefficient variation the new method is valid in terms of precision.

By above mentioned method, the percentage of CGA in various green coffee beans collected from different regions of Ethiopia were reported using the calibration equation of 5-caffeoylquinic acid mentioned in Section (4.3.2). The percentage of chlorogenic acids determined by this method is in the range of 6.05±0.33-6.25±0.23 $\%$ as shown in Table 4.6 . The contents of CGA reported by this method for green coffee beans are also with in the

range of values previously reported by HPLC techniques. The level of CGA in green coffee beans reported by various worker (Farah et al., 2006; Ky et al., 2001; Ky et al., 1997; Trugo et al., 1984b; Clifford et al., 1976) were about 4-8.4 % for arabica coffee type. And the contents of CGA in various green coffee beans (21 species) from Cameroon and Congo ranged from 0.8-11.9 % on dry matter basis as reported by (Campa et al., 2005). In addition, (Perrone et al., 2008) recently report a similar result with a total CGA contents for Arabica 6.3 and 5.5/100 g using Liquid Chromatography interfaced with Mass Spectrometry.

By similar method the contents of CGA were measured in coffee roasted at light, medium and dark temperature. There is a significant decline in CGA as the roasting temperature increases. For light and dark roasting the CGA present in green coffee beans were decreased by 25-30 %, and 45-52 % respectively, and these results agree with that reported by Fujioka et al. (2008) using HPLC for brewed coffee roasted at similar temperature.

4.4 Solvent Effects and Optical Transition Properties of 5-Caffeoylquinic and Caffeic Acids

4.4.1 Solvent Effects of 5-caffeoylquinic and Caffeic Acids

When absorption spectra of a solute measured in solvents of different polarity, it is found that the position, intensities and shapes of the absorption bands are usually modified by solvents (Reichardt, 1994; Lipty, 1969). These changes are results of physical intermolecular of solute-solvent interaction (such as ion-dipole, dipole-dipole, dipole-induced dipole, hydrogen bonding, etc.), which tend to alter the energy difference between the ground and excited state of the absorbing species containing the chromophores (Reichardt, 1994). The two types of intermolecular interaction responsible for the spectral change in a solvent medium are non-specific, which is due to the collective influence of the solvents, static dielectric constant, permanent dipole and refractive index. The other type of interaction are specific interaction arising from the formation of hydrogen bonds, complexes which result from molecular properties of solvent and solute (Reichardt, 1994).

At room temperature 5-caffeoylquinic and caffeic acids are not dissolved in solvents with low dielectric constant like in (dichloromethane, chloroform, cyclohexane etc) hence

there is no electronic transition, but it dissolves in solvents of large dielectric constants (ethanol, methanol, acetonitrile and water). Dielectric constant is one of the physical constants characterizing the solvents, it often runs parallel with the dissolving power of the solvents, and in case of polar solute it facilitates dissolution by separating the ions. Figs

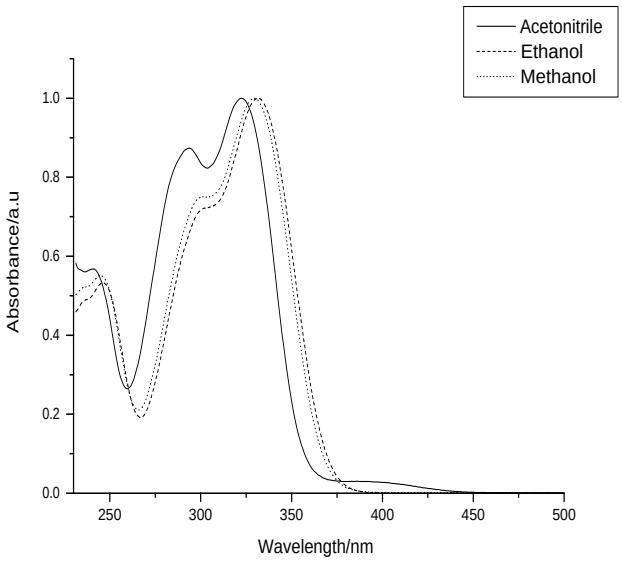

Figure 4.12: The Spectra of 5-caffeoylquinic acid in Different Polar Solvents (Acetonitrile, Ethanol, and Methanol).

4.12 and 4.13 show the overlapped spectra of 5-caffeoylquinic and caffeic acids in different polar solvents (water, methanol, ethanol and acetonitrile) in the wavelength regions of 200-500 nm. Generally a blue shift (hypsochromic) are observed as the polarity of the solvents increase. The blue shift can be interpreted, in terms of a general dipole-dipole interaction between the solvents and solutes in the ground state. The solute solvent interaction in the ground state is greater than in excited state and leads to a blue shift of the spectrum. Moreover a reasonable linearity was observed between maximum absorbance versus the dielectric constant of the solvent except acetonitrile which lacks hydrogen bonding. The deviation of maximum absorbance may be explained that a shift not only affected by dielectric constant of the solvent but also by hydrogen bonding. In this research, a shift due to solvent effects were 16 nm and 10 nm in caffeic and 5-caffeoylquinic acids respectively . Moreover in less polar solvents (methanol, and ethanol) there is a band observed at higher frequency, but obscured by strong band or appears as a shoulder in a more polar solvents

(water and acetonitrile).

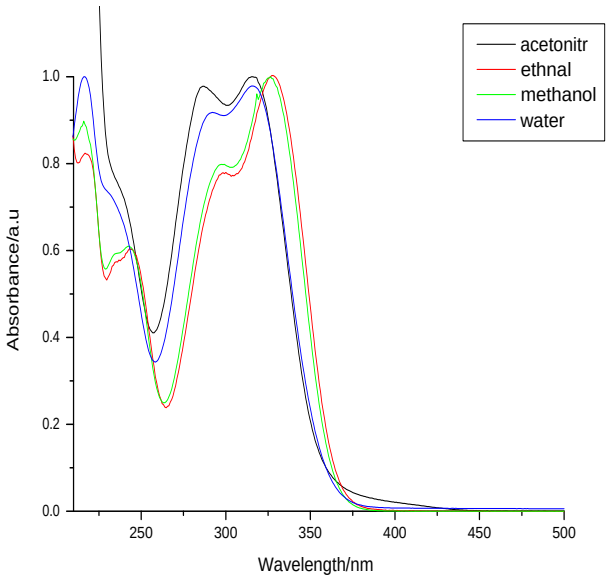

Figure 4.13: The UV-Vis Absorption Spectra of Caffeic Acid in Different Polar-Solvents (Ethanol, Methanol, Acetonitrile and Water).

4.4.2 Optical Transition Properties of 5-Caffeoylquinic and Caffeic Acids

From UV-Vis absorption spectra the optical transitional properties of 5-caffeoylquinic were calculated in solvents to compare the strength of transition shown in Table 4.7. The optical transition properties of the molecules are important to characterize an electron transition and to interpret the absorption spectra. The molar decadic absorption coefficient from measurement of the intensity of optical absorption at a given wavelength was calculated using Beer-Lambert's Law. The molar decadic absorption coefficient of 5-caffeoylquinic was calculated at λ_{max}=324, 322, 330 and 332 nm in water, acetonitrile, methanol and ethanol respectively and these results agree with the one reported by (Moridani et al., 2001).

The corresponding integrated absorption cross-section, transitional dipole moment and oscillator strength were also calculated in the wave number regions of 25000-38,109 cm^{-1} by Eqs of (2.67), (2.76) and (2.77) respectively . The results show 5-caffeoylquinic

Table 4.7: The Optical Transition (Integrated Absorption Cross-Section/$cmmolecule^{-1}$, Molar Decadic Absorption Coeffcient/m^2mol^{-1}, Oscillator Strength and Transitional Moment/Cm) of 5-caffeoylquinicin in various Solvents.

Solvents	σ	ϵ	f	μ
Ethanol	(216±2)×10^{-15}	2003±28	0.56±0.01	(20±0.15)×10^{-30}
Methanol	(204±2)×10^{-15}	1866±20	0.53±0.01	(19±0.10)×10^{-30}
Water	(217±2)×10^{-15}	1882±30	0.56±0.01	(20±0.10)×10^{-30}
Acetonitrile	(177±2)×10^{-15}	1476±28	0.46±0.01	(18±0.09)×10^{-30}

has less transition probability in acetonitrile than other solvents at room temperature as shown in Table 4.7. Moreover the values of oscillator strength calculated experimentally in this work are similar with the one reported by quantum chemical calculation by Cornard et al. (2008).

Similarly for comparison of the optical transition properties of caffeic acid in different polar solvents, the transition dipole moment, oscillator strength and integrated absorption cross-section and molar decadeic absorption coefficients were measured in water, methanol, ethanol and acetonitrile in similar concentration of caffeic acid in the wave number regions of 20, 000-37,993.92 cm^{-1} and the results are shown in Table 4.8. The molar decadic absorption coefficient of caffeic acid were also calculated at λ_{max}=314, 312, 324 and 327 nm in water, acetonitrile, methanol and ethanol respectively. The results show that at room temperature caffeic acid has high values of optical transition properties in methanol than other solvents and this has also reported by Cornard et al. (2004). Moreover the values of oscillator strength of the compound obtained in this research were in a good agreement with the one reported by Cornard et al. (2004) determined from theoretical and experimental methods. On the other hand caffeic acid has less optical transition properties in acetonitrile shows that the lack of hydroxyl of the solvent affects the optical transition properties of the compound.

Table 4.8: The Optical Transition (Integrated Absorption Cross-Section/$cmmolecule^{-1}$, Molar Decadic Absorption Coeffcient/m^2mol^{-1}, Oscillator Strength and Transitional Moment/Cm) of Caffeic Acid in Different Solvents.

Solvents	σ	ϵ	f	μ
Ethanol	(187±2)×10^{-15}	1670±26	0.49±0.01	(19±0.15)×10^{-30}
Methanol	(212±2)×10^{-15}	1847±20	0.55±0.01	(20±0.10)×10^{-30}
Water	(189±2)×10^{-15}	1542±30	0.49±0.01	(19±0.10)×10^{-30}
Acetonitrile	(176±2)×10^{-15}	1300±28	0.46±0.01	(18±0.09)×10^{-30}

4.5 Self-Association, Sodium Ions Complexation, Thermodynamic Properties of Caffeic and 5-Caffeoylquinic Acids

4.5.1 Self-Association of Caffeic Acid

Fig 4.14 shows the overlapped UV-Vis absorption spectra of caffeic acid measured in water for two different concentrations in the wavelength regions of 200-500 nm at room temperature. The dot shows the absorption spectrum of caffeic acid at concentration of 23.9×10^{-5} M. In this concentration, the main absorption peaks of caffeic acid are double band at 294 and 319 nm and other separate peak at 217 nm. On the other hand, the line shows the caffeic acid spectrum at the concentration of 1.49×10^{-5} M; in this case the peaks were observed at 287, 312 and 215 nm respectively. Generally a bathochromic shift of 2-7 nm, intensity variation at peaks and isobestic points were observed relevant to the concentration. For concentrations of caffeic acid greater than 5.31×10^{-5} M, the peak intensity at the wavelength (319 nm) is greater than the two peaks; however; for concentration less than 5.31×10^{-5} M, the peak intensity at the wavelength (215 nm) is greater than the two peaks found at higher wavelength. Moreover three isobestic points were observed for the spectra of the two concentrations. The shift of maximum absorbance, intensity variation and isobestic points, clearly suggests the existence of self-association of caffeic acid, most likely occurring due to hydrogen bonding by catechol or carboxylic groups. Previously this has been reported by, (Ge et al., 2006) that the dimer of caffeic is due to hydrogen bond of

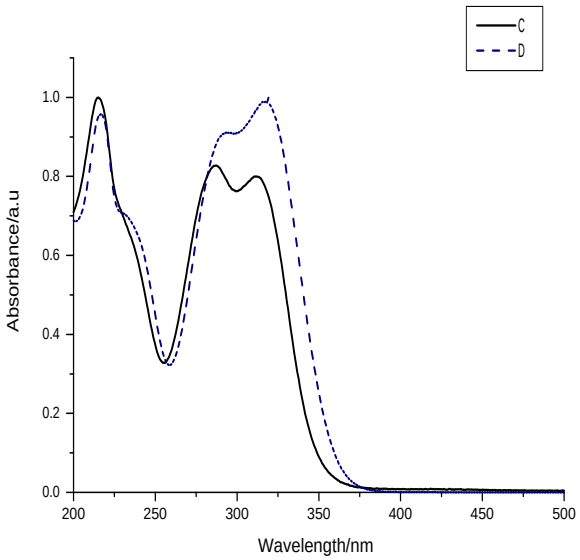

Figure 4.14: The UV-Vis Absorption of Caffeic Acid in Water for Two Different Concentrations.

hydroxyl and C=O, by theoretical method.

Usually a significant shift due to dimerization can be explained by excitation theory (Marme et al., 2005). The excitation theory predicts that equal excited-state energy levels of two monomer split into two different levels on dimerzation (Marme et al.,2005). The two resulting energy levels lie below and above the former energy levels of the excited state of monomer. The transition to the lower sate is forbidden for parallel sandwich dimers (H-type), whereas transition to the higher state is forbidden for head-to-tail dimer (J-type) (Marme et al.,2005). Therefore, blue-shifted absorption band are characteristic for H-type dimers and red-shifted bands for J-type dimers, respectively. According to the excitation theory the dimerzation of caffeic acid belongs to J-type in which the hydroxyl (OH) of caffeic acid is coordinated to the (C=O) of the other molecules.

The quantitative analysis of caffeic acid self-association was carried out using the concentration dependent molar extinction coefficient of the molecule. The values of molar extinction coefficient at the maximum of (215-217, 287-294 nm) bands decrease as the concentration increases, where as, the molar extinction coefficient at the maximum of (312-319 nm) increase as the concentration increases. The hypochromic and hyperchromic de-

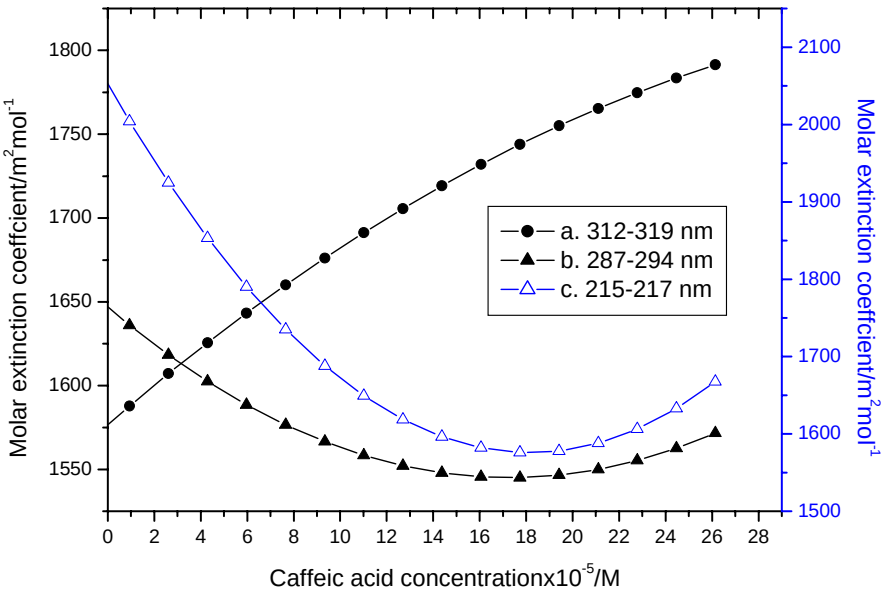

Figure 4.15: The Molar Extinction Coefficients vs Concentration of Caffeic Acid in the Wavelength Regions of a. 312-319 nm b. 287-294 nm c. 216-217 nm. The Circles and Triangles Show the Dimer Model Points Fitted to the Experimental Data.

viation from Beer-Lambert's law and dependence on concentration, suggest the existence of the process of self-association. The curves Fig 4.15a-c shows these effects, where the circles and triangles are the dimer model points fitted to the experimental data. For numerical analysis of the molar extinction coefficients of monomer, dimer and dimerzation constant the known dimer model was derived. The model was derived by considering the following molecular equilibrium in solutions

$$B_1 + B_1 \longrightarrow B_2 \tag{4.1}$$

Where, K_{dB} are the equilibrium dimerzation constant, B_1 and B_2 are monomers and dimers of caffeic acid respectively. The over all concentration of the dissolved molecules in solution is given by the mass conservation law,

$$[B_0] = [B_1] + 2[B_2] \tag{4.2}$$

Where,$[B_0]$ is the total concentration of caffeic acid, $[B_1]$ is the monomer concentration and $[B_2] = K_{dB}[B_1]^2$ is the concentration of the dimers. The contribution of the monomer and dimer to the molar extinction coefficient, of the solution is commonly considered to

Table 4.9: The Molar Extinction Coefficients/$Lmol^{-1}cm^{-1}$ and Dimerzation Constants/M^{-1} of Caffeic Acid at Three Peaks .

Wavelength/nm	ϵ_m	ϵ_d	K_d
215-217	2.40$\times 10^4$	1.30$\times 10^4$	2.80$\times 10^4$
287-294	1.83$\times 10^4$	1.50$\times 10^4$	9.09$\times 10^4$
312-319	1.57$\times 10^4$	2.73$\times 10^4$	5.49$\times 10^2$

be additive, and

$$\epsilon = \epsilon_m f_m + \epsilon_d f_d \tag{4.3}$$

Where, ϵ_m , ϵ_d are molar extinction coefficients of caffeic acid monomers and dimers respectively, $f_m = \frac{[B_1]}{[B_0]}$ is the equilibrium mole fraction of the molecules in the monomer and $f_d = \frac{2K_{dB}[B_1]^2}{[B_0]}$ is a mole fraction of the molecules with in the dimer concentration. Concentration can be determined from the solution of the mass conservation law of Eq (4.2). By substituting, the solution of Eq (4.2) in to Eq (4.3) the following dimer model is obtained

$$\epsilon = \epsilon_d + (\epsilon_d - \epsilon_m)\frac{1 - \sqrt{8[B_0]K_{dB} + 1}}{4[B_0]K_{dB}} \tag{4.4}$$

In Eq (4.4) there are three unknown parameters ϵ_m, ϵ_d and K_{dB} which can be obtained from numerical analysis of experimental concentration dependence of the molar extinction coefficients of caffeic acid Fig 4.15a-c. The values of these three quantities were computed by nonlinear regression based on the Lavenberg-Marquardt algorithm using origin software (Bolotin et al., 2006; Peral et al., 2000). They are serving as search parameters being adjusted in order to achieve the minimum discrepancy between the experimental data and Eq (4.4).

By this method the values of ϵ_m, ϵ_d and K_{dB}, were obtained at three peaks of caffeic acid and the values are shown in Table 4.9. The obtained values are quite reasonable and comparable with the results obtained by other workers in the UV region of the spectrum for similar molecules. The values of ϵ_m, ϵ_d determined for lauric acid in liquid of CCl_4 using FTIR were 2.8$\times 10^4$ and 8.66$\times 10^3$ $Lmol^{-1}cm^{-1}$ respectively (Lu et al., 1999). Moreover the self-association constant and molar extinction coefficient of monomer determined for ADP studied in aqueous solution at high pH value would be 1$\times 10^4$ M^{-1} and 1.5$\times 10^4$

$Lmol^{-1}cm^{-1}$ respectively (Peral et al., 2000). Similarly the dimerzation constant calculated and reported for Benzoic acid at room temperature was $1.4\times10^{4}\ M^{-1}$ which is expected to be due to hydrogen bonding.

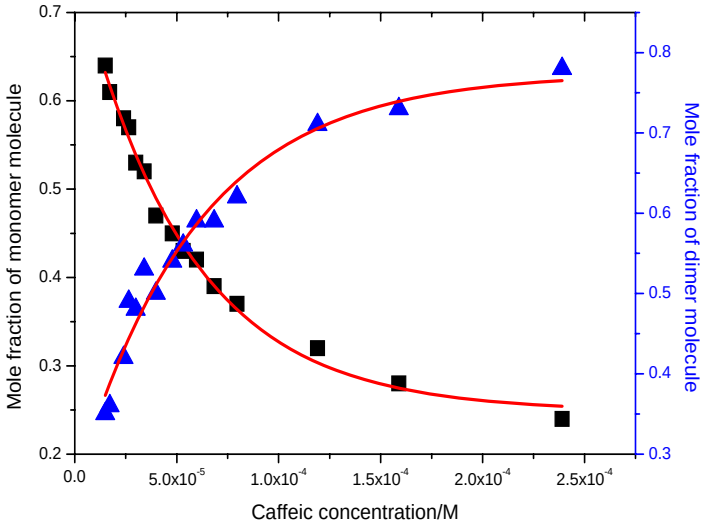

Figure 4.16: The Mole Fraction of Monomer and Dimer versus Total concentration of Caffeic Acid Under the Peak of 215-217 nm.

The concentration of monomer and dimer molecules under peaks of 215-217, and 312-319 nm were also determined as a function of total caffeic acid concentration in the range of (1.49-23.9) $\times10^{-5}$ M. The different values of dimerzation constants in Table 4.9 shows that the concentration of monomer and dimer at peaks are different. Fig 4.16 shows the mole fraction of monomer and dimer molecules versus concentration of caffeic acid at the peak of 215-217 nm. Generally the formations of dimer molecules are favored by high concentration, in which the mole fraction of monomer and dimer molecules decrease and increase exponentially respectively. Fig 4.17 on the other hand shows the mole fraction of monomer and dimer molecules versus the concentration of caffeic acid at the wavelength regions of 312-319 nm. In this case the percentages of dimer molecules contribution insignificantly to the total concentration of caffeic acids, which is only 1.8 $\%$ at the highest concentration; and this suggests us that the peak is due to the monomer of caffeic acid.

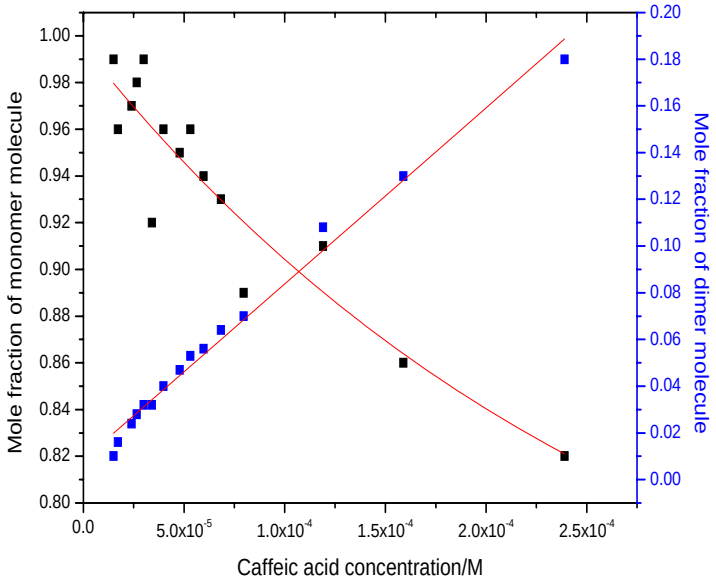

Figure 4.17: The Mole Fraction of Monomer and Dimer Versus Total Concentration of Caffeic Acid Under the Peak of 312-319 nm.

4.5.2 The Complexation of Sodium with Caffeic Acid

The electronic absorption spectrum of caffeic acid in distilled water is expressed in Fig (4.13). The addition of anhydrous of sodium hydroxide to caffeic acid solutions results in a shift of maximum peak to 343 nm and decrease in absorption intensity on increasing the concentrations of sodium hydroxide were observed. Fig 4.18 shows peak absorbance vs various concentration of sodium hydroxide in caffeic acid solutions or([NaOH]: [Caffeic acid] for the ratio of 07-87) at peak 343 nm. There are also isobestic points in the absorption spectra, which are indicative of the complex formation between sodium ions and caffeic acid by coordinate covalent bond.

The quantitative analysis of the complexation of sodium ions with caffeic acid was accomplished using Benesi-Hildebrand approaches (Benesi-Hildebrand , 1949), under the condition of $[B_0] >> [A_0]$. A caffeic acid solution of ($C_{CA} = [A_0] = 9.16 \times 10^{-5} M = constant$) and different concentrations of sodium hydroxide ($C_{NaOH} = [B_0] = (0.97 - 7.46) \times 10^{-3} M$) were used to calculate the equilibrium constant and molar extinction coefficient of the complex formation. The equilibrium constant for the complex formation K, derived as

follows,

$$A + B \rightarrow AB \tag{4.5}$$

For Eq (4.5) the equilibrium constant for the complex formation K can be defined as

$$K = \frac{[AB]}{[A][B]} \tag{4.6}$$

where $[A]$, $[B]$, and $[AB]$, are the equilibrium concentration of caffeic acid, sodium hydroxide and association of caffeic acid with sodium respectively. If the initial concentration of caffeic acid and sodium hydroxide designated as $[A_0] = [A] + [AB]$ and $[B_0] = [B] + [AB]$, substituting these in to Eq (4.6) it gives

$$K = \frac{[AB]}{([A_0] - [AB])([B_0] - [AB])} \tag{4.7}$$

For $[B] >> [A]$, then $[B_0] - [AB] \approx [B_0]$, so Eq (4.7) can be written as

$$K = \frac{[AB]}{([A_0] - [AB])([B_0])} \tag{4.8}$$

After re-arranging Eq (4.8) it gives

$$[AB] = \frac{K[A_0][B_0]}{1 + K[B_0]} \tag{4.9}$$

The absorbance (A) for concentration $[AB]$ according to Beer's law is

$$A = [AB]\epsilon l = \frac{\epsilon l K[A_0][B_0]}{1 + K[B_0]} \tag{4.10}$$

Eq (4.10) can be re-arranged as follows

$$\frac{[A_0]}{A} = \frac{1 + K[B_0]}{\epsilon l K[B_0]} \tag{4.11}$$

when the path length $l = 1cm$, Eq (4.11) can be written in the form of Benesi-Hildebrand equation

$$\frac{[A_0]}{A} = \frac{1}{\epsilon} + (\frac{1}{[B_0]})(\frac{1}{\epsilon K}) \tag{4.12}$$

The plot of $\frac{[A_0]}{A}$ vs $\frac{1}{[B_0]}$ gives a straight line with y-intercept $\frac{1}{\epsilon}$ and slope $\frac{1}{K\epsilon}$. By this method the equilibrium constant for the complex formation and molar extinction coefficient calculated at peak position were $K = 1.42 \times 10^3 M^{-1}$ and $\epsilon = 2.66 \times 10^3 M^{-1} cm^{-1}$ respectively. The equilibrium constant for the complex formation with sodium is with in the range of the dimerzation constants of caffeic acid calculated at different peaks.

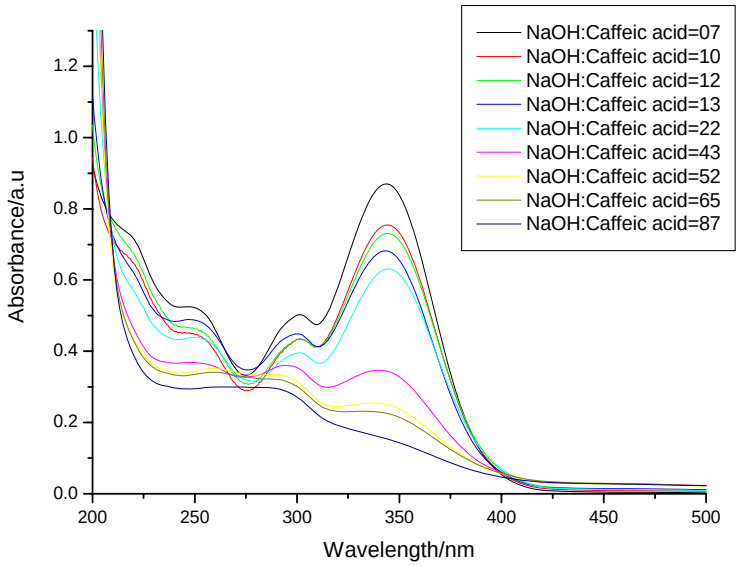

Figure 4.18: The Peak Absorbance vs Various Concentration of Sodium Hydroxide in Caffeic Acid Solutions ([NaOH]:[Caffeic Acid])for ratio of 07-87 at Peak 343 nm.

4.5.3 Thermodynamic Properties of the Self-Association of Caffeic Acid

Heating the aqueous solution of caffeic acid shows that the absorption spectra of the molecules are strongly dependent on the temperature in the range of (328-348 K). As temperature increases, the absorption intensity increases which indicate a dissociation of the molecular associated forms in the solutions. The equilibrium constant of caffeic acid at the above mentioned temperature were calculated at peak of 216 nm using Eq (4.3). Fig 4.19 shows the graph of lnK versus $f(\frac{1}{T})$, of caffeic acid, which is linear. It is an exothermic reaction since the slope is positive, it means that as temperature increases, the equilibrium constants decreases. A shift to the left leads to a decrease in product and an increases in reactants which decreases the equilibrium constant as predicted by the van't Hoff equation. The magnitude of the enthalpy was estimated from the slope of the approximating line according to van't Hoff's equation:

$$\frac{dln(K)}{d(\frac{1}{T})} = -\frac{\Delta H}{R} \tag{4.13}$$

where ΔH is the molar enthalpy change, $R = 8.31 Jmol^{-1}K^{-1}$ is the universal gas constant and T the temperature in Kelvin. The entropy was derived from Gibb's free energy and enthalpy. The Gibb's free energy and entropy can be expressed as

$$\Delta G = -RTln(K) \tag{4.14}$$

$$\Delta S = -\frac{\Delta G - \Delta H}{T} \tag{4.15}$$

The calculated values of the Gibbs free energy, enthalpy, and entropy of caffeic acid at

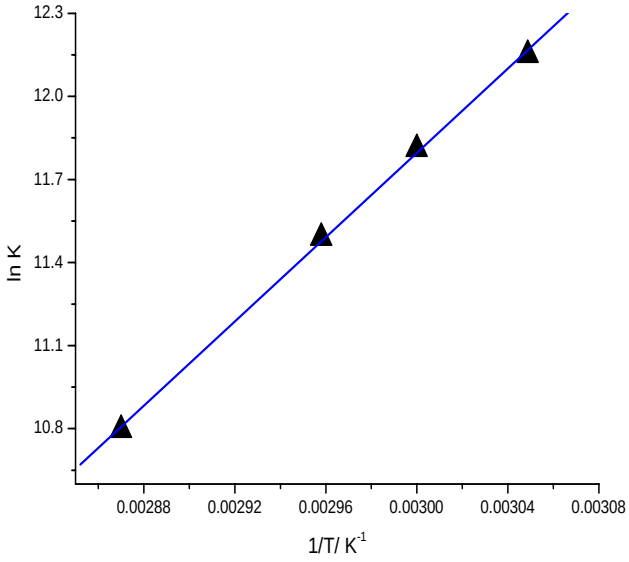

Figure 4.19: lnK_d vs $1/T$ of Caffeic Acid at Concentration (C=5.83$\times 10^{-5}$ M).

T= 328 K, are $\Delta G = -34.06 kJmol^{-1}$, $\Delta H = -63.20 kJmol^{-1}$ and $\Delta S = -88.84 JK^{-1}mol^{-1}$ respectively. The calculated thermodynamic parameters of caffeic acid are in a good agreement with the results recently reported. The thermodynamic molecular association process between malvidin-3-o-glucoside and caffeic acid for the stoichimetry of 1:1 are $\Delta H = -57.3 kJmol^{-1}$, $\Delta G = -31.90 kJmol^{-1}$ and $\Delta S = -87 JK^{-1}mol^{-1}$ respectively as investigated by fluorometric and quantum-chemical methods (Mate et al., 2006). The results show that the self-association of caffeic acid are exothermic which are characterized by relatively large negative enthalpy H.

4.5.4 Self-Association of 5-Caffeoylquinic Acid

Most organic acids are known to form strongly hydrogen bonded dimer and therefore can be found as a combination of monomer and dimer in liquid forms (Hintzes et al., 2001). The concentration dependent self-association of 5-caffeoylquinic acid is also expected to be hydrogen bonded due to interaction of hydroxyl and carboxylic groups of the compound. The graph of molar extinction coefficient vs concentration of the compound at the maximum of wavelength (324 nm) indicates increase in the concentration range $(3.02 - 17.50) \times 10^{-5}$ M decreases in the extinction coefficient (Fig 4.20). The deviation from Beer-Lambert's law and dependence on concentration suggest the existence of self-association process of the molecule. For numerical analysis of the molar extinction

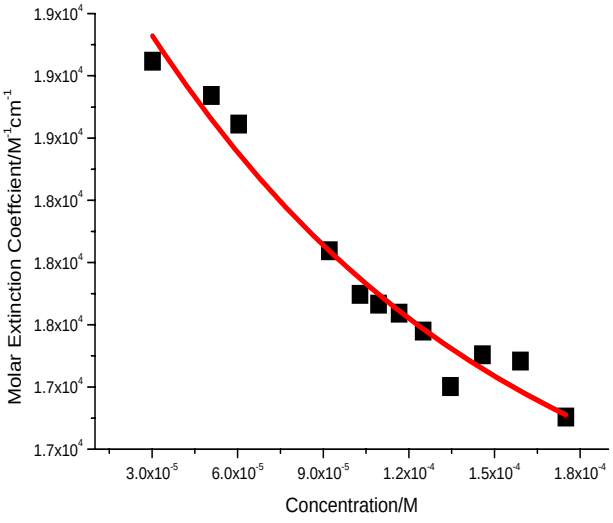

Figure 4.20: Molar extinction Coefficient vs Concentration of 5-Caffeoylquinic Acid at Absorption Maxima of 324 nm.

coefficients of monomer, dimer and dimerazation constant, the known dimer mode of Eq (4.4) was used.

The calculated values of ϵ_m, ϵ_d and K_{db} obtained for 5-caffeoylquinic acid at the wavelength 324 nm were 1.98×10^4, $1.14 \times 10^4 M^{-1}cm^{-1}$ and $1.81 \times 10^3 M^{-1}$ respectively. The dimerzation constant of 5-caffeoylquinic acid is within the range of previously calculated

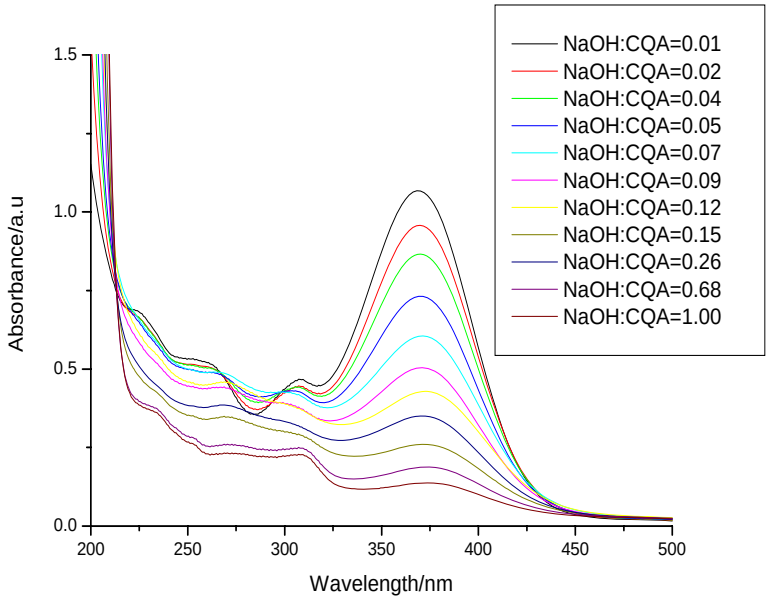

Figure 4.21: THe Peak Absorbance vs Various Concentration of Sodium Hydroxide in 5-caffeoylquinic Acid Solutions ([NaOH]:[5-caffeoylquinic Acid]) for ratio of 0.01 to 1.00 at Peak 370 nm.

for organic acid which are known to form strong hydrogen bond. The dimerzation constant calculated by Hintzes et al., (2001), for acetic acid and propionic acid at different temperatures are with in the range of (0.84 to 4.67)$\times 10^3$ and (0.46 to 2.63)$\times 10^3$ M^{-1} respectively. Similarly the dimerzation constants for acetic acid are reported by (Barrow and Yerger, 1954) using FTIR were (1.00-2.60)$\times 10^3$ M^{-1} for different concentrations.

The profile of monomer and dimer concentrations under peak was also determined as a function of total 5-caffeoylquinic acid concentration in the range of (3.02 to 17.50) $\times 10^{-5}$ M. In the concentrations regions, the formations of dimer molecules are favored by high concentration, in which the mole fraction of monomer and dimer molecules decreases and increases respectively as it is expected.

4.5.5 Complexation of Sodium with 5-Caffeoylquinic Acid

Fig 4.21 shows the effects of NaOH concentration on the UV-Vis absorption spectra of 5-caffeyloquinic acid solutions. The electronic spectrum of free 5-caffeoylquinic acid in distilled water is well expressed in Section (4.3.1). The addition of anhydrous of sodium hy-

droxide to 5-caffeoylquinic acid solutions results in important spectral modification with band shift to higher wavelength (370 nm). The peak absorbance versus molar ratio of (NaOH:5-Caffeoylquinic acid) decrease as the ratio of (NaOH:5-Caffeoylquinic acid) increases. Moreover, the existences of isobestic points were observed in the absorption spectra, which indicate the formation of complexes between sodium ions and 5-caffeoylquinic acid.

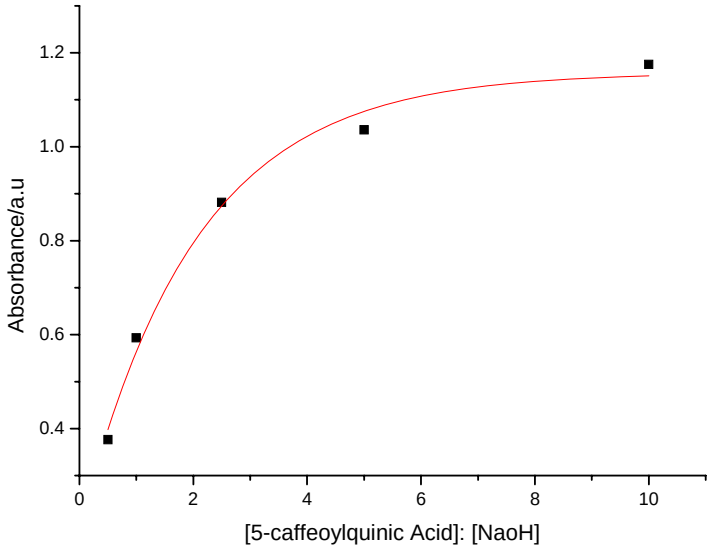

Figure 4.22: Mole Ratio Method for 5-Caffeoylquinic Acid and Sodium hydroxide

The quantitative analysis of the association of sodium hydroxide with 5-caffeoylquinic acid was accomplished by Benesi-Hildebrand equation, under the condition of [NaOH] $>>$ [5-Caffeoylquinic acid]. A solution of sodium hydroxide, whose concentration ranges are (1.13-22.46)$\times 10^{-3}$ M and 5-caffeoylquinic acid 2.20$\times 10^{-5}$ M solution were prepared in distilled water to calculate the equilibrium constant and molar extinction coefficient. The equilibrium constant for the complex formation and molar extinction coefficient calculated using Eq (4.12) at 370 nm were $5.52 \times 10^{3} M^{-1}$ and $2.40 \times 10^{4} M^{-1} cm^{-1}$ respectively . The equilibrium constant for the complex formation for 5-caffeoylquinic acid is greater than its dimerzation constant. On the other hand, the equilibrium constant for the complexation with sodium is in a good agreement with the one reported by (Cornard et al., 2008) for the complexation of this compound with Pb (II).

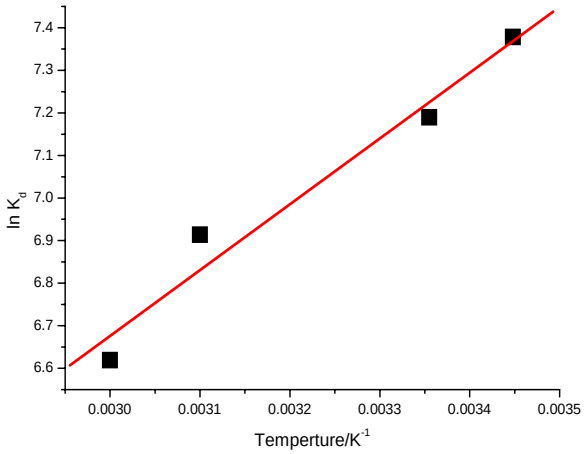

Figure 4.23: lnK vs $1/T$ of 5-Caffeoylquinic Acid

The stoichiometry of the complexes was also obtained by mole ratio method. Sodium hydroxide solutions (1 ml) were added to the 5-caffeoylquinic acid solution (0.5-20 ml) in separate steps. Ratios of 0.5:1 to 1:10 sodium hydroxide to 5-caffeoylquinic acid solution were prepared and the absorbance recorded at 370 nm. The graph of these absorbance vs mole ratios was plotted in Fig 4.22. As a result the stoichiometry was found to be 1:1 sodium to 5-caffeoylquinic acid.

4.5.6 Thermodynamic Properties of the Self-Associated 5-Caffeoylquinic Acid

Heating the aqueous solution of 5-caffeoylquinic acid shows that the absorption spectra strongly depend on the temperature in the range of (290-328 K). As temperature increases, the absorption intensity increases which indicate a dissociation of the molecular associated forms in the solutions.

Fig 4.23 shows the graph of lnK versus $f(1/T)$ of 5-caffeoylquinic acid, which is linear. The magnitude of the enthalpy was estimated from the slope of the approximating line according to Van't Hoff's equation and Gibbs free energy entropy were calculated at temperature $298K$. The enthalpy, Gibbs free energy and entropy of 5-caffeoylquinic acid

were calculated by Eqs (4.13), (4.14) and (4.15) respectively. The calculated values of enthalpy, Gibbs free energy and entropy of 5-caffeoylquinic acid self-association were $\Delta H = -12.89 kJmol^{-1}$, $\Delta G = -17.78 kJmol^{-1}$ and ,$\Delta S = 16.60 JK^{-1}mol^{-1}$ respectively. These values are in good agreement with the results recently reported by, (Dearden and Bresnen, 2005) for benzoic acid which are $\Delta H = -12.50 kJmol^{-1}$, $\Delta G = -18.00 kJmol^{-1}$ and ,$\Delta S = 18.90 JK^{-1}mol^{-1}$. Moreover, (Gornas et al., 2009) determined the thermodynamic parameters of Beta-Cyclodextrin complexes with chlorogenic acids and the obtained values are similar with $\Delta H = -12.70 kJmol^{-1}$, $\Delta G = -14.80 kJmol^{-1}$ respectively. From calculated thermodynamic parameters, the self-association reaction of 5-caffeoylquinic acid is exothermic reaction and results in higher entropy ($\Delta S > 0$) and decrease in enthalpy ($\Delta H < 0$).

5

Conclusion

The methods of measuring major bioactive compounds in coffee beans, their optical transition properties, self-association, hetero-association and thermodynamic properties of the compounds have been investigated by UV-Vis spectrophotometry. The developed methods for analysis of caffeine and chlorogenic acids are simple, fast, cheap and highly sensitive. Moreover chemicals and equipments to carry out the analysis are mostly available in common laboratories.

The optical transition properties (oscillator strength, dipole moment, integrated absorption cross-section and peak absorption cross-section) calculated in different solvents are the intrinsic ability of molecules to absorb light and they are proportional to the intensity of transition. Experimental determination of the optical transition probabilities are important for direct applications to absorption, emission and dispersion radiation. On the other hand it is also useful for providing stringent tests of atomic structure calculations in theoretical works.

The equilibrium constants, the concentration profile of monomer and dimer, and thermodynamic properties calculated for caffeic and 5-caffeoylquinic acids are useful for analyzing and interpreting the spectroscopy and study kinetic of chemical reaction. Moreover Understanding the mechanism of self-association and hetero-association of 5-caffeoylquinic and caffeic acids are useful in order to design the advanced and controllable carriers of drugs, food components and in extraction alkali metals ions selectively from natural environment. Thus, it may be concluded that the investigated results have wider applications in terms of economic and scientific utility.

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I hereby declare that this PhD dissertation is my original work and has not been presented for a degree in any other universities, and that all sources of material used for the dissertation have been duly acknowledged.

Name: Abebe Belay

Signature: ———————————

This PhD dissertation has been submitted to for examination with my approval as university advisor.

Name: Prof.A.V. Gholap

Signature: ———————————

Place and date of submission:

Department of Physics

Addis Ababa University

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