OPTICAL AND THERMAL PROPERTIES OF COFFEE AND CAFFEINE

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Contents

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Abstract

In this project, the thermal and optical properties of coffee and caffeine are investigated. Characteristics involved are solvatochromic effect, fluorescence, chemical structures (bonds) and thermal properties of both coffee and caffeine. The UV/Vis absorption band of caffeine in different solvents shows very small solvatochromic effect, indicating only local transitions of electrons. This founding is similar to that found for QM-calculations. Fluorescence measurements revealed no fluorescence emission by caffeine, but deactivation of fluorescien fluorescence intensity. However, simple correlation between the deactivation produced by caffeine and coffee on the fluorescence of fluorescein could not be drawn with out the detailed knowledge of other components. From FT-IR results, the fluorescence deactivation mechanism is found to incorporate carbonyl groups of both caffeine and coffee. The observed thermal (TGA) results showed coffee is thermally unstable and decomposes steadly.This shows the inherent discrepancy of the temperature dependent measurement, which makes exact measurement of caffeine at various temperature intervals unrealistic. The DSC result shows crystallization, heat absorption melting and heat release of coffee at various temperature regions.

Key Words: Coffee, Caffeine, Fluorescence, Solventochromism, Thermal, UV/Vis

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1. Introduction

Coffee, the second-most-traded commodity after petroleum, produced in over 60 countries around the world accounts for the livelihood of up to 100 million people. Many of these countries are heavily dependent on coffee, which can account over 80% of their export earnings [1,2].Thus it is not surprise that coffee and caffeine have been the subject of extensive scientific study during the past quarter of a century, with 1500-2000 papers published ever year on the topic. [1]

The two commercially important species of coffee available in the market are known as "Arabic a" and "Robust a". The word "Coffee" originated from the place "kaffa" in Ethiopia. The "Arabic a", which provides 70% of the world market, have the same origin. The coffee "Robust a" was discovered growing wild in Uganda, in Congo basin and near the cost of West Africa only during the last hundred years. This variety accounts for some 20% of the world export [3].

It is likely that, long before their use as the base of the drink, the coffee fruit and beans were chewed and found to be stimulating; later the people of Ethiopia were discovered using crushed dried coffee beans mixed with fat and rolled into balls, as food to sustain them on their journeys. In those early days the juice of the fruit may also well have been fermented and used as a beverage [3,4].

In the early 15^{th} century, it was introduced to Yemen then to many parts of the Islamic world. Turks introduced this drink to Europe around 16^{th} century. By the middle of the 17^{th} century, the habit of drinking coffee was well established through out the Europe and North America [3,4].

Generally, coffee is a tropical plant. With in the tropics altitude is the main factor for coffee growth, since near the equator the higher the altitude at which there is a risk of frost, so the plant may be found growing at 2500 m on the equator but not above about 100 m at altitudes of 25° N and 25° S. There is also with respect to temperature an upper limiting factor. Since the plant will not tolerate temperatures approaching 30° C, especially in the condition of low humidity. Ideally for coffee annual rainfall should not be less than about 1500 mm and in some areas producing excellent coffee it may exceed 2500 mm [3, 5].

In somewhat general review of the coffee soil, one ideal for coffee would be a deep slightly acidic, well-drained loam, and it should be rich in nutrients, especially potash, with an ample supply of humus. "Robust a" coffee can be grown in a wide range of environments than

"Arabic a", it thrives at lower, generally warmer altitudes, and it is resistant to coffee leaf rust [4].

The flavor and quality of coffee vary generally depending up on the maturity of coffee berries at harvest, the environment in which the crop is grown, especially altitude and temperature, differences between cultivators, the skill and care with which the barriers are processed and the methods used, and the temperature and length of time the beans are roasted [5].

The major coffee producing countries are Brazil, Vietnam and Colombia. Their export constitutes according to the data collected by ICO in the production years 1985-87, to be more than 50% of the world coffee production. According to ICO 1985-86 coffee production statistics, Ethiopia is the 7^{th} coffee producer. Coffee accounts for 65% of Ethiopian export earning [6, 7].

On the other hand, according to the statistics from 1985-1988, by UN café, coffee consumption (in kg/person) is highest in Finland, Sweden, Denmark and Norway. From the coffee producing countries Colombia, Brazil, Guatemala and Salvador are the highest consumers while USA, Japan and Germany are the leading importers [8].

Green beans vary in mass from below 100 mg to over 200 mg with considerable overlap between "Arabic a" and" Robust a". Such variations may be associated with the amount of water available in the bushes. The true relative density of the green beans ranges from 1.15-1.42. The average water content of the green beans (8-12%) and roasted beans (0-5%) [1,9].

In roasting process 'Arabic a' and' robust a' ultimately swell to a similar extent data recorded by Dalla Rosa, Lerici, Riva and Fini (1980) suggested those two species behave differently early in the roasting process. The data implies the 'Arabic a' and 'robust as' differ in their thermo physical behavior [9].

Knowledge of water content of green and roasted coffee products required because it influences water acidity and stability during storage. It provides a monitor of coffee extracts drying process, and there are often limits set by national and international legislation. [9]

The main compositions of coffee are: ash and minerals, free amines, amino acids, proteins, carbohydrates, polysaccharides, lipids, acids, volatile components and caffeine [3]. Of these components, caffeine is believed to be the component responsible for the stimulating effect of coffee.

Caffeine is a white compound, which melts at 236 °C although this is relatively unimportant as it sublimates at the much lower temperature of 178 °C it is very weak base, forming unstable salts, e.g. acetate from which the acetic acid may be readily volatilized. [3, 9].

Caffeine is moderately soluble in water even at low temperatures and has a considerable increase in solubility at higher temperatures. Caffeine is moderately soluble in organic solvents like chloroform, benzene and dichloromethane [3,9]. Other soluble constituents of coffee in order of their abundance are: Phenol polymer, Polysaccharides, Chlorogenic acid, minerals, organic acid, sugar, lipids and aroma [10].

In the 1950s and 1970s many believed that caffeine could be a series cause of cancer in human because of the studies in plant and showing chromosome breaks, inhibition of mitosis and formation of chromatin breaks after high dose caffeine testament [11]. A 1994 review, concluded that caffeine at the doses experienced in coffee drinking is not mutagenic in humans. A review of the studies in human and animals found no conclusive evidence for any incidence of cancer in any organ due to coffee [12]. In the study of nearly fifty thousand male health professionals showed no increase of cardiovascular disease due to coffee drinking [13]. Neither caffeinated nor decaffeinated (coffee that has had most of the caffeine removed) coffee is associated with an associated risk of myocardial infection [14].

In Chinese medical dietary system, the green bean of coffee would be classified as a herb that regulate liver qi, which is therapeutic route to strong energy stimulation (attributed chemically to the caffeine action on the nervous system). By vigorously edging the stagnated liver qi a strong sense of mental and physical vitality is experienced. The early use of coffee beans to regulate menstruation is consistent with Chinese medical approach of regulating menstruation by dredging stagnant liver qi. The green bean also cools the constrained liver qi when the bean is roasted, but transfer from the cooling herb [15]. Coffee not only regulates the liver qi but also purges the gall bladder. The liver (and gall bladder) regulating properties of coffee explains its property to protect against formation of gallstones and its property to alleviate constipation. This action is attributed to chlorogenic acid and other constituents found in coffee, thus coffee assists digestion. Coffee helps a meliorate liver-spleen disharmony associated with liver qi stagnation and spleen weakness. To all these effects 1-3 cups of coffee per day is about the correct range [15]. New studies reveal the linkage between coffee drinking and a reduced risk in developing Parkinson's disease. The study deals mainly with men, where the results are very positive. Trials with woman have been inconclusive [16]. According to research in Germany, drinking coffee may help to prevent colon cancer. A study revealed a potent antioxidant that boosts cancer [17].

However, heterocyclic amines from roasting and alkyl carbonyls in coffee are found to be mutagenic [18]. Further it was pointed out coffee in excess of 8 cups per day may aggravate cardine arrhythmias [19] and plasma homocy stein [20]. Coffee regularly increase energy

metabolism through out the brain while decreasing cerebral blood flow. Administered acutely, moderate doses of caffeine can increase blood pressure and decrease heartbeat rate [9].

Caffeine, which is found in coffee and other foods (coca, tea), is that substance responsible for the stimulation effect of coffee. Caffeine content for "Robust a" which is 2.4 to 2.8 percent and "Arabic a" which is not exceeding 1.5 percent by weight [3, 15].

Unfortunately, to some people this and other effects of caffeine are not welcome. As mentioned earlier, decaffeinated coffee is coffee most of its caffeine removed. Today decaffeinated coffee accounts for approximately 12% of total worldwide coffee consumption. [3,21]

Chemically, there are three main decaffeinating processes currently in use. They have some basic similarities. In all the three approaches, the green and roasted beans are first moistened, making the caffeine soluble so that it can be drown out. In addition, they all decaffeinate green coffee at moderate temperatures typically ranging from 10 °C to 100 °C. In all these processes, i.e. water (Swiss), ethylene chloride (European) and carbon dioxide 94-97% caffeine is removed. Although there is virtually no trace of any chemicals left in the beans after roasting, some peoples are uncomfortable knowing that the coffee they are drinking was chemically processed [21]. The other problem associated with artificial decaffeination is the coffee aroma and test decreases due to these processes.

Scientists recently came out with two new findings that address the above problems. The first finding is by genetic engineering i.e. by RNA interference repressing thyobromine synthesis, which successively adds methyl groups to xanthosne in converting it in into caffeine. The caffeine content of these plants is reduced up to 70% indicating that it should be feasible to produce coffee beans that are intrinsically deficient in caffeine [22].

The second is naturally decaffeinated coffee varieties found by Paulo Mezzefera and his team from university of Campinos (Brazil). The researchers, after screening 3000 Ethiopian coffee trees, discovered three naturally decaffeinated varieties, which they named AC1, AC2 and AC3. Analysis of these varieties showed they contain only 0.07% caffeine compared to the caffeine found in natural coffee [23].

For the determination caffeine from coffee many analytical methods have been reported, techniques including tritrimetry, spectrophotometry, polarography, GC, HPLC [24], Mass spectrometries [25] have been reported. Of all these methods, spectroscopic methods are the most suitable for on line monitoring. However, in the past their applications were not efficient and were not widely implemented due to the expensiveness of the instruments. These days, due

to the rapid development of new technologies based on diode array, fiber optics and charged coupled devices (CCD) these instruments are no longer expensive, even cheaper, than most of the instruments used in caffeine analysis. Therefore, our aim is to monitor the quality and quantity of caffeine in coffee beans based on optical methods. This will include the development of simple methodologies that can be adopted for a fast and selective determination, especially when routine determination is required.

In this study, thermal and optical methods are employed to study the optical and thermal properties of coffee and caffeine. The thermal property is investigated using Thermal gravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC). The optical properties are studied using: UV/VI spectrophotometer, FT-IR absorption spectrophotometer and Fluorescence spectrophotometry.

The specific objectives are to investigate the effect of solvent on the position and nature of the caffeine spectrum (Solventochromism effect) using UV/ VIS spectroscopy. The effect and consequence of temperature will be investigated using TGA and DSC. The interaction of caffeine and soluble component of coffee with florescent dyes as an indirect approach in determining the caffeine content in coffee beans will be investigated using fluorescence spectroscopy. The structure of caffeine and coffee that undergone a change in structure due to interaction with the fluorescein will be investigated using FT-IR spectrometer.

2. Spectroscopy and Thermal Properties

2.1 Spectroscopy

2.1.1 Introduction

The word spectroscopy is derived from the Latin word "spectron" and means "ghost or sprit "According to IUPAC, Spectroscopy is defined as the study of physical systems by electromagnetic radiation with which they interact or that they produce. In simple words one may define spectroscopy as a part of science that deals with the interaction of electromagnetic radiation with matter, i.e. it deals with the exchange of molecular (or atomic) quanta with electromagnetic quanta (photons) [26]. The interaction of radiation with matter can cause reradiation of the radiation and / or transition between the energy levels of the atoms or molecules. A transition from a lower level to a higher level with transfer of energy from the radiation field to the atom or molecule is called absorption. A transition from a higher level to a lower level is called emission if energy is transferred. A transition is called nonradiative decay if no radiation is emitted. Redirection of light due to its interaction with matter is called scattering, and may or may not occur with transfer of energy, i.e. the scattered radiation has a slightly different or the same wavelength [27].

2.1.2 Molecular quanta (Energy)

According to Born-Oppenheimer (BO) approximation the electronic, vibrational, rotational and translational energies are considered independent of each other, i.e. transitions between different energy levels may occur independently. A change in the total energy of a molecule could then be written as

$$\Delta E_{\text{total}} = \Delta E_{\text{electronic}} + \Delta E_{\text{vibrational}} + \Delta E_{\text{rotational}} + \cdots \qquad (2.1)$$

The approximate orders of magnitude of these changes are

$$\Delta E_{\text{electronic}} \approx 1000 \cdot \Delta E_{\text{vibrational}} \approx 1000000 \cdot \Delta E_{\text{rotational}} + \cdots \tag{2.2}$$

While rotational spectra are observed only on molecules that have a permanent dipole moment, vibrational spectra are only observed when the dipole moment of the molecule changes during the vibration. Electronic spectra can be obtained from all molecules, since changes in the electron distribution in a molecule are always causing a change in the dipole moment of the molecule. This means that homonuclear molecules like H_2 or O_2 that do not show rotation or rotation-vibration spectra, do give an electronic spectrum and show vibrational and rotational in their spectra that can be used to derive rotational constants and bond vibration frequencies [28,29].

2.1.3 Photon Energy

All radiation is characterized by a wavelength, an amplitude and velocity of propagation, Energy (E), frequency (ν), wavelength (λ), and wave number ($\tilde{\nu}$) are related by the Planck's equation

$$E_{ph} = \mathrm{h}\nu = \mathrm{h}c_0\tilde{\nu} = \mathrm{h}c_0/\lambda = \hbar\omega = \hbar c_0 k \tag{2.3}$$

where h is the Planck's constant (h = $2\pi\hbar$ 6.626×10^{-34} J s), c_0 is the velocity of electromagnetic radiation in vacuum ($c_0 = 2.998\times10^8$ m s^{-1}) and k is the magnitude of the wave vector. The photon spectroscopy covers the wavelength range from < 10 pm to > 100 mm.

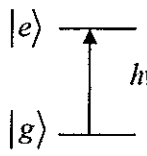
2.1.4 Molecule-Electromagnetic Field Interaction

We describe the interaction in a semi-classical way; Molecule-quantized and emf-classical. Using the so called 'electric dipole approximation' for E, one can express the perturbing operator $\hat{H}'(t)$

$$\hat{H}'(t) = -\underline{\hat{\mu}} \cdot \underline{E} = -\underline{\hat{\mu}} \cdot \underline{E}_0 \cos\omega t \tag{2.4}$$

where $\hat{\underline{\mu}}$ is the dipole operator and $\underline{E}$ the electric field vector. Further for a spectroscopic transition must apply:

1. The Bohr frequency criteria



$$h\nu = \hbar\omega = E_e - E_g \tag{2.5}$$

2. The electric transitional dipole moment must be different from zero

$$\underline{\mu}_{eg} = \int \psi_e^* \hat{\underline{\mu}} \psi_g d\tau = \langle e|\hat{\underline{\mu}}|a\rangle \neq 0 \tag{2.6}$$

2.1.5 Electromagnetic Radiation and Its Interaction with Matter

2.1.5.1 Nature of Electromagnetic Radiation

2.1.5.1.1 The Macroscopic Maxwell Equations

All macroscopic aspects of the statics and dynamics of the electromagnetic field in the presence of material media are described by Maxwell's equations. The differential form of these axioms in the international system of units (SI) or rationalized MKS system is given:

$$\underline{\nabla} \cdot \underline{D}(\underline{r},t) = \rho(\underline{r},t) \qquad \text{Gauss's Law} \tag{2.7}$$

$$\underline{\nabla} \cdot \underline{B}(\underline{r},t) = 0 \qquad \text{Gauss's Law} \tag{2.8}$$

$$\underline{\nabla} \times \underline{E}(\underline{r},t) = -\dot{\underline{B}}(\underline{r},t) \qquad \text{Faraday Law} \tag{2.9}$$

$$\underline{\nabla} \times \underline{H}(\underline{r},t) = \underline{D}(\underline{r},t) + \underline{J}(\underline{r},t) \qquad \text{Ampere's Law} \tag{2.10}$$

For a given position vector $\underline{r}(\mathrm{m})$ and a time $t(\mathrm{s})$ the Maxwell's equations couple the dielectric displacement vector $\underline{D}(\mathrm{C\,m^{-2}})$, the charge density $\rho(\mathrm{C\,m^{-3}})$, the magnetic induction vector $\underline{D}(\mathrm{T;T=V\,s\,m^{-2}})$, the electric field strength $\underline{E}(\mathrm{V\,m^{-1}})$, the magnetic field strength $\underline{H}(\mathrm{A\,m^{-1}})$ and the total current density $\underline{J}(\mathrm{A\,m^{-2}})$.

The response of the medium to the electric and magnetic fields may be expressed by the so-called constructive or material equations:

$$\underline{D}(\underline{r},t)=\varepsilon_0\underline{E}(\underline{r},t)+\underline{P}(\underline{r},t) \tag{2.11}$$

$$\underline{B}(\underline{r},t)=\mu_0\underline{H}(\underline{r},t)+\mu_0\underline{M}(\underline{r},t) \tag{2.12}$$

$\underline{P}$ is the dielectric polarization vector (electric dipole moment per volume, $\mathrm{C\,m\,m^{-3}=C\,m^{-2}}$) induced by the electric field, $\underline{M}$ is the magnetization vector (magnetic dipole moment per volume, $\mathrm{A\,m^{-1}}$) induced by the magnetic field, ε_0 is the vacuum permittivity ($\varepsilon_0=8.854\times10^{-12}\ \mathrm{C\,V^{-1}\,m^{-1}}$) and μ_0 is the vacuum permeability ($\mu_0=4\pi\times10^{-7}\ \mathrm{V\,s^2\,C^{-1}\,m^{-1}}$). $\underline{P}$ and $\underline{M}$ are linked with $\underline{E}$ and $\underline{H}$ through susceptibility tensor $\underline{\underline{\chi}}_e$ (electric susceptibility) and $\underline{\underline{\chi}}_m$ (magnetic susceptibility) respectively:

$$\begin{aligned}\underline{P}&=\varepsilon_0\underline{\underline{\chi}}_e\,\underline{E},\\ \underline{M}&=\mu_0\underline{\underline{\chi}}_m\,\underline{H},\end{aligned} \tag{2.13}$$

In an isotropic medium $\underline{\underline{\chi}}_e$ and $\underline{\underline{\chi}}_m$ may be expressed through scalar quantities χ_e and χ_m:

$$\begin{aligned}&\underline{D}=\varepsilon_0(\chi_e+1)\underline{E}=\varepsilon\underline{E};\ \varepsilon=\varepsilon_0\varepsilon_r;\ \varepsilon_r=\chi_e+1,\\ &\underline{B}=\mu_0(\chi_m+1)\underline{H}=\mu\underline{H};\ \mu=\mu_0\mu_r;\ \mu_r=\chi_m+1\end{aligned} \tag{2.14}$$

ε_r is the relative permittivity ('dielectric constant') and μ_r is the relative permeability. For diamagnetic substances $\mu_r\approx1$ (e.g.: for cyclohexane $\mu_r=0.999999373$).

2.1.5.1.2 Electromagnetic Waves

In any experiment with photons, light energy is transported in the form of electromagnetic waves. Equations for the electromagnetic waves result from the Maxwell's equations. For isotropic medium without free charge carriers ($\rho = 0$) and current density ($J = 0$) follows:[32]

$$\begin{aligned} \underline{\nabla} \times \underline{E} &= -\mu \underline{H}, \\ \underline{\nabla} \times \underline{H} &= \mu \underline{E} \end{aligned} \tag{2.15}$$

For a wave polarized in x-direction $E_y = 0$ and $H_x = 0$ (the polarization is determined by the direction of $\underline{E}$):

$$\Rightarrow \left(\frac{\partial}{\partial z} E_x\right) = -\mu \left(\frac{\partial}{\partial t} H_y\right) \quad \text{and} \quad \left(\frac{\partial}{\partial z} H_y\right) = \varepsilon \left(\frac{\partial}{\partial t} E_x\right) \tag{2.16}$$

$$\Rightarrow \frac{\partial^2}{\partial z^2} E_x = \varepsilon\mu \frac{\partial^2}{\partial z^2} H_y \quad \text{and} \quad \frac{\partial^2}{\partial z^2} H_y = \mu\varepsilon \frac{\partial^2}{\partial z^2} E_x \tag{2.17}$$

The above equations are second order differential equations with mixed variables and can be solved using separation method. The solutions are:

$$\begin{aligned} E(z,t) &= E_0 \underline{e}_x \cos(\omega t - kz + \varphi), \\ H(z,t) &= E_0 \underline{e}_y \cos(\omega t - kz + \varphi) \end{aligned} \tag{2.18}$$

where E_0 and H_0 are the amplitudes of the electric and the magnetic fields, and are not independent from each other.

$$H_0 = \left(\varepsilon_0 / \mu_0\right)^{1/2} E_0 = c_0 \varepsilon_0 E_0$$

2.1.5.1.3 Macroscopic Polarization and Molecular Polarizations

Susceptibilities characterize the medium response to the applied field. The field can be electric (electric susceptibility) or magnetic (magnetic susceptibility). We only consider the electric susceptibility.

For $M = 0$, $\rho = 0$ and $J = 0$ and we may express the wave equation as follows

$$\underline{\nabla} \times \underline{\nabla} \times \underline{E}(\underline{r},t) = -\mu_0 \frac{\partial^2}{\partial t^2}\left\{\varepsilon_0 \underline{E}(\underline{r},t) + \underline{P}(\underline{r},t)\right\} \tag{2.20}$$

- **Linear Susceptibility $\underline{\underline{\chi}}^{(1)}$**

Optically linear medium are characterized by linear response of the medium to the electric field. Considering an alternating electric field at position $\underline{r}$, which varies sinusoidally with time $\underline{E}(t)(\underline{E}(t) = \underline{E}_0 \cos(\omega t))$. In the electric dipole approximation, the dielectric polarization $\underline{P}(t)$ is created by local response in the medium.

$$P(t) = \varepsilon_0 \underline{\underline{\chi}}^{(1)}(-\omega;\omega) \cdot \underline{E}_0 \cos(\omega t) \tag{2.21}$$

Where the linear susceptibility $\underline{\underline{\chi}}^{(1)}(-\omega;\omega)$ characterizes the first order (linear) response of the medium and is frequency-dependent. The argument in parenthesis describes the nature of this dependence. In general $\underline{\underline{\chi}}^{(1)}$ is a second order tensor and is a 3×3 matrix (nine components). In an isotropic medium $\underline{\underline{\chi}}^{(1)}$ may be represented with a scalar quantity $\chi^{(1)}$, since only one independent component remains. For optically linear medium $\underline{\nabla} \cdot \underline{E} = 0$, and applying the identity $\underline{\nabla} \times \underline{\nabla} \times \underline{E} = \underline{\nabla}(\underline{\nabla} \cdot \underline{E}) - \Delta \underline{E}$:

$$\Delta \underline{E}(\underline{r},t) - \mu_0 \varepsilon_0 \left\{\chi^{(1)}(-\omega;\omega) + 1\right\} \frac{\partial^2}{\partial t^2} \Delta \underline{E}(\underline{r},t) = 0 \tag{2.22}$$

For a wave propagating in $+z$-direction in a weakly absorbing medium:

$$\underline{E}(\underline{r},t) = \underline{E}_0 \exp\left(-\frac{1}{2}\alpha z\right)\cos(\omega t - kz) \tag{2.23}$$

where α is the natural absorption coefficient and $k(k = 2\pi n/\lambda)$ is the magnitude of the wave vector. Both α and n represent the linear response of the medium and linked to the imaginary and real part of $\chi^{(1)}(-\omega;\omega)$:

$$\alpha = \frac{\omega \,\mathrm{Im}\left\{\chi^{(1)}(-\omega;\omega)\right\}}{c_0} \tag{2.24}$$

$$n = \sqrt{1 + \mathrm{Re}\left\{\chi^{(1)}(-\omega;\omega)\right\}} \tag{2.25}$$

The real and the imaginary part of the susceptibility $\chi^{(1)}(-\omega;\omega)$ are coupled through Kramers-Kronig relations:

2.1.6 Methods of Optical Spectroscopy

There are various spectroscopic methods used to study different aspects of a physical system. They are absorption, emission, scattering and reflection spectroscopies. UV/Vis and IR are categorized under absorption spectroscopy. Fluorescence, Phosphorescence, chemo-luminescence, Bioluminescence and thermo luminescence are the different forms of emission spectroscopes. Raleigh scattering (elastic, λ=constant), Raman-scattering (inelastic $\lambda \neq$ constant) are the two forms of scattering spectroscopy. Reflection spectroscopies are transmission, photo acoustic and photoelectron. In the following sections infrared, UV/Visible and fluorescence spectroscopy will be considered [33].

2.1.7 UV/Visible absorption spectroscopy

Electrons are promoted to a higher orbital by ultra violet or visible light. Ultraviolet and visible light extends in the spectral region from 10 nm to 780 nm of the electromagnetic radiation. When electromagnetic radiation interacts with a molecule, the oscillating electric field of the radiation

can in some cases disturb the potential energy of the molecule and allow it to escape from the initial stationary state. The electric field of the radiation oscillates at the point occupied by the molecule with a frequency v given in exponential form [34].

$$E_x = E_x^0(e^{i2\pi\nu t} + e^{-i2\pi\nu t}) \tag{2.26}$$

The electric field E_x can act on a dipole moment component μ_x to produce a change in energy. This term adds to the potential energy of the system and is responsible for a change in H that occurs.

$$H' = E_x\mu_x = E_x^0(e^{i2\pi\nu t} + e^{-i2\pi\nu t})\mu_x \tag{2.27}$$

Substituting this equation in induced quantum transition equation gives

$$\frac{d}{dt}a_m = -\frac{2\pi}{\mathrm{h}}E_x^0\int_{-\infty}^{\infty}\psi_m^*\mu_x\psi_l dx(e^{i2\pi/\mathrm{h}(\varepsilon_m-\varepsilon_l+\mathrm{h}\nu)t}e^{-i2\pi/\mathrm{h}(\varepsilon_m-\varepsilon_l+\mathrm{h}\nu)t}) \tag{2.28}$$

$$|\mu_{xlm}| = \int_{-\infty}^{\infty}\psi_m^*\mu_x\psi_l dx \tag{2.29}$$

Integration of equation (2.28) over the time interval 0 to t, one obtains

$$a_m(t) = |\mu_{xlm}|E_x^0\left[\frac{1-e^{i2\pi/h(\varepsilon_m-\varepsilon_l-h\nu)t}}{\varepsilon_m-\varepsilon_l+h\nu} + \frac{1-e^{i2\pi/h(\varepsilon_m-\varepsilon_l-h\nu)t}}{\varepsilon_m-\varepsilon_l-h\nu}\right] \tag{2.30}$$

Since absorption process are investigated, the energy of the m state is greater than that of the l state, and it is only necessary to retain the final term of equation (2.30)

$$a_m(t) = |\mu_{xlm}|E_x^0\left[\frac{1-e^{i2\pi/h(\varepsilon_m-\varepsilon_l-h\nu)t}}{\varepsilon_m-\varepsilon_l+h\nu} + \frac{1-e^{i2\pi/h(\varepsilon_m-\varepsilon_l-h\nu)t}}{\varepsilon_m-\varepsilon_l-h\nu}\right] \tag{2.31}$$

$$\begin{aligned} a_m^*(t)a_m(t) &= |\mu_{xlm}|^2 E_x^0\left[\frac{2-e^{i2\pi/h(\varepsilon_m-\varepsilon_l-h\nu)t}e^{-i2\pi/h(\varepsilon_m-\varepsilon_l-h\nu)t}}{(\varepsilon_m-\varepsilon_l-h\nu)^2}\right] \\ &= 4|\mu_{xlm}|^2\left(E_x^0\right)^2\left[\frac{\sin(\pi/h)(\varepsilon_m-\varepsilon_l-h\nu)t}{(\varepsilon_m-\varepsilon_l-h\nu)^2}\right] \end{aligned} \tag{2.32}$$

The above expression shows the effect of a given frequency of radiation. Integration over all frequencies, treating E_x as a constant since the absorption usually occur over a narrow frequency range, gives, with the definite integral.

$$\int_{-\infty}^{\infty} \frac{\sin^2 x}{x^2} dx = \pi \tag{2.33}$$

The result becomes

$$a_m^*(t)a_m(t) = \frac{4\pi^2}{h^2}\mu_{xlm}^2\left(E_x^0\right)^2 t \tag{2.34}$$

This is the absorption of a given molecular system. In terms of energy density, the three components of the radiation-dipole interaction are equal, and one writes.

$$\begin{aligned}\frac{d}{dt}\left(a_m^* a_m\right) &= \frac{8\pi^3}{3h^2}\left[(\mu_{xlm})^2 + (\mu_{ylm})^2 + (\mu_{zlm})^2\right]\rho \\ &= \frac{8\pi^3}{3h^2}\mu_{lm}^2\rho\end{aligned} \tag{2.35}$$

where the energy density $\rho = (6/4\pi)E_0^2$. Equation (2.35) is the rate of change of the system as a result of absorption of radiation under the perturbing effect of the electric field of the radiation. This is usually written as B_{lm} called Einstein's coefficient of induced absorption. This equation can be re-written as

$$\frac{d}{dt}\left(a_m^* a_m\right) = B_{lm}\rho;\ B_{lm} = \frac{8\pi^3}{3h^2}\mu_{lm}^2 \tag{2.36}$$

2.1.8 Infrared Absorption spectroscopy

Inferred radiation extends from780 nm to 1000μm of the spectral region. Molecular vibrations are excited by inferred radiations. A molecule can absorb energy from the oscillating field of a beam of infrared radiation if and only if it possesses an oscillating dipole. The frequency of

oscillation of the dipole must be the same as that of the applied electric field. All molecules except homonuclear diatomic have some vibrations that produce a change of dipole moment. These vibrations can be treated by classical mechanics using a simple ball and spring model, were as vibrational energy levels and transition between them are concepts taken from quantum mechanics [35]. Both approaches are useful infrared absorption spectroscopy.

2.1.8.1 Molecular vibration (classical mechanics)

- **Vibration of diatomic molecules**

The vibration of an organic molecule involving stretching of bonds can be described based on Simple Harmonic model. In this model, the atoms can be considered connected by a spring. Consider motion along the x-direction. The frequency of vibe ration is given by

$$\nu = \frac{1}{2\pi}\sqrt{\frac{k}{\mu}}; M = m_1 + m_2; \frac{1}{\mu} = \frac{1}{m_1} + \frac{1}{m_2} \tag{2.37}$$

μ is called effective mass. If one of the atoms is very light in comparison to the other for example, hydrogen ($m_2 = m_H$) coupled with oxygen or chlorine equation (2.37) is reduces to

$$\nu = \frac{1}{2\pi}\sqrt{\frac{k}{m_H}} \tag{2.38}$$

- **Vibration of tri atomic molecules**

For tri atomic molecules, in predicting the frequency of vibration of adjacent groups that have similar' diatomic' frequencies because such vibrations are mechanically coupled. In the case of such adjacent groups, two new vibrations will result, which can be described as in phase and out of phase combinations of the original two diatomic vibrations.

$$\bar{\nu}_1 = \tilde{\nu} = \frac{1}{2\pi c}\sqrt{\frac{k}{\mu}} \quad \text{(Symmetric)} \tag{2.39}$$

$$\tilde{\nu}_2 = \frac{1}{2\pi c}\sqrt{\frac{3k}{\mu}} \qquad \text{(Anti symmetric)} \quad \text{if } m_1 = m_2 \tag{2.40}$$

2.1.8.2 Molecular vibration (Quantum Mechanics)

As mentioned earlier, quantum mechanics accounts for vibrational energy levels and the transition between them. To calculate the energy levels we use time independent Schrödinger equation.

$$\hat{H} = -\frac{h^2}{8\pi^2\mu}\frac{d^2}{dq^2} + V \tag{2.41}$$

where μ is the effective mass, q is the vibrational coordinate, h is the Planks constant and V is a potential energy function.

$$V = \frac{1}{2}kq^2 \tag{2.42}$$

Schrödinger equation for the vibrational energy levels of a diatomic molecule is

$$-\frac{h^2}{8\pi^2\mu}\frac{d^2}{dq^2}\psi_v + \frac{1}{2}kq^2\psi_v = E\psi_v \tag{2.43}$$

The wave functions ψ of equation (2.43) are Hermite polynomials. The first two of which are:

$$\begin{aligned} \psi_{v=0} &= \left(\frac{\alpha}{\pi}\right)^{1/4} e^{-\alpha q^2/2};\ v = 0 \text{ the ground state,} \\ \psi_{v=1} &= \left(\frac{4\alpha^3}{\pi}\right)^{1/4} q e^{-\alpha q^2/2};\ v = 1 \text{ the first excited state} \end{aligned} \tag{2.44}$$

The energy levels of diatomic molecules are obtained by solving the preceding value of ψ_v in equation (2.44), which gives

$$E_v = h\nu(v+\frac{1}{2}) = \hbar\omega(v+\frac{1}{2}); v = 0,1,2,\cdots \quad (2.45)$$

The quantum mechanical treatment predicts a set of equally spaced levels with a separation between two levels of $h\nu$. For poly atomic molecules the energy levels are found

$$E(v_0, v_1, v_2, \vdots) = \sum_{i=1}^{3N-6} h\nu(v_i + \frac{1}{2}) \quad (2.46)$$

For large number of molecules we see that there are very large numbers of energy levels.

2.1.8.3 Transitions-Fundamentals, overtones, and combinations

Transitions between energy levels give rise to the observed infrared spectra. Most molecules are in the ground state (all v_i=0) at room temperature (Boltzmann distribution) and only for which $\Delta v = \pm$ 1 are allowed by the harmonic oscillator selection rules. Transitions described as fundamentals are those between the ground state and the levels for which only one of the $v_i = 1$. Transitions from the ground state to a state in which only one of the v_i=2, 3, and all others are zero, are known as overtones. Transitions from the ground state to a state for which v_i= 1 and v_j=1 simultaneously are known as binary combinations. Other combinations, with $v_i, v_j and v_k \neq 0$ are also possible. Overtones and combinations are not allowed, but may appear weakly because of anharmonicity or Fermi resonance. The intensities overtones and combinations in polyatomic molecules are usually very small because the values of the integrals are small compared with the integrals for the fundamentals.

2.1.9 Fluorescence Spectroscopy

Atoms or molecules that are excited to higher energy levels can decay to lower energy levels by emitting radiation (emission or luminescence). If the transition is between states of the same

spin, it is called Fluorescence and phosphorescence if the transition occurs between states of different spin. For simplicity, we use the term fluorescence to mean both fluorescence and phosphorescence [36].

2.1.9.1 Radiative and non-radiative transitions between electronic states

The Jablonski diagram is convenient for visualizing in simple way the possible processes: photon absorption, internal conversion, fluorescence, intersystem crossing, phosphorescence, delayed fluorescence and triple- triple transition. The singlet electronic states are denoted $S_0, S_1, S_2, \cdots$ and triple states; $T_1, T_2, T_3, \cdots$ vibrational levels are associated with each electronic state.

Emission of photons accompanying the $S_0 \leftarrow S_1$ relaxation is called fluorescence. Fluorescence spectrum is located at higher wavelength (low energy) than the absorption spectrum because of the energy loss in the excited state due to vibrational relaxation.

In general, the difference between the vibrational levels is similar in the ground and excited states, so that the fluorescence spectrum often resembles the first absorption band (mirror image' rule). The gap (expressed in wave numbers) between the maximum of absorption band and the maximum of fluorescence is called stocks shift. Stocks shift increases with solvent polarity.

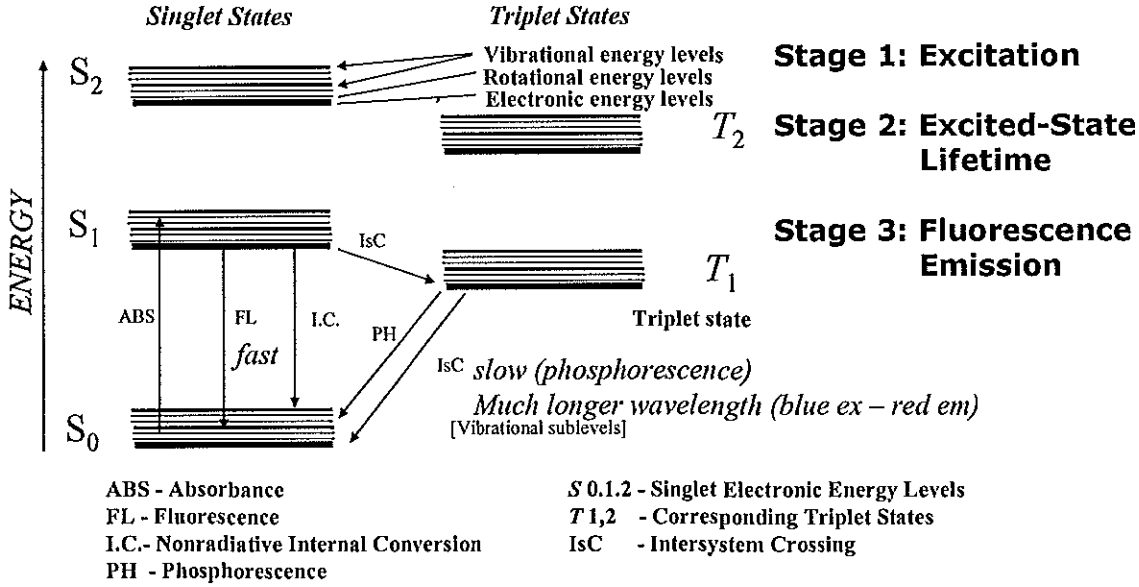


Fig. 2.1: Jablonski diagram and illustration of the relative position of absorption, fluorescence and phosphorescence

The characteristic times of the different processes: absorption $<10^{-15}$, Vibrational relaxation $10^{-12}-10^{-10}$, Lifetime of the excited state S_1 $10^{-10}-10^{7}$, Intersystem crossing $10^{-10}-10^{-7}$, Internal conversion $10^{-11}-10^{-9}$ and Lifetime of the excited state $10^{-6}-10^{0}$ s.

2.1.9.2 Quantum yield

The fluorescence quantum yield Φ_F is the ratio of the number of emitted photons (over the whole duration of the decay) to the number of absorbed photons.

$$\Phi_F = \frac{k_r^s}{k_r^s + k_{nr}^s} = k_r^s \tau_s . \tag{2.47}$$

With k_r^s the rate constant for fluorescence emission, $k_{nr}^s (k_{nr}^s = k_{ic}^s + k_{isc}^s)$ the rate constant for non-radiative deactivation, k_{ic}^s rate constant for internal conversion and k_{isc}^s rate constant for intersystem crossing.

Using the radiative lifetime, the fluorescence quantum yield can be written as.

$$\Phi_F = \frac{\tau_s}{\tau_r}; \tau_s = \frac{1}{k_r^s + k_{nr}^s}, \tau_r = \frac{1}{k_r^s} \tag{2.48}$$

where τ_s is the emission life time and τ_r is the non radiative life time.

2.1.9.3 Factors affecting fluorescence

Lifetime and quantum yields are characteristics of major importance. The larger the fluorescence quantum yield, the easier it is to observe a fluorescent compound, especially in the fluorescent probe. Many parameters can affect the quantum yields and life time: Temperature, an increase in temperature generally decrease in the fluorescence quantum yield and the life time because non-

radiative processes related to thermal agitation are more efficient at high temperatures. Other factors are PH, polarity, viscosity, and hydrogen bonding [37].

2.1.10 Fluorescence quenching and Solvantochromism

2.1.10.1 Fluorescence Quenching

Fluorescence quenching is a process, which decreases the intensity of the fluorescence emission. Quenching may occur by several mechanisms. Collisional quenching or dynamic quenching, static quenching by energy transfer, charge transfer reactions are the major quenching mechanisms.

The quenching agent (Q) can form complexes with either the ground state of the florescent molecule (static quenching) or with its excited state (via collusions during the excited state life time hence dynamic quenching). In either case, the quantitative result can be expressed by the Stern-Volmer equation.

$$F_0 - \frac{F}{F_0} = k(Q) \tag{2.49}$$

In the case of static quenching the constant k is related the equilibrium constant of the complex In the case of dynamic quenching k is the product of the rate constant of the quenching process and the natural fluorescence life time.

The deactivation or activation changes introduced in the fluorescing substance can be given by equation (2.50). Grynkiewicz *et al.* proposed an expression for the fluorescence enhancement produced by caffeine on Ca^{2+} [38]

$$\frac{\Delta F}{F_0} = \frac{\Delta\varepsilon_{\lambda,b}[Cf_t]}{\varepsilon_{\lambda,f}[D_t][Cf_t] + k_d^{Cf}} \tag{2.50}$$

Here $\Delta F(\Delta F = F - F_0, F_0 = \varepsilon_{f,\lambda}[D_t])$ is the fluoresce intensity in the absence of caffeine, and $K_d^{Cf}i$ is the apparent caffeine dissociation constant. The coefficients $\varepsilon_{\lambda,b}, \varepsilon_{\lambda,f}$ describe caffeine-bound and caffeine-free states, respectively.

Differential Scanning Calorimeter (DSC) measures heat flow to or from a sample as a function of temperature and time. The heat flow and temperature of the sample are monitored in comparison to the reference material. The amount of energy absorbed (endothermic) or evolved (exothermic) as the sample undergoes physical or chemical changes (e.g. melting, crystallization, curing) is measured in calories as a function of the temperature change. Any material reactions involving change in heat capacity (e.g. glass transition) are also detected. DSC can be performed from ambient to 1500 °C

DSC used to determine melting temperature, heat of fission and crystallization, entropies of fusion and crystallization, thermal stability, specific capacity and glass transition temperature, analysis of polymer bends and copolymer, determine cure temperature for thermally cured polymer, reaction rate and temperature evaluation. TGA performed simultaneously with DSC a lot of information can be extracted about the thermal properties of a material under investigation [40].

Figue2.3 illustrates the thermal properties of calcium oxalate. It is a very good example to explain the thermal properties of substances. As can be seen from the figure, different components are driven out at each stage with an increase in temperature. The straight-line shows thermally stable regions, from these results it is possible to analyze the amount of each component indicated. The temperature at which reaction takes place between each reactant, decomposition temperature and the chemical formula of each component can be found.

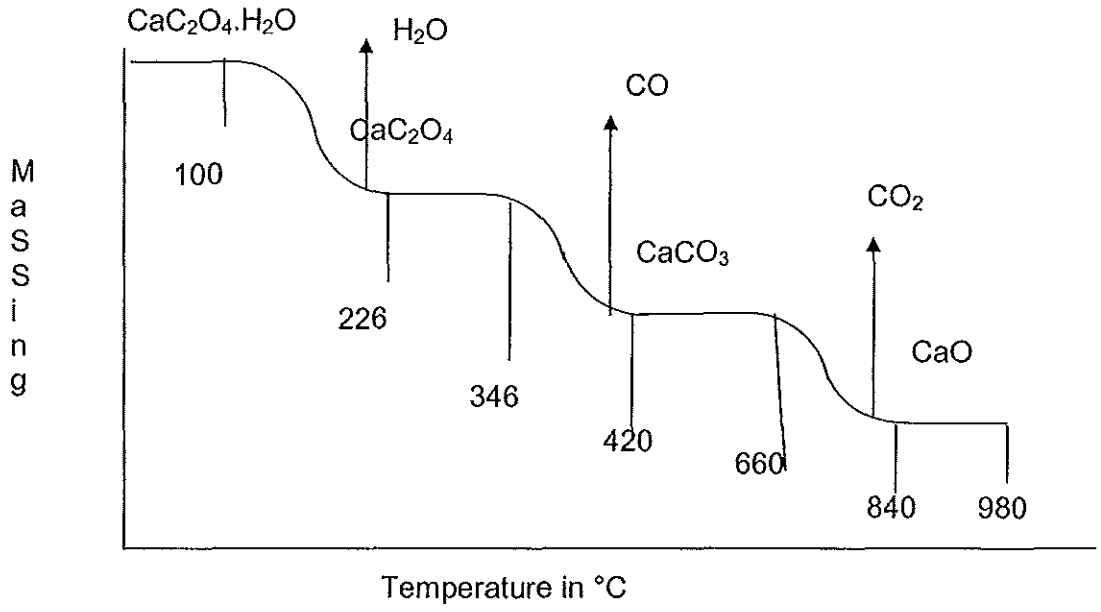


Fig 2.2 A thermo gram of calcium oxalate.

3. Experimental Part

In this chapter, equipments, materials and chemicals used for the experiment are presented. Further, sample preparation, experimental procedures, and theoretical backgrounds used to analyze the data are also discussed.

3.1 Equipments

For the study of the thermal and optical properties of coffee and caffeine, five instruments were used. For the study of thermal properties Thermal Gravimetric Analysis (TGA) and (DSC) were used. UV/ Vis absorption spectroscopy, FT-IR absorption spectroscopy and Fluorescence spectroscopy were employed to study the optical properties. In the following sections brief descriptions of the equipments are given.

3.1.1 UV/Visible Spectrophotometer

UV/ Visible spectrophotometer is an instrument used to measure absorption in the region between ultraviolet to visible part of the electromagnetic radiation. In this experiment computer-interfaced SPECTRONIC® GENESIS™ 2PC spectrophotometer with a wavelength specification 200-1100 nm, accuracy ±1nm and absorbance percentage ±1 was used.

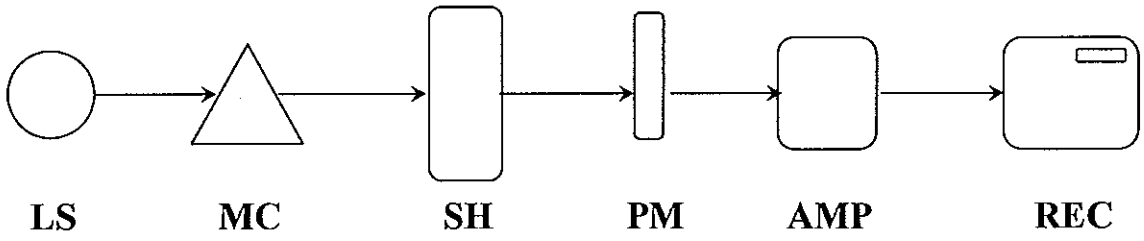


Fig. 3.1: Schematic diagram of UV/Visible Spectrophotometer. Light source (LS), monochromator (MC), sample holder (SH), photomultiplier (PM), Ampl ifier (AMP) and recorder (REC).

There are two types of light sources for ultraviolet and visible light. The electrical excitation of deuterium or hydrogen at low pressure produces a continuous UV spectrum in the range of 160-375 nm. Tungsten filament is employed as a source of visible light. This lamp is used in the wavelength range of 350-2500 nm. Polychromatic radiation from the light sources passes

through a wavelength selector (monochromator) through the entrance slit. Radiation of only one wavelength leaves the monocromator through the exit slit. The monochromatic light linearly polarized by the polarizing prism and pass through the sample holder. The sample holder is called cuvette. A cuvette must be transparent in both spectral regions. Quartz or fused silica cuvettes are required for spectroscopy in the UV region while plastic cuvettes can be used in the visible region of the radiation. The modulated signal is detected by the pohtomultiplier and then feed to a phase sensitive amplifier and then to the recorder [41].

3.1.2 Infrared Spectroscopy

Infrared spectroscopy is a very powerful tool for the determination of molecular structure. Direct information about the presence of functional groups is immediately available from an infrared spectrum, and comparison of the infrared spectrum of an unknown material with a reference spectrum, or with the spectrum of a known compound, can give an empirical proof of the intensity of the unknown substance. The normal infrared range will be assumed betwen 4000-650 cm^{-1}. Many infrared spectrometers, however, cover a wide range; overlapping the far infrared to 400 or even 200 cm^{-1} . In this experiment computer interfaced Perkin Elmer spectrum Bx FT-IR spectrophotometer in the range of 4000-400 cm^{-1} was used.

3.1.3 Fluorescence spectroscopy

Fluorometers achieve 10^3-5×10^5 times better limits of detection as compared to spectrophotometers. Fluorometers are highly specific and less susceptible to interference because it is rare that a non- target compound to absorb and emit the same wavelength of light as target compound . There are two primary kinds of instruments that measure fluorescence: filter fluorometers and spectroflorometer.

In this experiment computer interfaced spectrofluorometer, Model RF-5301PC (light source-150 W xenon lamp) wavelength range 220-900 nm and zero order light (EX & EM) slit with 1.5/3/5/10/15/20/30 nm (emission side) wave length truth ± 1.5 nm, sensitivity High/ Low selectable were used.

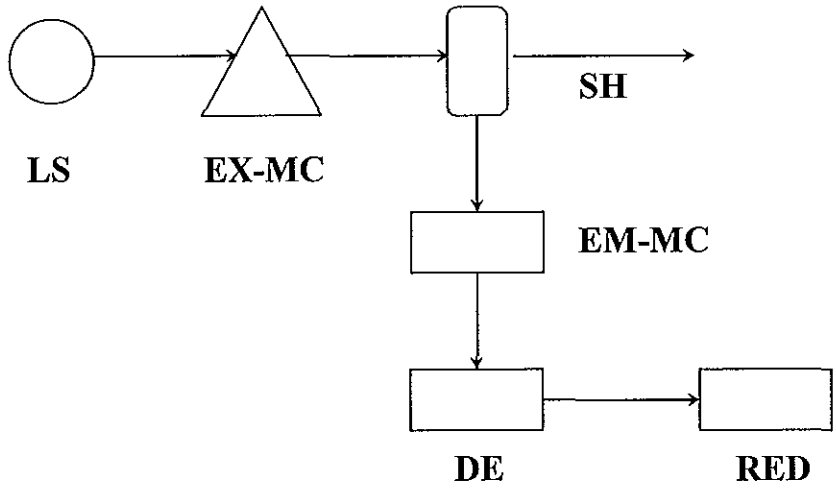


Fig. 3.3: Schematic diagram of fluorescence spectroscopy. Light source (LS), excitation monochromator (EX-MC), sample holder (SH), emission monochromator (EM-MC), detector (DE), read out (RED)

The lamp or light source provides the energy that excites the compound of interest. Xenon lamps are very versatile and powerful, providing the light out put from 190-1200 nm. The excitation monochromator is used to screen out the wavelengths of light not absorbed by the target compound. The monochromator allows a selected band of light energy to pass through and excite the sample; it blocks other wavelengths, especially in the emission spectrum .The sample cell or cuvette holds the sample of interest. The cuvette material must allow the compound's absorption and emission light energy to pass through. Cuvettes are made from borosilicate or quartz glass as

well as various plastics that can pass the selected wavelength of light.1 cm square plastic cuvettes was used in this experiment.

Stray light, Rayleigh and Raman scatter is emitted from the sample. In addition, stray back ground light may be present that has not passed through the sample. The emission monochromator, screen out these components allowing primary wave lengths of light specific to the compound to pass through. The light detector is most often a photo multiplier tube, through photodiodes are increasingly being used. The light passing through the emission monochromator is detected by photomultipliers or photodiodes. The light intensity, which is directly proportional (linear) to the compound's concentration, is registered as a spectrum.

A photomultiplier tube (PMT) contains a material which crates an anode current proportional to light intensity. A silicon photodiode (PD) converts incident light into an electric current [43].

3.2 Materials and chemicals

The materials and chemicals were used in the optical and thermal analysis of coffee and caffeine are: n-Hexane (GLC major pick 995 BDH Limited Pool, England), carbon tetrachloride, dichloromethane (99.6% A.C.S, reagent, ALDRICH), chloroform (Gehalt lassy, GC 99.0-99.4%-Rledle-dellaen), de-ionized water, Electrical beam balance for measuring mass of coffee powder (SA.120, number 14510,a 10^{-4} g level, BOULDER company), Mechanical beam balance for measuring mass of caffeine (M5.SA, number 6500355, micro gram level), electric- motor grinder for grinding the coffee beans, 250 μm sieve (England),magnetic stirrer, glass filter, filter paper, Quartz and plastic cuvettes, lab boy, beakers, flasks, separatory funnel, funnel, pipates and micro pipates.

3.3 Samples

Two different types of sample were used in this experiment: coffee beans and caffeine. Caffeine powder (Evan Pharmaceutical Company, England). Coffee bean sample (Yirga Cheffe washed export standard) collected from Ethiopian coffee and tea quality and liquoring center on 16/02/2005.

3.4 Experimental Methods

3.4.1 Solvent Effect on the UV/Vis absorption band of Coffee and Caffeine.

Five different solvents were used: they are distilled water, n-Hexane, Carbonteterachloride, dichloromethane and chloroform. Stock solution of Caffeine dissolved in each solvent was prepared. The concentration of each solution was controlled by UV/Vis absorption spectrometer. The absorption was made to fall with in 1-1.5 on the absorption scale. The absorption spectra found for each solution was collected.

- **Sample Preparation with Coffee Powder.**

Stock solution of coffee powder dissolved in water, dichloromethane and chloroform were prepared. Each solution was filtered using glass filter. The concentration was controlled using UV-Vis- absorption spectrometer. The absorption for each solution was made about 1 on the absorption scale. All the absorption spectra found for the three solutions were collected.

3.4.2 The Quenching Effect of Caffeine and Dissolved Components of Coffee on The Fluorescence Spectrum of Fluorescein

A 167 mg caffeine powder was dissolved in 10.20 ml of distilled water which gives concentration of the order of (c=84.766 mM) .Similarly, fluorescein dissolved in water was also prepared. The concentration of fluorescein solution was adjusted using UV-Vis absorption spectroscopy at about 1.6 on the absorption scale. By keeping 2 ml of fluorescein solution in a plastic cuvette using micropipate, the fluorescence spectrum of fluorescein was taken. This serves as a base line for the first measurement. The first measurement was taken by poring 1 ml of caffeine solution (84.766 mM) on top of the fluorescein solution .The response of fluorescein to caffeine was determined as a function of caffeine concentration between 0 and 84.777 mM. 1 ml of caffeine at different concentration mixed with 2 ml of fluorescein solution was used at each stage.

3.5.3 The effect of fluorescein on the IR-spectrum of coffee and caffeine.

The four IR-spectrums found caffeine in chloroform, coffee in chloroform, caffeine in chloroform plus fluorescein in chloroform and coffee in chloroform plus fluorescein in chloroform solutions will be compared. The structure observed in each IR-spectrum and the effect of caffeine and coffee interaction with fluorescein will be interpreted.

3.5.4 TGA, DSC Results

The two separate graphs plotted one for DSC the other for TGA results were analyzed. Each graph found was divided into different regions of similar physical phenomenon. The regions found at different points of temperature were interpreted.

4. Results and Discussion

Based on the experimental measurements and procedures described in the previous sections, the results obtained are presented in this chapter.

4.1 Solvent dependence of the position of the electronic bands

The UV- absorption spectra of caffeine, dissolved in five different solvents are shown below. All the spectra of caffeine are recorded between 235 and 350 nm and the absorption maximum normalized at 1.234. The solvents are n-Hexane, carbon tetrachloride, chloroform, dichloromethane and water.

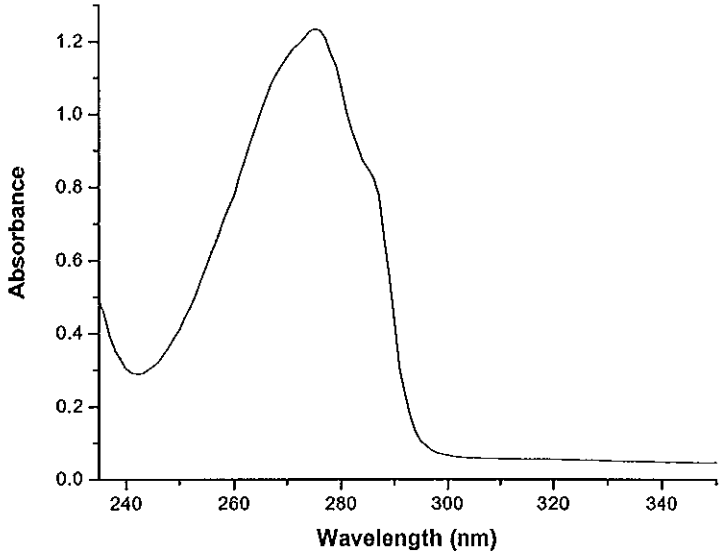


Fig. 4.1: UV/Vis absorption spectrum of caffeine in *n*-Hexane.

The absorption spectrum of caffeine in n-Hexane exhibited practically no absorption above 300 nm and strong absorption below 300 mn. The band gap extends between 241-300 nm. The absorption spectrum showed an intense absorption band at 275 nm, which is due to $\pi \rightarrow \pi^*$ electron transition. As can be seen from Figure 4.1 the spectrum curves in to the absorption band in the region between 282-290 nm. This is due to vibrational progression as expected for a polar molecule dissolved in non-polar solvent. In the region below 241 nm shows the region of solvent absorption. The absorption increases with a decrease in wavelength.

From the equation described in section (3.5.1) for polar dissolved molecule, a relationship between the dielectric constant and the wave number of the maximum absorption spectrum can be set. The graph-plotted $\tilde{\nu}^{sol}$ verses $(\varepsilon_r - 1)/(2\varepsilon_r + 1)$ below indicate a linear relationship between the wavenumber at maximum absorption and the dielectric constant of the solvents used. This relationship holds true for carbon tetrachloride, chloroform and dichloromethane. Water and n-Hexane fall out of the linear relationship. This is because water forms strong hydrogen bonding.

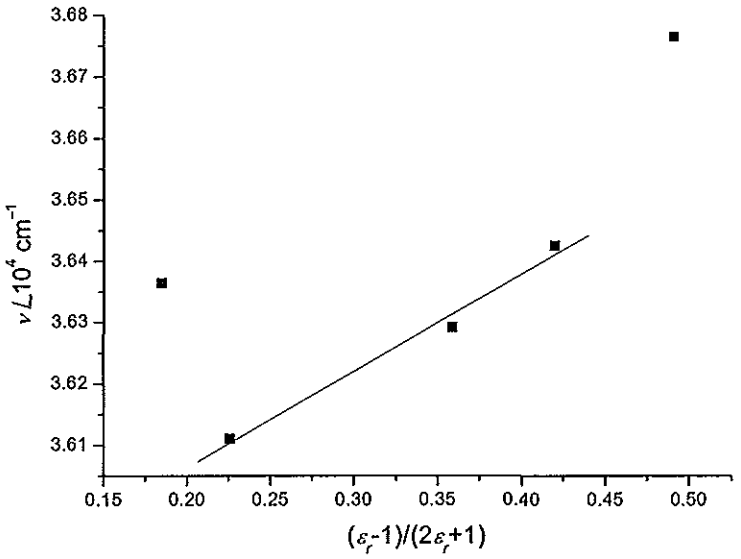


Fig. 4.6: A plot of the wave number $\tilde{\nu}^{sol}$ against $(\varepsilon_r - 1)/(2\varepsilon_r + 1)$ of caffeine dissolved in different solvents

The vertical-axis intercept is equal to the absorption maximum of caffeine in gaseous state (expressed in wave number). This value is obtained by extrapolating the points where the linear relationship between the wave number at maximum absorption and the dielectric constant of the solvent applies. This value is found to be 3.5748× 10^4 cm^{-1} (279.7 nm). This implies caffeine in gaseous state absorbs at greater wavelength than in solution. Further, the shift in $\lambda_{max}(\tilde{\nu})$ is not significant as would be expected for a charge transfer transition with larger $\Delta\mu$. This indicates the transition is not accompanied with a strong charge transfer, i.e. the excitation is a local excitation one. This is illustrated in a normalized graph figure 4.7 shown below.

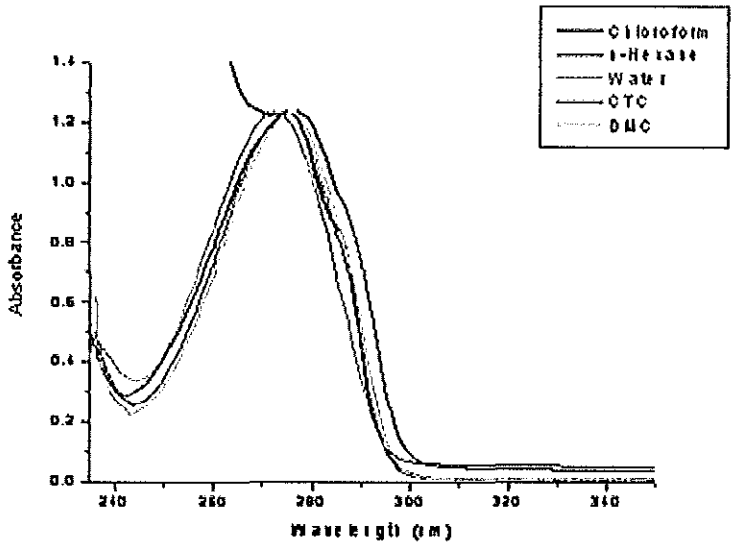


Fig. 4.7: The normalized spectrum of caffeine dissolved in carbon tetrachloride, chloroform, dichloromethane, *n*-Hexane and water.

On the bases of the observations and experimental results found above and the caffeine solubility data collected from literature (45), three solvents were chosen for the quantitative study of caffeine in coffee using UV-Vis absorption spectroscopy. The results of the experiment are shown below. All the spectra are in the region between 235-350 nm and normalized at the absorption maximum of 1.4 nm.

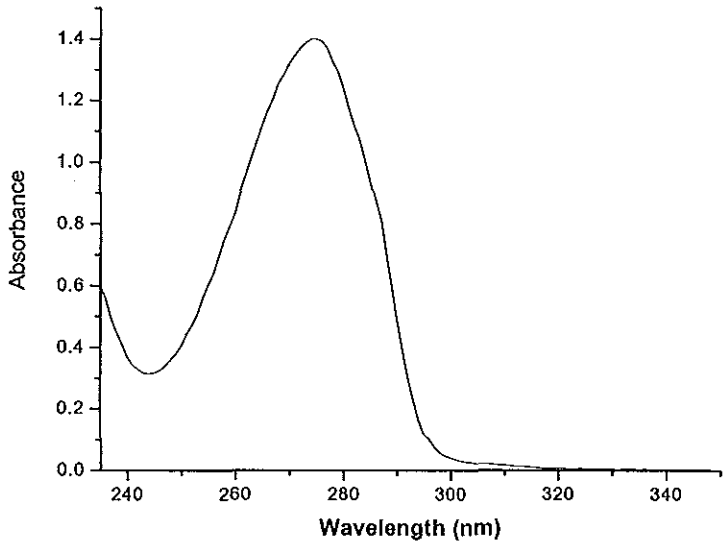


Fig. 4.8: UV/Vis absorption spectrum of coffee in dichloromethane solution.

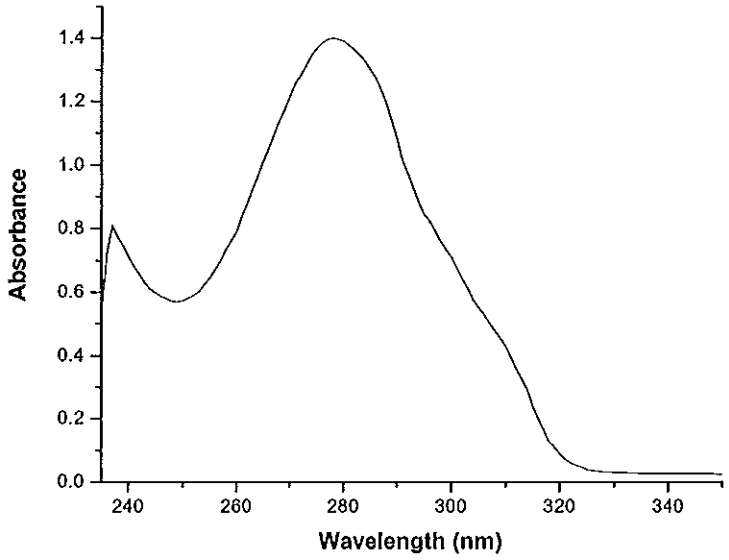


Fig. 4.9: UV/Vis absorption spectrum of coffee in Chloroform.

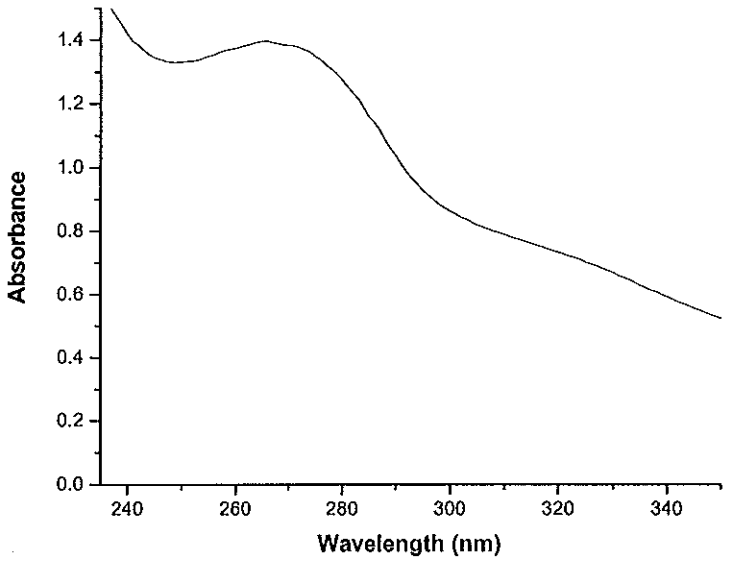


Fig. 4.10: UV/Vis absorption spectrum of coffee in water.

From the three UV-absorption spectrums shown above, the caffeine-dichloromethane band shown (Figure 4.8) indicates a relatively clear Gaussian curve; other dissolved components of coffee have almost no interfering effect on the caffeine band. The caffeine-chloroform UV-absorption band shown (Figure 4.9) is asymmetrical Gaussian cure and as seen at the base of the

band the spectrum encountered an interference effect due to the spectrum of other dissolved components of coffee. Water is the cheapest and easily available solvent. It is preferable to use water in the experiment. Figure 4.10 shows the UV- absorption band of caffeine in water. As can be seen from the graph the caffeine spectrum is highly interfered by the bands of other components. This indicates it is practically impossible to use water as a solvent for the quantitative study of caffeine in coffee using UV- Visible absorption spectroscopy [46]. From the experimental results shown above dichloromethane is recommended for the quantitative study of caffeine in coffee by UV-Vis absorption spectroscopy.

4.2 Study of caffeine content in coffee by fluorescence spectroscopy.

The other method employed to study the amount of caffeine content in coffee beans was fluorescence spectroscopy. As pointed out earlier, it is preferable to use water as a solvent especially, when routine measurements are required. The technique involves here is the selective excitation of the band needed (caffeine) by varying the excitation wavelength. Most commonly, excitation is made at 15 nm less than the maximum absorption wavelength to avoid the interference of Rayleigh scattering. By this method the caffeine spectrum will be resolved from the overlapping bands (figure 4.10) and a separate caffeine band can be achieved. This used for the comparative determination of caffeine in different coffee beans.

The first step was to see whether caffeine is fluorescence or not. No fluorescence spectrum of caffeine was observed. Therefore, it is impossible to measure caffeine amount using direct fluorescence intensity.

Following this result, another method was employed to measure the amount of caffeine in coffee. It was an indirect method using fluorescence deactivation of fluorescein first by caffeine then by dissolved and filtered coffee solution and then after setting a relationship between the two. The results of the experiment are as indicated in Figure.4.11 and 4.12.

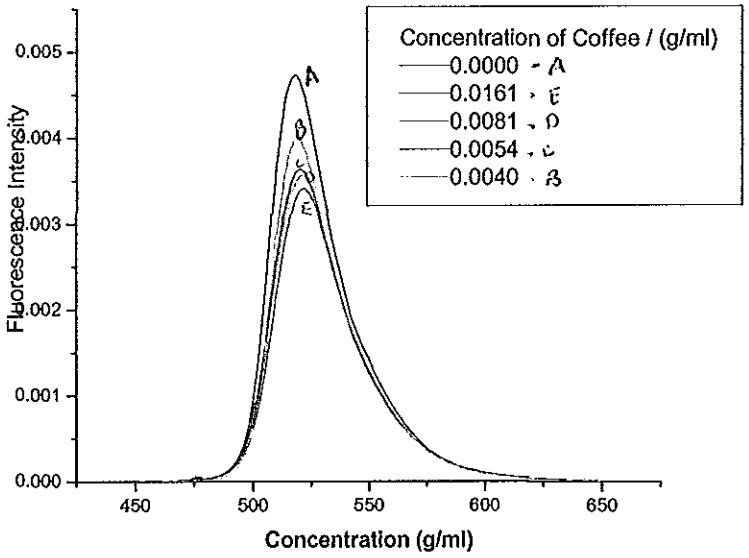


Fig. 4.11: Normalized quenched fluorescence spectrum of fluorescein in water. The spectra are de-activated by different concentration of caffeine

The fluorescence measurement figure 4.11 reveals that 84.766 mol/cm^3 caffeine reduces the intrinsic dye fluorescence by 22 % accompanied with a small wavelength $\lambda_{max,em}$ shift from 518 nm to 522 nm. Similar effect was observed [38]. The increase in $\lambda_{max,em}$ indicates there is an electronic structure alteration of fluorescein.

Figure 4.12 documents the maximum change induced by dissolved components of coffee (2.5×10^{-3} g/ml) on the fluorescence spectra of fluorescein. It reduces the dye fluorescence intensity by 29.69%.

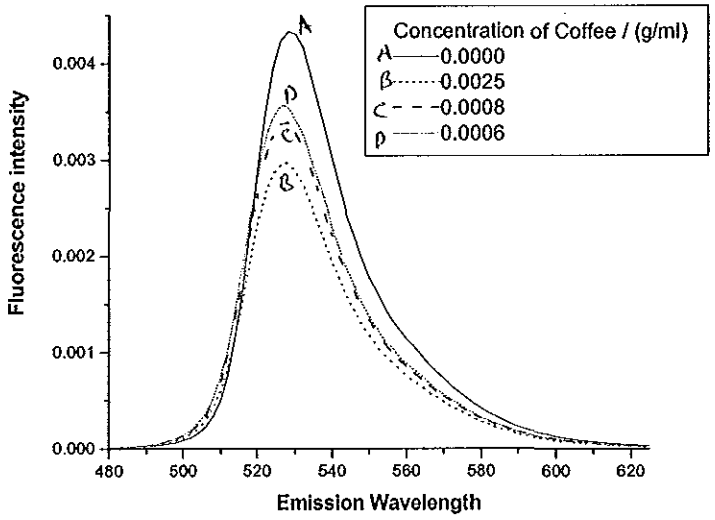


Fig. 4.12: Normalized quenched fluorescence spectrum of fluorescein-water solution at different concentration of coffee.

Caffeine of mass 167.9 mg was dissolved in 10.20 ml of de-ionized water (C= 84.766mole/ml). 16.766 g/ml.1 ml of this solution added on top 2 ml fluorescein. 1 ml of this solution mixed with 1 ml of de-ionized water (C=42.383 ml/cm^3, 8.0686 g/ml). 1 ml of caffeine solution mixed with 2 ml of de-ionized water (C=28 mole/cm^3 = 5. 379 g/ml). The fourth sample was 1 ml caffeine solution + 3 ml of water (C=21.192 mole/cm^3=4.0343 g/ml).

Tab. 4.3: Change in fluorescence spectrum and the corresponding concentration of caffeine and coffee.

Change in Fluor. intensity; Caffein	Conc. Caffein / mol/cm^3	Mass Caffeine / (g/ml)	Change in Fluor. intensity; Coffee	Mass Coffe / (g/ml)
0.2197	84.766	0.0161	0.6326	0.005
0.1854	42.383	0.0081	0.4203	0.0025
0.167	28.225	0.0054	0.2425	0.0008
0.087	21.192	0.0040	0.1801	0.0006

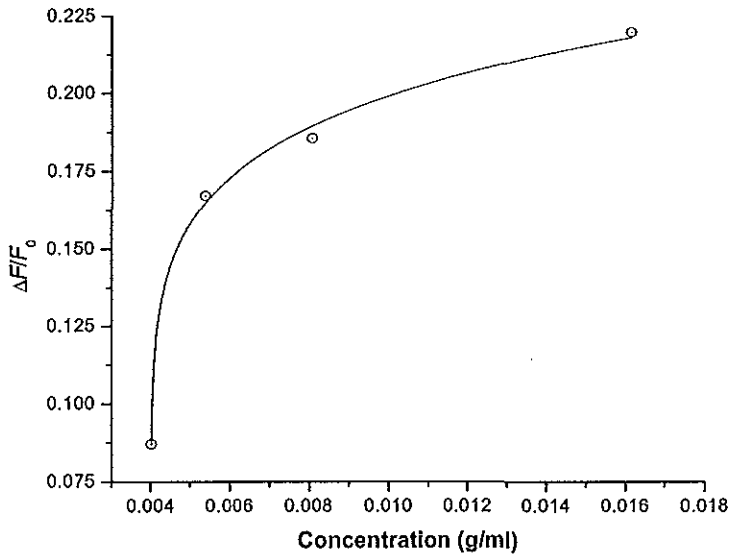


Fig. 4.13: Change in fluorescence intensity verses concentration of caffeine.

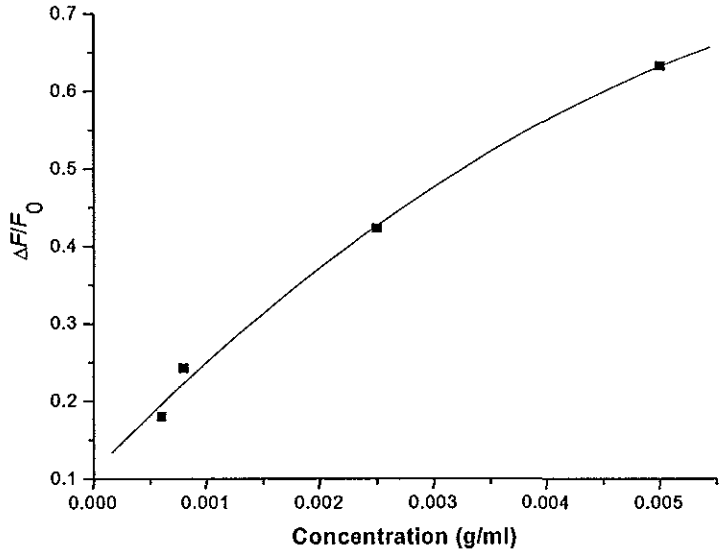


Fig. 4.14: Change in fluorescence intensity against concentration of coffee.

Comparing the two figures 4.13 and 4.14 it is apparent that the stronger quenching effect is produced by small concentration of the soluble components of coffee than caffeine. This indicates other soluble components have stronger quenching effect than the caffeine that is found

in coffee. Therefore, it is not possible to tell the amount of caffeine in coffee beans by correlating the two graphs.

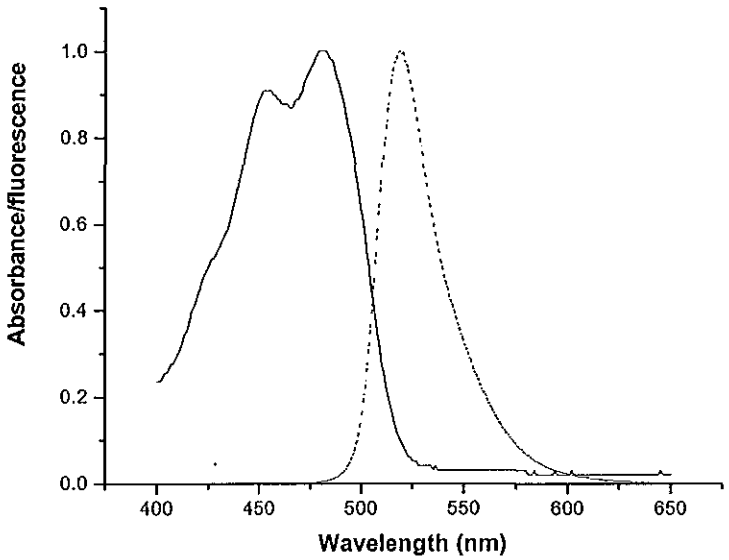


Fig. 4.15: Absorption (——) and fluorescence (----) spectrum of fluorescein.

The absorption and fluorescence spectra of fluorescein spectra are illustrated in figure 4.15.The structure shown at the pick of the absorption band is due to vibrational progression. The band gap for absorption band is wider than the fluorescence. The linear distance between the two picks is called stocks shift. The stock shift increases with solvent polarity.

4.3 A structure responsible for the deactivation of the fluorescent dyne

As can be seen in the theory part, according to selection rule, overtone and combinations of overtone are not allowed. Even if they appear, the intensities are very week. Figure 4.16 shows the IR-spectrum of caffeine, coffee, caffeine+fluorescein and coffee+fluorescein solutions. In this graph the structure of these compounds are seen. The major sharp picks are found at 760 cm^{-1}, 1224 cm^{-1}, 1718 cm^{-1}, 2417 cm^{-1}, and 3026 cm^{-1}. These picks are observed for both caffeine and coffee. These are –C-Cl, -C-N- (amines), -C=O (carbonyl), P-H phosphinesans and CH stretches. Out of these structures, as can be seen from the figure the carbonyl band found both in coffee and

caffeine decrease in absorbance. Sharp IR- spectra were seen between 1650-1870 nm, which is a carbonyl structure[47]. In the figure 4.17, the structure of this band is shown clearly.

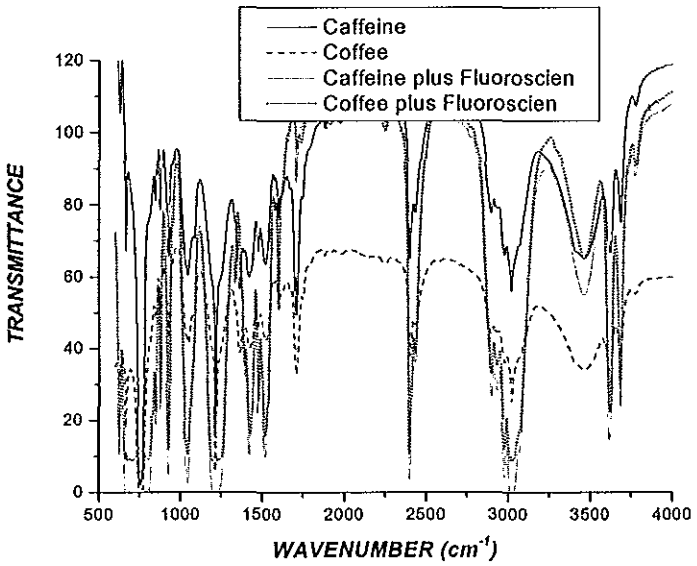


Fig. 4.16: The normalized IR-spectrum of caffeine, coffee, Caffeine plus fluorescein and coffee and fluorescein in water.

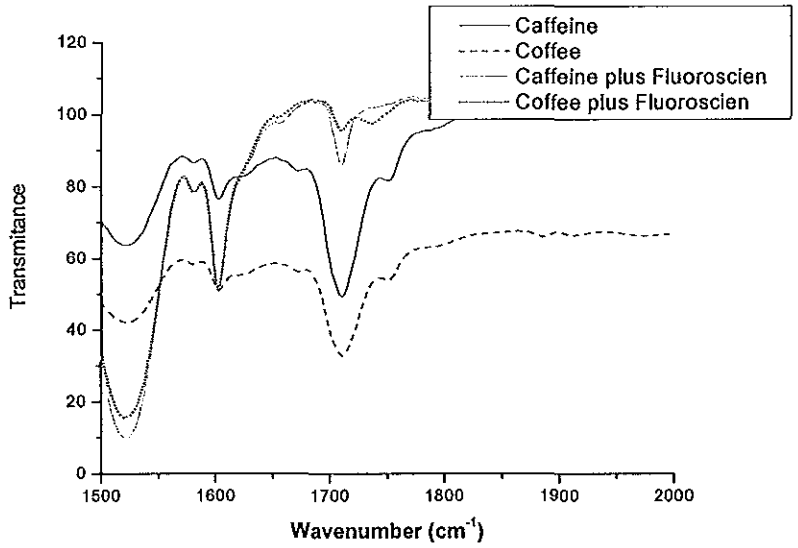


Fig. 4.17: The normalized IR-spectrum of caffeine, coffee, Caffeine plus fluorescein and coffee plus fluorescein in water.

Figure 4.17 the strong carbonyl absorption band shown. The strong carbonyl band was observed both in pure caffeine and coffee. The absorption intensity decreases for both caffeine and coffee

when interacted with fluorescing dyne. This indicates strong interaction of the carbonyl structure with the fluorescent dyne. This also explains the 4 nm shift in $\lambda_{max,em}$ observed in fluorescence spectra of fluorescein when caffeine was added.

4.4 Thermal properties of coffee

Figure 4.18 records the TGA result of a10 mg green powder coffee. As can be seen from the graph the mass of coffee changes progressively with an increase in temperature. In the region, A from 26.4 °C to 194 °C there is a steady decrease in the mass of coffee due to the removal of water. The mass change is 6.958% (powdered green coffee). Literature value is 8-12% (for green bean) [44]. In region, B from 194 °C to 393 °C 61% of the mass of coffee is removed while in the third part, C from 393 °C to 786 °C 28% disappear. The component of coffee removed in these regions is beyond the scope of this thesis. As can be seen from the graph, there is no thermally stable position (a continuous mass change). Therefore, it is not possible to tell exactly the amount of caffeine or other components in a given temperature region.

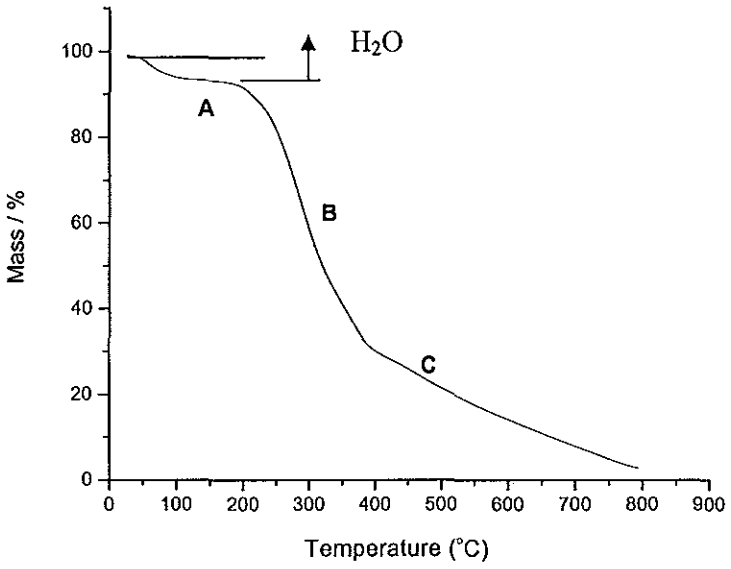


Fig. 4.18: Mass change in percent of green powder coffee against temperature.

Figure 4.19 documents the DSC result of grounded coffee. In region A, 27-148 °C the sample crystallizes (heat is released from the sample). In region B of the figure, from 294 –372 °C

heat is absorbed. The two picks (region C) 370-540 °C melting occurs. The sample gives off heat in region D (630-770 °C). TGA result shows that coffee undergoes a continuous change in its mass for a continuous change in temperature. The DSC result indicates different state changes and heat absorption and release by the sample at different temperature regions.

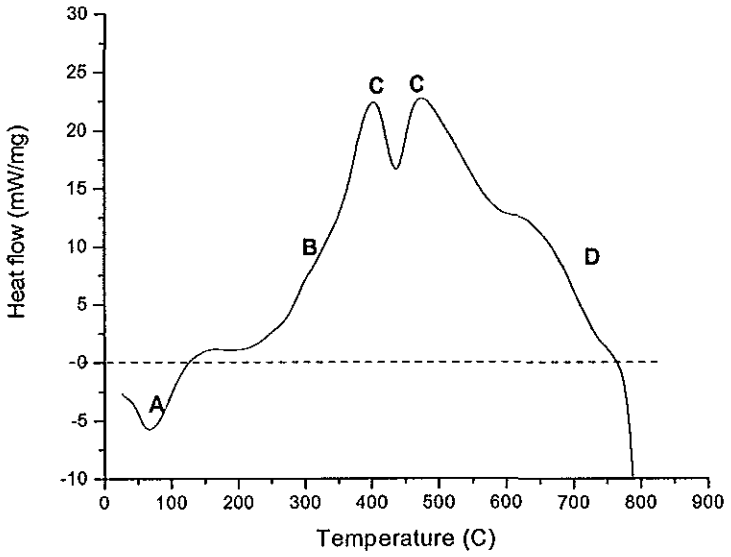


Fig. 4.19: Heat flow against temperature of powder green coffee.

5. Conclusion

The optical and thermal properties of coffee and caffeine were investigated. The objectives of the study were to select an appropriate solvent used to study the caffeine content in coffee beans by electronic spectroscopy and solventochromic effect of caffeine in different solvents. To make comparative study of the caffeine content of the different coffee beans by fluorescence spectroscopy., to observe chemically active structures found both in coffee and caffeine and the structural changes occurred due to the introduction of host compound by FT-IR spectroscopy and the thermal properties of coffee by both TGA and DSC. The following conclusions are drown from the results of the experiment.

Five different solvents were used in the investigations. Since the concentration of caffeine related to the area under the integral absorption, the shape of the absorption band and its intensity determine the results. Clear cut single absorption band was observed for with negligible interference effect of other matrix elements for dichloromethane. Therefore, dichloromethane is a recommended solvent for the quantitative study of caffeine in coffee using UV-Vis spectroscopy.

It was observed that caffeine exhibited no fluorescence emission spectrum. An indirect approach was made to produce a comparative study of the caffeine content by correlating fluorescence quenching produced by soluble components of coffee and caffeine. The experiment result showed strong quenching that can not be assigned for caffeine solely, and there are other components in coffee which have fluorescence deactivation like caffeine. This made the quantitative determination of caffeine, with out the detail knowledge of all the components impossible. However, quantitative mechanistic study of deactivation was possible, for this purpose IR-Spectra was taken.

The FT-IR study revealed strong carbonyl bands found in both coffee and caffeine. This structure was seen to change its absorption intensity when both coffee and caffeine added in fluorescein. This is an indication that carbonyl is strongly interacting with the host compound.

TGA results of coffee shows that the mass of coffee continuously decreases with an increase in temperature .The mass change was relatively slow up to 200 °C, the change in mass was very high above 200 °C.The result also reveals that coffee is thermally unstable, which makes temperature dependent analysis of caffeine content unrealistic. DSC results record that different thermal phenomenon that undergone by coffee; crystallization, heat absorption, melting and heat releasing with an increase in temperature. In the future detailed

analysis on coffee components is necessary so as to fluorescence spectroscopy determine the caffeine content.

6. References

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