TIME DEPENDENCE OF CAFFEINE IN COFFEE USING OPTICAL METHOD

A Thesis

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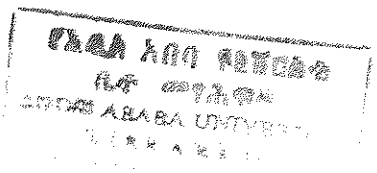
The School of Graduate Studies

The Faculty of Science



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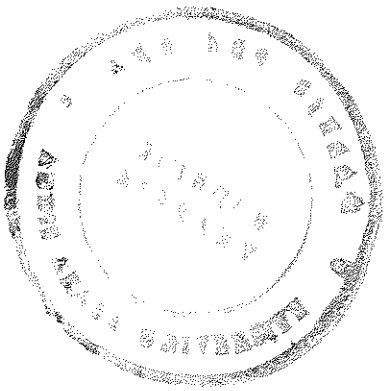

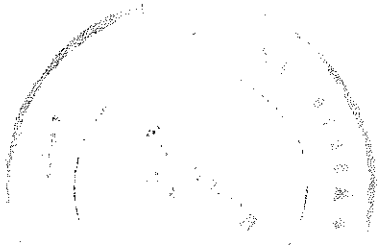

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Abstract

In this project optical properties of pure caffeine, i.e., time dependency of caffeine in coffee powder and solution kept under different conditions and temperatures were inyestigated. For the pure caffeine molar decadic absorption coefficient and transitional dipole moment were computed and found to be (1095 ± 37) m^2 mol^{-1} and (11.3 ± 0.2)·10^{-30} C m. Respectively in all the systems and temperatures caffeine in coffee was investigated , a decrease in caffeine concentration as a function of time was observed. For the solution both in closed and open storage conditions the pseudo zero order kinetics was observed. The rate constant of decomposition $k_d = 2.41667\text{x}10^{-5}$ mol m^{-3} hr^{-1} for closed system and $k_d = 5.50417\text{x}10^{-5}$ mol $m^{-3}hr^{-1}$ for open system were determined. The linear regression coefficient values were above $R = 0.99$ in both cases. The half-life time of caffeine were 1177 hr and 517 hr for closed and open systems, respectively. In the contrary, for open powder system *the* kinetics obeyed the expected first order rate expression. For open powder coffee the monoexponential fits resulted in rate constant of decay at room temperature (23 °C) and in ambient atmosphere is computed to be $k_d = 2.4\times10^{-4}$ hr^{-1} and half-life time of caffeine is 289 hr, which implies again chemical reaction during half-life was fastest for powder form storage than other conditions. Reaction rate was faster, as expected, for storage at 23 °C than 8 °C.

Key words: Caffeine, Coffee, Spectrophotometer, Pseudo Zero-Order, Monoexponential fit

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1. Introduction

Coffee is a tropical plant, which requires for its growth temperature of less than 30 °C; annual rain falls of greater than 1500 mm and a deep slightly acid and well-drained loam soil [1]. It is highly traded commodity next to petroleum and one of the most popular drinks with enormous commercial and social importance [1].

The history of coffee has been and still is an agenda of great controversy among many plant scientists. Some attach its origin with African and strongly argue in favor, while other denies the fact of its indigenousity to Africa. Major source of controversy was the "Arabica" coffee due to the "miss-namer" of its qualifying species name "Arabica" which gave a lee-way for some to argue that this qualifying term was to indicate its origin. According to Harris, the term coffee derives from Kaffa (a region in South Western Ethiopia) where the flower was first discovered blossoming [2].

Raw coffee beans are the seeds of plants belonging to the Rubiaceae family, which comprises at least species of the genus coffee. The two species that are commercially exploited are coffee Arabica, which accounts for two thirds of world production, and coffee canephora, often-called Robusta coffee, with one third of global output. Robusta coffee plants and all wild coffee species have 22 chromosomes, whereas Arabica has 44. Therefore, Arabica and other coffee species cannot be crossed to produce a hybrid plant [2].

Robusta is a high – yielding and disease- resistant tree standing up to 12 meters tall that grows best in warm, humid climes. It has elevated caffeine content that ranges from 2.4 to 2.8 percent by weight. Although many purveyors sell Robusta, it does not give rise to the highest quality coffee [2].

Arabica, which originated in the Ethiopia highlands, is a medium-to low – yielding, rather delicate tree five to six meters tall that requires a temperate climate and considerable growing care. Coffee made from Arabica beans has an intense, intricate aroma that can be reminiscent of flowers, fruit, honey, chocolate, caramel or roosted bread. Its caffeine content never exceeds 1.5 percent by weight. Because of its superior quality and taste, Arabica sells for a higher price that it is hardy [3].

With in any area, broadly designated as coffee production, there is always a wide variation in suitability, not only from farm to farm, but also from land to land within individual farm. Suitable condition for coffee production depends on the basic environmental variables namely climate (temperature, light and water) and soil. These ecological factors have a major effect on coffee because the trees cannot grow if a certain number of conditions are not met [3].

Coffee production through out Ethiopia can be put in to two categories based on their level of production as major and minor coffee growing regions.

Illubabor, Wellega, Kaffa, Sidamo, Gedeo and Harrarge are the major coffee growing regions in Ethiopia, since their coffee contribution is 95% of the total coffee production in Ethiopia. Arssi, Shoa, Gojjam, Gonder and Wollo are where coffee is not produced as major production or in a large scale as the previous ones at least and if they produce, it only satisfies the local consumption. This region of the country is considered minor coffee growing regions [4].

Coffee is a very important crop for the least developed countries in Africa. For some of them it is vital. In countries like Ethiopia, Burundi, central Africa Republic, D.R. Congo, Madagascar, Rwanda, Sierra Leone, Togo and the United Republic of Tanzania, coffee is one of the main export goods. The performance of the coffee sector in these countries affects the overall performance of their exports and their economic growth [4]. The long term trend in coffee prices has been poor and today coffee prices are at their lowest for the last 30 years for a brief period during the mid's 90's, coffee prices recovered and were instrumental in helping coffee exporting Least Developed countries (LDCS) improve export and economic performance. Unfortunately, coffee price collapsed again after 1997.

Although there is little to suggest that, overall, the coffee market will recover to previous levels, there are still opportunities for LDC is exporting to the world coffee market. To realize these opportunities, export development policies and programs are required to reduce the impact of shocks provoked by the vagaries of the international coffee market. These policies and programmes should aim to increase return to producers, improve the quality of their products, position themselves in attractive market

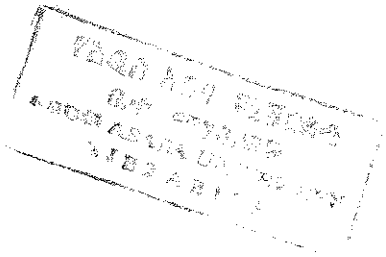
segments and encourage the processing of coffee in their countries by foreign and local companies [4].

Coffee is sold green, roasted processed into soluble form. Most least developed countries export coffee in its green form. Global least developed exports of green coffee during the period 1995 to 1999 [5], while their exports of roasted coffee and of coffee soluble and extracts did not exceed 0.1% of the total world trade respectively. Processing even simple roasting is not prevalent in LDCS.

About half the LDC countries produce and export mostly Robusta coffee, with the exception of Ethiopia, Yemen and Benin which cultivate and export Arabica varieties, and Rwanda, Burundi and Tanzania- which export both Arabica (over 80% total exports in quality) and Robusta coffees] [5].

Although about half of all LDC countries produce and export coffee, a few only are significant world exporters. The largest exporters of green coffee are Uganda (32%), Ethiopia (24%), Tanzania (9%), DRC (7%), Burundi (6%), and Madagascar (6%).
Coffee producing countries consumes just about a quarter of the world output. The significant coffee producers and consumers are Brazil (the world largest coffee producer and exporter followed by Colombia and Vietnam) [6], Columbia, Costa Rica, Ethiopia, Mexico, Venezuela, India and Indonesia. Their domestic consumption averaged over 24.6 million bags. About 76% of world import trade invalid consisted of green coffee, 13% were extracted coffee and 11% was roasted coffee [7] .The total annual world production of green coffee is about six million tons; with an estimated cost of six milliard us dollars.
The world's largest coffee consumers are developed and industrialized developing countries. The European Union is the largest consuming region. German, France and Italy are the largest individual EU importers. More than 7% of coffee consumed in EU is decaffeinated coffee. Japan and US are again good importers [8].

Coffee and tea consumption are wide spread in the United States, with more than 50% of the population using these beverages. According to the national coffee association, the average cup of coffee or tea in the United States is reported to contain between 40 and 150 mg caffeine [9] although specialty coffees may contain much higher doses [10]. About 54% of adults in the US drink coffee with average per capital daily consumption of 1.9 cups for men and 1.4 cups for women [11]. Coffee is a beverage that



is consumed daily worldwide and is one of the most popular beverages in the world. Because of its popularity and wide use, there are numerous studies concerning its safety and implications to health [12].

Although coffee consumption has been studied in relation to the risk of several diseases, the reproduceability and validity of the information on its intake, as obtained by a food frequency questionnaire (FFQ). Thus, information on coffee, decaffeinated coffee and tea provided by this FFQ is a satisfactory, reliable and valid measure of intake of these beverages for the purpose of epidemiological inference [12].

Coffee contains other active compounds rather than caffeine formed during roasting procedures that could contribute to the in vitro genotoxic effect of coffee [13]. Coffee is one of the most widely consumed psychoactive beverages in our society [14]. It is a well-known and extensively utilized psychotropic agent with effects on mood, cognitive performance, and motor activity [15]. Coffee is a complex mixture of potential "neutriceuticals" coffee's chemical composition is determined by a complex interaction of agricultural factors, roasting, blending, and brewing. The main constituents of coffee in order of their abundance typical values for the water soluble constituents are phenolic polymers (pulp) 8%, poly saccharides 6%, chlorogenic acids 4%, minerals 3 %, water 2%, caffeine 1%, organic acids 0.5%, sugars 0.3%, lipids 0.2%, and aroma 0.1%. [16]. The discrimination between coffee beverages from pure Arabica, pure Robusta and blends of these two varieties was investigated by near- infrared (NIR) reflectance spectroscopy, the basis for this discrimination appears to involve caffeine and / or other alkaloids [16].

Out of this coffee's constituents the psychoactive effects of caffeine have been well documented [17,18]. Caffeine is the world's most popular drug. The white, bitter tasting, crystalline, substances found in coffee, tea, cola drinks, chocolate etc. caffeine was first isolated from coffee in 1820 and first isolated from Tea leaves in 1827 and named "theine" because it was believed to be a distinctly different compound from the caffeine in coffee. Tea leaves contain about 3.5% caffeine, but a cup of tea usually contains less caffeine than a cup of coffee, because much less tea than coffee is used during preparation [19].

Caffeine taken in beverage form begins to reach all tissues of the body within five minutes. Peak blood levels are reached in about 30 minutes. Half of a given dose of caffeine is metabolized in about four hours and more rapidly in smokers and less rapidly in new born infants, in women in late pregnancy, and in sufferers from liver disease. [20]. Normally, almost all ingested caffeine is metabolized less than 3% appears unchanged in urine, and there is no day –to-day accumulation of the drug in the body [21].

A potentiometer enzyme sensor can be used successfully for the direct detection of caffeine in coffee beverage samples [22]. It has been estimated that adults living in western societies have an average all sources daily caffeine in take of about 200-300 mg [22], even though coffee represents the vast majority of caffeine intake [23,24].

There have been an increasing number of investigations that have focused in individual differences in caffeine reactivity. Some authors suggest that expectancies about the psycho stimulant effects of caffeine may play apart in the behavioral responses to caffeine, i.e. expectancies could play a role in the individual variability in caffeine reactivity [25,26].In addition, more recent study suggests that no sex differences in the pharmaco-kinetics of caffeine, as measured in salivary concentration of caffeine using high- performance liquid chromatography [27,28].

Caffeine in widely distributed in pharmaceutical preparations and beverages. The need for a fast and selective determination method is obvious, especially when routine determinations are required. For the determination of caffeine in beverages, various analytical techniques including titrimetry, spectrophotometer, paleography, GC, HPLC and derivative spectrometer have been reported [29,30,31].

In order to avoid fussy separation procedures, some researchers used mathematical processing to determine caffeine (CF) by UV or IR to eliminate the interference. Although this method could improve selectivity, mean while, it caused the reduction of sensitivity [32], assuming that factors that can affect the nature of caffeine spectra during experiment like environmental variables fluctuate and fluctuation in voltage is controlled [33,34].

There has been considerable interest in the effects of caffeine on human performance and mood. The majority of studies on caffeine intake and sleep have examined this substance used near bed time, whereas coffee is more often taken in the

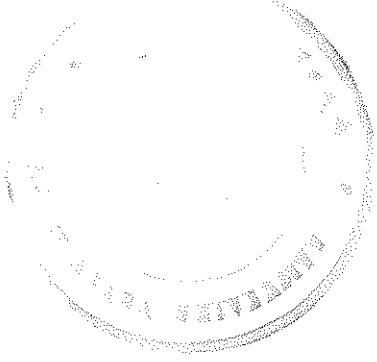
morning or early after noon, e.g. to counteract sleep inertia in the morning or a post-lunch dip in alertness [35,36].

Results have often shown that caffeine increases alertness and improves the ability to maintain attention [37,38]. The most common medicinal use of caffeine is as apart of head ache pain curer tablet preparations and other pain relievers. Caffeine is added both for its specific ability to relieve headache, including that caused by caffeine with drawal [39]. The ability of caffeine to stimulate breathing is used in the treatment of apnea (cessation of breathing) in newborn babies and as an antidote against the depression of breathing by over doses of heroin and other opiate drugs. More controversial therapeutic uses of caffeine are these to kill skin funguses; to improve sperm mobility, to enhance the toxic effects of chemicals used in cancer therapy and to facilitate the production of seizures during electrovusive therapy [40]. It has been again reported that chronic consumption of caffeinated coffee or decaffeinated coffee reduces insulin secretion [41], in other way of saying caffeinated and decaffeinated coffee consumption might prove to be an effective strategy for reducing insulin resistance, especially in over weight women.

In light of recent findings caffeine consumption are negatively associated with the risk of diabetes [42]. There is promise that these beverages, or some of their constituents, may prove useful in their development of techniques for the prevention or treatment of diabetes.

In contrast to this caffeine is thought to increase alertness, reduce sleep propensity and produce adverse effects in the central nervous system, such as insomnia. Infact, many investigators have shown that the adverse effect of coffee is mostly due to caffeine, which has powerful effects on a wide range of organ systems.

Patients with insomnia are hyper aroused, which prevents them from falling asleep and maintaining sleep. The hyper arousal theory is further supported by reports that some individuals with insomnia avoid taking caffeine or caffeine-containing beverages. The main pharmacological action of caffeine is the blockade of adenosine receptors [43]. Epidemiological studies suggests that regular consumption of caffeine is associated with adverse cardiovascular out comes [44]. Within 50 minutes, oral intake of a common caffeine dose corresponding to two cups of coffee (200 mg) significantly



decreases bicycle exercise and is even more pronounced during exposure to simulated altitude [45].

Caffeine has been linked to various adverse pregnancy out comes, including fetal loss, birth defects, and fetal growth retardation, thus, coffee consumption during pregnancy has been subject to preventive action in some countries. Caffeine is rapidly absorbed by the digestive system. It crosses the placenta freely, which implies that caffeine concentration in the fetus is the same as those in the mother's plasma.

Moreover, the fetus has low levels of enzymes that metabolize caffeine. Caffeine increases levels of cellular cyclic adenosine monophosphate, which may influence cell development [46]. Approximately, 90% of the caffeine in a cup of coffee is absorbed from the stomach within 20 minutes with peak plasma concentrations occurring approximately 40-60 minutes later [46].

Lastly, coffee has been studied as a risk factor for neurological disorders, and is associated with a reduced risk of Parkinson's disease [46,47]. Eventhough consumption of coffee in women does not appear to increase the risk of developing hypertension, both sugared cola and diet cola beverages were associated with and increased risk of hypertension. Hence, it is possible to speculate that it is not caffeine but perhaps some other compound contained in soda- type of soft drinks that may be responsible for the increased risk in hypertension [47].

Finally, some investigators have reported that caffeine and /or heavy caffeine intake may serve as risk factors for life style- related diseases including heart disease and osteoporosis as well as periodontal diseases, which are intimately associated with nutrition exercise, alcohol, smoking, and several other life style factors [47] .

Concern over the potential risk of uncontrolled caffeine intake and its adverse effects resulted in recommendation that certain sections of the human population should reduce caffeine consumption. This prompted coffee companies to develop and market decaffeinated coffee. A German chemist, Runge in 1820, achieved the first successful extraction of caffeine from coffee beans [48]. Since then, coffee consumers always have had the choice of normal and / or decaffeinated coffee.

Commercial decaffeinating of coffee commenced in Germany early in the twentieth century and a little later in USA. The adverse side effects of caffeine have

increased the market for decaffeinated coffee to about 10-12 % of coffee consumption world wide, despite the loss of key flavor compounds in the industrial decaffeinating process.

Decaffeination of coffee by chemicals changes the taste and aroma of coffee. Moreover risks of chemicals are involved in this process so for this and other reasons, scientists start to search for naturally decaffeinated plant. Brazilian scientists discovered a naturally, decaffeinated plant, which is modified one than the artificially decaffeinated coffee already on the market from coffee Arabica plant of Ethiopia, a species normally recognized for the high quality of its beans. Brazilian researcher bred 300 Ethiopian coffee plants as part of the program to produce low caffeine strains. They found three bushes all derived from the same plant that was virtually caffeine free [48].

The other hot news on scientific literature on "Coffee" at recent time is investigation of properties of coffee and caffeine including time dependence of caffeine and coffee. Investigation about time dependence of coffee is pre-knowledge to half-life determination.

Time dependence of caffeine and coffee can be analyzed from a kinetic standpoint with focus on the effect of oxygen (O_2), water activity (a_w) and temperature on the acceleration of deterioration. Optical half-life decreases with the increase in percentage of oxygen, water activity and temperature.

Among the constituents of coffee, the most extensively studied is caffeine, under the assumption that it is responsible for the effects of coffee. Thus, it might selected to be right active ingredient for quantitative as well as qualitative prediction of time dependence of complex coffee system.

It has been reported that many analytical methods have been developed for the investigation of time dependence of caffeine half-life time of caffeine in coffee. For the investigation of a time dependence as well as half life time caffeine in coffee and other dry beverages various analytical techniques including HPLC, FTIR spectroscopy, Digital image processing, Astree electronic tongue, TTI, weibull Hazard analysis, Algorithms have been reported [48]. Most of the reported studies suffer from disadvantages such as sensory evaluation, which is affected by individual differences in which the result is not reproduce able and not valid.

Of all these methods, spectroscopic method is simple, sensitive, rapid, producible, valid and the most suitable for on line monitoring. Even though in the past the application of optical instruments was not efficient and not widely implemented due to their expensiveness. These days the rapid development of new techniques based on diode array, fiber optics and charged coupled devices create comfortable conditions to use optical instruments.

At present, there is a need of investigation of caffeine as function of time associated with the factors affecting reaction rate of caffeine in coffee and standard procedures suitable for time dependence study and determination of half-life of caffeine in coffee using spectroscopic methods.

Thus, the purpose of this study is to provide standard procedures for investigation of time dependence of caffeine and coffee such as half-life and to investigate characterization of caffeine as function of time associated with factors affecting reaction rate of coffee using optical methods.

2. Theory

2.1 Spectroscopy

2.1.1 Introduction

The word spectroscopy is derived from the Latin word "spectron" and means "ghost or spirit". According to IUPAC spectroscopy is defined as the study of physical systems by the 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 quanta of the electromagnetic quanta (photons)[49].

$$M \xrightarrow{h\nu} M^{*} \qquad (2.1)$$

According to Born-Oppenheimer (BO) approximation the electronic, vibrational, rotational and translational energies are considered to be independent of each other, i.e. transitions between different energy levels may occur independently [50]. A change in the total energy of a molecule could then be written as

$$\Delta E_{\text{total}} = \Delta E_{\text{electronic}} + \Delta E_{\text{vibrational}} + \Delta E_{\text{rotational}} + \ldots \qquad (2.2)$$

The approximate orders of magnitude of these changes are

$$\Delta E_{\text{electronic}} \approx 1000 \cdot \Delta E_{\text{vibrational}} \approx 1000000 \cdot \Delta E_{\text{rotational}} \qquad (2.3)$$

While rotational spectra are observed only on molecules that have a permanent dipole moment and 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.

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

$$E_{ph} = h\nu = hc_0\tilde{\nu} = \frac{hc_0}{\lambda} = \hbar\omega = \hbar c_0 k \qquad (2.4)$$

where h is the Planck's constant c_0 is the velocity of electromagnetic radiation in free space and k is the magnitude of the wave vector.

2.1.2 Molecule-Electromagnetic Field Interaction

The interaction electromagnetic radiation (EMR) with matter (atom or molecule) can be conveniently describe in a semi-classical way; molecule-quantized and EMR-classical. Using the so called 'electric dipole approximation' the perturbing Hamiltonian $\hat{H}^{(1)}(t)$, which results from the interaction of EMR with matter, may expressed as:

$$\hat{H}^{(1)}(t) = -\hat{\mu}\cdot E = -\hat{\mu}\cdot E_0 \cos\omega t \qquad (2.5)$$

where $\hat{\mu}$ is the dipole operator and $\underline{E}$ the electric field vector. From the above equation result that for a spectroscopic transition must the Bohr frequency criteria, which states

that to the energy of the electromagnetic radiation $\hbar\omega_{eg}$ should be equal the energy difference between the transitional states of the molecule E_{eg}

$$\Delta E_{eg} = \hbar\omega_{eg} \tag{2.6}$$

and the electric transitional dipole moment μ_{eg} must be different from zero

$$\mu_{eg} = \int \psi_e^* \hat{\mu} \psi_g d\tau = \langle e|\hat{\mu}|g\rangle \neq 0 \tag{2.7}$$

2.1.3 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[51] is given:

$$\nabla \cdot \boldsymbol{D}(r,t) = \rho(r,t) \tag{2.8}$$

$$\nabla \cdot \boldsymbol{B}(r,t) = 0 \tag{2.9}$$

$$\nabla \times \boldsymbol{E}(r,t) = -\frac{\partial}{\partial t}\boldsymbol{B}(r,t) \tag{2.10}$$

$$\nabla \times \boldsymbol{H}(r,t) = \frac{\partial}{\partial t}\boldsymbol{D}(r,t) + \boldsymbol{J}(r,t) \tag{2.11}$$

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

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

$$\nabla \cdot \boldsymbol{D}(r,t) = \varepsilon_0 \boldsymbol{E}(r,t) + \boldsymbol{P}(r,t) \tag{2.12}$$

$$\nabla \cdot \boldsymbol{B}(r,t) = \mu_0 \boldsymbol{H}(r,t) + \mu_0 \boldsymbol{M}(r,t) \tag{2.13}$$

$\boldsymbol{P}$ is the dielectric polarization vector (electric dipole moment per volume, C m m^{-3} = C m^{-2}) induced by the electric field, $\boldsymbol{M}$ is the magnetization vector (magnetic dipole moment per volume, A m^{-1}) induced by the magnetic field, ε_0 is the vacuum permittivity ($\varepsilon_0 = 8.8542 \cdot 10^{-12}$ C V^{-1} m^{-1}) and μ_0 is the vacuum permeability ($\mu_0 = 4\pi \cdot 10^{-7}$ V s^2 C^{-1} m^{-1}). $\boldsymbol{P}$ and $\boldsymbol{M}$ are linked with $\boldsymbol{E}$ and $\boldsymbol{H}$ through susceptibility tensor χ_e (electric susceptibility) and χ_m (magnetic susceptibility) respectively:

$$\boldsymbol{P} = \varepsilon_0 \chi_e \boldsymbol{E} \tag{2.14}$$

$$\boldsymbol{M} = \mu_0 \chi_m \boldsymbol{H} \tag{2.15}$$

In an isotropic medium the χ_e and χ_m may be expressed through scalar quantities χ_e and χ_m. Thus, combining the Eqs. one gets

$$\boldsymbol{D} = \varepsilon \boldsymbol{E}; \quad \varepsilon = \varepsilon_0 \varepsilon_r; \quad \varepsilon_r = \chi_e + 1 \tag{2.16}$$

$$\boldsymbol{B} = \mu \boldsymbol{H}; \mu = \mu_0 \mu_r; \mu_r = \chi_m + 1 \tag{2.17}$$

ε_r is the relative permittivity ('dielectric constant') and μ_r is the relative permeability. For diamagnetic substances $\mu_r \approx 1$.

2.1.4 Electromagnetic Waves

2.1.4.1 Electromagnetic Waves in Free Space

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[51]. For isotropic medium without free charge carriers ($\rho = 0$) and current density ($J = 0$) follows:

$$\nabla \times E = -\mu_0 \frac{\partial}{\partial t} H \tag{2.18}$$

$$\nabla \times H = \varepsilon_0 \frac{\partial}{\partial t} E \tag{2.19}$$

Considering a plane wave propagating in the $+z$-direction and x-polarized the above equations can be solved and the possible solutions are:

$$E(z,t) = E_0 \vec{e}_x \cos(kz - \omega t + \varphi) \tag{2.20}$$

$$H(z,t) = H_0 \vec{e}_y \cos(kz - \omega t + \varphi) \tag{2.21}$$

where E_0 and H_0 are the amplitudes of the electric and the magnetic fields. The two equations are the wave equations in free space.

2.1.4.2 Electromagnetic Waves in Medium

In this work only optically linear medium considered. Optically linear medium are characterized by linear response of the medium to the electric field. Considering an alternating electric field at position r, which varies, sinusoidally with time

$E(t)$ ($E(t) = E_0 \cos(\omega t)$). In the electric dipole approximation, the dielectric polarization $P(t)$ is created by local response in the medium.

$$P(t) = \varepsilon_0 \chi^{(1)} \cdot E_0 \cos(\omega t) \tag{2.22}$$

Where the linear susceptibility $\chi^{(1)}$ characterizes the first order (linear) response of the medium and is frequency-dependent. The argument in parenthesis in equ. 2.22 above describes the nature of this dependence. In general $\chi^{(1)}$ is a second order tensor and is a 3×3 matrix (nine components). In an isotropic medium $\chi^{(1)}$ may be represented with a scalar quantity $\chi^{(1)}$, since only one independent component remains. For optically linear medium $\nabla \cdot E = 0$, and applying the identity $\nabla \times \nabla \times E = \nabla(\nabla \cdot E) - \Delta E$:

$$\Delta E - \mu_0 \varepsilon_0 \left(\chi^{(1)} + 1\right) \frac{\partial^2}{\partial t^2} E = 0 \tag{2.23}$$

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

$$E = E_0 \exp\left(-\frac{1}{2}\alpha z\right) \cos(\omega t - kz) \tag{2.24}$$

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 $\chi^{(1)}$:

$$\alpha = \frac{\omega \operatorname{Im}\left\{\chi^{(1)}\right\}}{c_0} \tag{2.25}$$

$$n = \sqrt{1 + \operatorname{Re}\left\{\chi^{(1)}\right\}} \tag{2.26}$$

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

$$\mathrm{Re}\left\{\chi^{(1)}(\omega_{eg})\right\} = \frac{2}{\pi}\int_0^{+\infty} d\omega \frac{\omega\,\mathrm{Im}\left\{\chi^{(1)}(\omega)\right\}}{\omega_{eg}^2 - \omega^2} \qquad (2.27)$$

where ω_{eg} is the frequency of maximum absorption.

2.1.5 Electromagnetic Spectrum

The electromagnetic spectrum is the entire wavelength, wavenumber, frequency, energy or some other property range of electromagnetic waves. The different regions of the electromagnetic spectrum correspond to different transition possible in the molecular (atomic) system[52]. In the following table the electromagnetic spectrum with the corresponding spectroscopic method is given.

Table2.1. Electromagnetic spectrum with the corresponding spectroscopic methods

Spectral Region	*Wavelength*	*Excitation, Transition*	*Spectroscopic Method*
γ-Rays	< 10 pm	Nuclei, Core	γ-Ray
X-Rays	10 pm – 10 nm	Inner Electrons	X-ray
Vacuum UV	10 nm – 200 nm		Optical Absorption
Near UV	200 nm – 380 nm	Valence Electrons	+
Visible	380 nm – 780 nm		Fluorescence
Near IR	780 nm – 2500 nm	Valence Electrons, Overtones	IR
IR	2.5 μm – 50 μm	Vibration, Scattering	+
Far IR	50 μm – 1000 μm	Vibration, Rotation	Raman
Microwaves	1 mm – 100 mm	Rotation, Electron Spin	IR, ESR
Radio waves	> 100 mm	Electron Spin	NMR

2.1.6 Absorption and Emission of Radiation

Consider two state systems: The initial state wave function and energy being ψ_g and E_g, and the final state wave function and energy are given to be ψ_e and E_e. According to the time-independent perturbation theory the system Hamiltonian H may be given as the sum of the unperturbed $H^{(0)}$ and the perturbing $H^{(1)}$ Hamiltonians:

$$H = H^{(0)} + H^{(1)} \tag{2.28}$$

We consider the perturbation due to the applied electric field $E(t)$, then:

$$\hat{H}^{(1)} = \hat{\mu} \cdot E(t) \tag{2.29}$$

where $\hat{\mu}$ is the dipole operator. By taking only the first-order correction term, one may get the expression for the coefficient a_{eg}:

$$a_{eg} = \frac{\langle e|\hat{H}^{(1)}|g\rangle}{E_e - E_g} \tag{2.30}$$

The expression $\langle e|\hat{H}^{(1)}|g\rangle$ is known as the transition dipole moment is denoted as μ_{eg}. From the time-dependent perturbation calculation one can show that the coefficient a_{eg} will be small unless the frequency corresponding to the transition ν_{eg} is equal to the energy difference between the two states (ψ_g and ψ_e):

$$\nu_{eg} = \frac{E_e - E_g}{h} \tag{2.31}$$

Eq. 2.31.is the Bohr's resonance criteria. Further, a_{eg} is proportional to the rate of probability of transition P_{eg} :

$$P_{eg} \propto \left|a_{eg}\right|^2 \tag{2.32}$$

Since P_{eg} depends on $\left|a_{eg}\right|^2$, it will also be proportional to $\left|\mu_{eg}\right|^2$:.

$$\begin{aligned} P_{eg} &= \underbrace{\frac{1}{6\varepsilon_0\hbar^2}\left|\mu_{eg}\right|^2}_{B_{eg}} \rho(\nu_{eg}) \\ &= B_{eg}\rho(\nu_{eg}) \end{aligned} \tag{2.33}$$

where B_{eg} is the Einstein coefficient of stimulated absorption.

Now we relate P_{eg} (microscopic, molecular) to the molar decadic absorption coefficient (macroscopic). Since $I(z) \propto \left\langle \left(E(z,t)\right)^2 \right\rangle_T$:

$$\begin{aligned} I(z) &\propto \left\langle \left(\underline{E}_0 \exp\left(-\frac{1}{2}\alpha z\right)\cos(\omega t - kz)\right)^2 \right\rangle_T \\ &\propto E_0^2 e^{-\alpha z} \end{aligned}$$

$$\frac{I(z)}{I(z=0)} = e^{-\alpha z} \tag{2.34}$$

where α is the natural absorption coefficient. α is an extensive quantity. Thus it is usually expressed using an intensive quantity called molar decadic absorption coefficient ε :

$$\alpha = \ln(10)\varepsilon c \tag{2.35}$$

Combining Eq. 2.34 and Eq.2.35 rearranging, it results

$$\log \frac{I(z=0)}{I(z)} = \varepsilon c z = A \qquad (2.36)$$

Eq 2.36 is commonly known as Lambert-Beer's law. Considering a dilute solution or a very short optical path length $|\alpha z| \square 1$, and substituting for the concentration c with $c = N / N_A$ it results for the infinitesimal change of the intensity

$$-dI(z) = I_0 \ln(10)\varepsilon(\nu) c \, dz \quad \text{or} \quad -d\rho(\nu_{eg}) = I_0 \ln(10)\varepsilon(\nu) c \, dz \qquad (2.37)$$

In addition, if P_{eg} is the rate of probability for a single molecule, then $P_{eg} N dz$ is the number of molecules excited in a layer dz with an energy of absorption $h\nu_{eg}$. Therefore, the loss in intensity:

$$\begin{aligned} -dI &= P_{eg} N \mathrm{h} \nu_{eg} dz \\ \Rightarrow -d\rho(\nu_{eg}) &= \frac{1}{c_0} P_{eg} N \mathrm{h} \nu_{eg} dz \end{aligned} \qquad (2.38)$$

Combining the equations and:

$$P_{eg} = \frac{\ln(10) c_0 \varepsilon(\nu)}{N_A \mathrm{h} \nu_{eg}} \rho(\nu_{eg}) \qquad (2.39)$$

In order to obtain the rate of probability of transition for the entire absorption band P'_{eg} one has to integrate over the entire frequency range:

$$P'_{eg} = \int_{\text{band}} P_{eg} d\nu = \frac{\ln(10) c_0}{N_A h \nu_{eg}} \int_{\text{band}} \rho(\nu) \frac{\varepsilon(\nu)}{\nu} d\nu \qquad (2.40)$$

Assuming $\rho(\nu)$ to be constant throughout the band:

Assuming $\rho(\nu)$ to be constant throughout the band:

$$P'_{eg} = \frac{\ln(10)c_0}{N_A h\nu_{eg}} \rho \int_{band} \frac{\varepsilon(\nu)}{\nu} d\nu = \frac{1}{6\varepsilon_0 \hbar^2} |\mu_{eg}|^2 \rho \qquad (2.41)$$

Rearranging we get for the transitional dipole moment:

$$|\mu_{eg}|^2 = 3 \cdot \frac{2\ln(10)c_0\varepsilon_0 h}{2\pi^2 N_A} \int_{band} \frac{\varepsilon(\nu)}{\nu} d\nu \qquad (2.42)$$

The factor 3 in the above equation is due to the orientation averaging of the transitional dipole in isotropic medium. Since the direction of the electric field and $\underline{\mu}_{eg}$ may not be the same. The integral in the right part of Eq. 2.42 is called integral absorption I_A:

$$I_A = \int_{band} \frac{\varepsilon(\nu)}{\nu} d\nu = \int_{band} \frac{\varepsilon(\tilde{\nu})}{\tilde{\nu}} d\tilde{\nu} = \frac{1}{3} \frac{2h\pi^2 N_A}{\ln(10)c_0\varepsilon_0} |\underline{\mu}_{eg}|^2 = \frac{1}{3} S |\underline{\mu}_{eg}|^2;$$
$$S = \frac{2h\pi^2 N_A}{\ln(10)c_0\varepsilon_0} = 2.93521 \cdot 10^{60}\ \mathrm{C^{-2}\ mol^{-1}} \qquad (2.43)$$

2.2 Rate Laws and Factors Affecting the Rate Constant

The reaction rate for a reactant or product in a particular reation is defined as the amount of the chemical that is formed or removed (in moles or mass units) per unit time per unit volume. Knowledge of these rates is essential in, among other disciplines, physical chemistry, chemical engineering and environmental engineering. Chemical kinetics is the part of which studies reaction rates.

A rate law is an equation that relates concentrations of reactants to the reaction rate. The rate law can be used in a mass balance to describe how the system changes. For

simplicity, we here assume that the system is a closed, homogeneous system with only one reaction[53]. For the reaction

$$aA + bB \rightarrow cC + dD \tag{2.44}$$

The rate law is

$$\upsilon = -\frac{d[A]}{dt} = k\,[\mathrm{A}]^{\mathrm{m}} \tag{2.45}$$

where k is the reaction rate constant, and the exponents are reaction order. The reaction is of order m in A and of order n in B. The overall reaction order is $m+n$. Usually, reaction orders are 0, 1, or 2, but they can be fractions or even negative numbers.

2.2.1 Zero-Order Reactions

A zero-order reaction is independent of the concentration of the reactants. Increasing the concentration of the reacting species will not speed up the rate of the reaction. Zero-order reactions are typically found when a material required for the reaction to proceed, such as a surface or a catalyst, is saturated by the reactants[53]. The rate law for a zero-order reaction is

$$\upsilon = k \tag{2.46}$$

If, and only if, this zero-order reaction, then it occurs in a closed system, there is no net build-up of intermediates and there are no other reactions occurring, it can be shown by solving a mass balance for the system that

$$\upsilon = -\frac{d[A]}{dt} = k \tag{2.47}$$

The above expression is known as the differential rate equation, and integrating it gives an equation that is often called the integrated zero-order rate law

$$[A] = [A]_0 - kt \tag{2.48}$$

where $[A]$ represents the concentration at a particular time and $[A]_0$ represents the initial concentration. A reaction is zero order if concentration data are plotted versus time and the result is a straight line. The slope of this resulting line is the zero order rate constant k.

The half-life of a reaction describes the time needed for half of the reactant to be depleted (same as the half-life involved in nuclear decay, which is a first-order reaction). For a zero-order reaction the half-life is given by

$$t_{1/2} = \frac{[A]_0}{2k} \tag{2.49}$$

An example of zeroth-order reaction is the decomposition of ammonia to hydrogen and nitrogen in the Haber process.

2.2.2 First-Order Reactions

A first-order reaction depends on the concentration of only one reactant (a unimolecular reaction). Other reactants can be present, but each will be zero-order. The rate law for a first-order reaction is

$$\upsilon = k[A] \tag{2.50}$$

k is the first order rate constant that has units of 1/time. The derivative form of the first order reaction is given as

$$-\frac{d[A]}{dt} = k[A] \tag{2.51}$$

The integrated first-order rate law is

$$[A] = [A]_0 \exp(-kt) \quad or \quad \ln[A] = \ln[A]_0 = -kt \tag{2.52}$$

A plot of $\ln[A]$ vs. time gives a straight line with a slope equal to negative of the reaction rate constant. The half-life of a first-order reaction can be determined using the equation

$$t_{1/2} = \frac{\ln 2}{k} \tag{2.53}$$

Characteristic of a first-order reaction is that all the half-lives are equal.

2.2.3 Second-Order Reactions

A second-order reaction or bimolecular reaction depends on the concentrations of one second-order reactant, or two first-order reactants. For a second order reaction, its reaction rate is given by:

$$\upsilon = k[A]^2 \quad or \quad \upsilon = k[A][B] \tag{2.54}$$

The integrated second-order rate laws are respectively

$$\frac{1}{[A]} = \frac{1}{[A]_0} + kt \tag{2.55}$$

$$\frac{[A]}{[B]} = \frac{[A]_0}{[B]_0} \exp\left[\left([A]_0 - [B]_0\right) \times kt\right] \tag{2.56}$$

The half-life equation for a second-order reaction dependent on one second-order reactant is

$$t_{1/2} = \frac{1}{k[A]_0} \tag{2.57}$$

Characteristic of a second-order reaction is that the half-lives progressively double.
Another way to present the above rate laws is to take log on both sides:

$$\ln \upsilon = \ln k + 2\ln[A] \tag{2.58}$$

Note that if either [A] or [B] remains constant (or infinitely large) as the reaction proceed, then the reaction is called pseudo first order reaction.

2.2.4 Half-life

The half-life of a quantity subject to exponential decay is the time required for the quantity to fall to half of its initial value. The concept originated in the study of radioactive nuclei but it now also occurs in many other fields [53].

Quantities that are subject to exponential decay are commonly denoted by the symbol *N*. (This convention suggests a decaying *number* of discrete items. This interpretation is valid in many, but not all, cases of exponential decay.) If the quantity is denoted by the symbol *N*, the value of *N* at a time *t* is given by the formula:

$$N(t) = N_0 e^{-kt} \tag{2.59}$$

where N_0 is the initial value of N at $t = 0$ and k is a positive constant (the decay constant). When $t = 0$, the exponential is equal to 1, and $N(t)$ is equal to N_0. As t approaches infinity, the exponential approaches zero. In particular, there is a time $t_{1/2}$ such that:

$$N(t_{1/2}) = N_0 \times \frac{1}{2} \tag{2.60}$$

Substituting into the formula above, we have:

$$N_0 \times \frac{1}{2} = N_0 e^{-kt_{1/2}}$$

$$-kt_{1/2} = \ln \frac{1}{2} = -\ln 2$$

$$t_{1/2} = \frac{\ln 2}{k} \tag{2.61}$$

This expression for the half-life is the same as the half-life given to the first-order reaction, i.e. the natural decaying of radioactive nuclei is the first-order type reaction.

2.2.5 Effect of Temperature on the Rate of Reaction

There are several factors that affect the rate of reaction. Some of them are Temperature, oxygen, water activity, pressure and light. Their effect is in increasing the reaction rate. The effect of temperature is described by the emprical equation known as Arrhenius equation. The Arrhenius equation is a simple, but remarkably accurate, formula for the temperature dependence of a chemical reaction rate. It was first proposed by the Dutch chemist J. H van't Hoff in 1884; five years later, the Swedish chemist Syante Arrhenius provided a physical justification and interpretation for it [53].

According to Arrhenius in order for reactants to be transformed into products, they first need to acquire enough energy to form an "activated complex". This minimum energy is called the "activation energy" E_a for the reaction. In thermal equilibrium at an absolute temperature T, the fraction of molecules that have a kinetic energy greater than

E_a can be calculated from the Maxwell-Boltzmann distribution of statistical mechanics, and turns out to be proportional to $\exp(-E_a / RT)$, where E_a is measured in molar units and R is the gas constant. This leads to the Arrhenius formula for the reaction rate constant k:

$$k = Ae^{-\frac{E_a}{RT}} \tag{0.1}$$

If the energy is given in molecular units (e.g. joules per particle or per molecule), then R, the gas constant, should be replaced by Boltzmann's constant k_B. The A-factor or the frequency factor A is a constant specific to a particular reaction.

It can be seen that either increasing the temperature or decreasing the activation energy will result in an increase in rate of reaction. As a rule of thumb, the reaction rate doubles for every 10 degrees Celsius (Kelvin) increase in temperature. While remarkably accurate in a wide range of circumstances, the Arrhenius equation is not exact, and various other expressions are sometimes found to be more useful in particular situations. One example comes from the "collision theory" of chemical reactions. In this theory, molecules react if they collide with a relative kinetic energy along their line-of-centers that exceeds E_a. This leads to an expression very similar to the Arrhenius equation, with the difference that the preexponential factor "A" is not constant but instead is proportional to the square root of temperature. This reflects the fact that the overall rate of all collisions, reactive or not, is proportional to the average molecular speed which in turn is proportional to $T^{1/2}$. In practice, the square root temperature dependence of the preexponential factor is usually very slow compared to the exponential dependence associated with E_a.

Another Arrhenius-like expression appears in the Transition State Theory of chemical reactions commonly known as Eyring equation. This takes various forms, but one of the most common is[53]:

$$k = \frac{k_B T}{h} e^{-\frac{\Delta G^{\ddagger}}{RT}} \tag{2.63}$$

where $\Delta G^{\mp}$ is the Gibbs free energy of activation, k_B is Boltzmann's, and h is Planck's constant.

At first sight this looks like an exponential multiplied by a factor that is linear in temperature. However, one must remember that free energy is itself a temperature dependent quantity. The free energy of activation includes an entropy term as well as an enthalpy term, both of which depend on temperature, and when all of the details are worked out one ends up with an expression that again takes the form of an Arrhenius exponential multiplied by a slowly varying function of T. The precise form of the temperature dependence depends upon the reaction, and can be calculated using formulas from statistical mechanics, which involves the partition functions of the reactants and of the activated complex. Taking the natural logarism of the Arrhenius equation yields

$$\ln k = -\frac{E_a}{R}\left(\frac{1}{T}\right) + \ln A \qquad (2.64)$$

So, when a reaction has a rate constant which obeys the Arrhenius equation, a plot of $\ln k$ versus T^{-1} gives a straight line, whose slope and intercept can be used to determine E_a and A.

2.2.6 Effect of Moisture on the Rate of Reaction

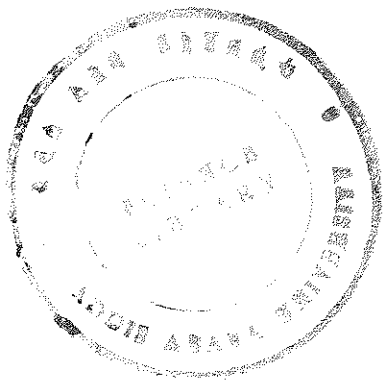
In the mid 1970s, water activity came to the forefront as a major factor in under standing the control of the deterioration systems. It was found that the general modes of deterioration namely physical and physicochemical modification, microbiological growth and both aqueous and lipid phase chemical reaction, were all influenced by the thermodynamic availability of water termed as (water activity) as well as the total moisture content of the system. It is the difference in the chemical potential of water (μ in joules/ mole) between two systems that results in moisture exchange and above a certain

chemical potential as related to the a_w of a system there is enough water present to result in physical and chemical reactions[53] .

The physical structure of a food or biological product important from both functional and sensory standpoints is often altered by changes in water activity due to moisture gain or loss.

2.2.7 Effect of Oxygen on the Rate of Reaction

Many fresh foods as well as dry and semi-moist foods packaged in oxygen permeable packaging are sensitive to oxygen, losing shelf life faster as the amount of oxygen increases. An example of this is the oxidation of fat in potato chips or the lipids of fresh roasted ground (FRG) coffee reacting with oxygen permeating through the package film. Above a certain critical oxygen level, an increase in oxygen does not change in the rate of degradation as seen for FRG maintain low oxygen in level[53].



3. Materials and Methods

In this chapter the laboratory apparatus, chemicals and samples are presented. Further sample preparations, storage conditions and experimental procedures used during the experiment and vital points of data analysis will be discussed.

3.1 Laboratory Apparatus and Instrumentation

The total laboratory apparatus used during the study are beakers, measuring cylinders, pipettes, volumetric flasks, spatula, magnetic stirrer with hot plate, funnel, separatory funnel, glass filter quartz cuvette, 250 μm sieve, electronic balances (SA 120, Boulder company) and (S M 1600 , Sauter company) and Incubator. In addition, the absorption spectra were recorded with UV-Vis spectrophotometer (Perkin-Elmer Lambda 19) with a spectral range of measurement to be 200-800 nm, and with abscissa accuracy of ± 0.15 nm(UV-Vis range) and ± 0.6nm (NIR range). The spectrophotometer was interfaced with personal computer, which is operated by software UVCSS.

3.1.1 Operational Principle of the UV-Vis Spectrophotometer

In this portion, the use of spectrophotometer specification accuracy working principle and instrumental components of the spectrophotometer will be discussed.

UV-Vis spectrophotometer is spectroscopic instrument used to measure absorption of electromagnetic radiation in the region between ultra violet to visible range. The ultra violet visible region of the electromagnetic radiation is extending from roughly 200-800 nm. Absorption of ultra violet or visible light by molecules will occur when the energy of the incident radiant radiation is the same as that of a possible electronic transition in the molecules involved. Such absorption of energy is termed electronic

excitation and corresponds to promotion of an electron from the ground state to an excited electronic state. It measures the intensity of transmitted, reflected, or absorbed light as a function of wavelength or energy. The relation between absorption of light, light path (cell thickness) and concentration is described by the Lambert- Beer's law.

In double beam instruments, the beam of light leaving the exit slit of the spectrometer is split in to two parts. One part is passed through the cell containing the solvent while the other part is passed through the sample cell. Both cells are placed in the instrument together. After the two parts of the beam have passed through the cells, they are brought together by mirrors and strike the photocell together.

The photocell receives first one beam and then the other, giving a current that alternates in a step- like fashion .The current is then amplified and the ratio is registered on the measuring instrument as the percent transmittance or by means of an electronic converter directly as absorbance.

UV-Vis spectrophotometer consists of a radiant energy source, monochromator, sample holder and detector. The wavelength is scanned by the monochromator at a constant rate (geared to chart speed of recorder) while the detector responds to light beams, and discriminates between the two alternating beams, one from the sample and the other from the attenuator.

3.2 Chemicals

The chemicals used for investigation of caffeine character as a function of time and determination of half-life of caffeine in coffee include; dichloromethane (99.6% A.c.s, reagent ALDRICH), de-ionized water and pure caffeine .

3.3 Samples

In this study, two different types of samples, caffeine and raw coffee were used. Pure caffeine was bought from Evan pharmaceutical company, England. The coffee bean

sample (Ayera Guliso washed export standard), 93.92% (dry matter), 6.08%(moisture content), collected from Ethiopian Coffee and Tea quality and Liquoring center.

3.4 Methods of Experiment

3.4.1 Sample Preparations

The coffee sample used in this research was Ethiopian coffee Arabica of Ayera Guliso, selected due to its maximum caffeine content and low moisture content relative to others. Fresh raw coffee beans were ground in a McGarveys commercial grinder to much less than drip brewing size to a diameter of 250 μm sieve size (Mc Garveys, Minnea Polis, MN).

Samples of 600 mg of raw ground coffee were weighed by 0.1 mg (Mettler Toledo Inc., Hightstown, NJ) and mixed with 250 ml de-ionized water. The solutions were hermetically sealed with rubber stoppers then stirred for 1 hour using magnetic stirrer.

3.4.2 Storage and Sampling

After flushing, the internal pressure coffee solution was stirred and equilibrated for 5 minutes to atmospheric pressure. The first bottle was kept horizontally in incubator at controlled temperature of 8 °C. The depth of the raw coffee solution layer was approximately 40 mm. A second bottle containing coffee solution was stored open at 23 °C. In a third bottle, the coffee powder was stored open again at 23 °C.

Table 3.1 Shows storage conditions used for this study. Levels of O_2 partial pressure, temperature (T °C) and water activity (a_w) were selected to be representative of real world situations frequently found in the storage of Raw Ground Coffee(RGC).

Table3.1. Storage conditions for half-life determination of caffeine in raw ground coffee using UV-Vis Spectroscopy.

Sample type	Temperature
Coffee solution (closed system)	8 °C (in Incubator)
Coffee solution (open system)	23 °C (open air)
Coffee powder (open system)	23 °C (open air)

The samples from different storage conditions were taken once every three days and caffeine was extracted from coffee samples by chemical extraction method [54]. The ratio between coffee and water was 60:25 in weight. The stirring time as well as the extraction time was equally 10 minutes. Finally, absorbance measurement was taken using spectrophotometer.

3.4.3 Experimental procedure

1) Collecting coffee from local source.
2) Grinding green coffee beans and sieve to a sieve size of 250 μm.
3) 60 mg sieved coffee is measured and added to 25 ml of de-ionized water.
4) Slowly heating coffee solution and stirring for 1hr using magnetic stirrer.
5) Storing the coffee solution at different conditions and levels
6) At expected time of change, (in this case 3 days), 25 ml of coffee solution measured and added to 25ml of dichloromethane.
7) The solution formed under procedure '6' stirred using magnetic stirrer for 10 min.
8) After stirring, the solution added to a separatory funnel since density of dichloromethane is greater than density of water caffeine easily extracted from water solution.
9) The above three steps (6,7,8) repeated for three times (for the same sample).
10) The dichloromethane, which consists of caffeine stored in non-plastic flask, absorbance of the solution taken using spectrophotometer.

11) The above procedures repeated until 50% loss in concentration of caffeine for each sample.

12) At 50% loss of concentration, half-life time of caffeine was determined by extrapolation programme from simulation time and experimental time coffee sample stored at three different storage conditions.

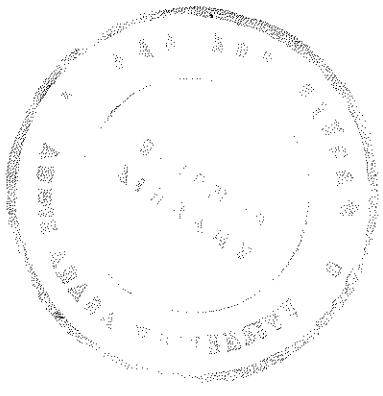
The physical quantity directly measured in this experiment using PC-interfaced spectrophotometer is absorbance as a function of wavelength. The other quantities like concentration of caffeine, transmittance, and molar decadic absorption coefficient of caffeine transitional dipole moment of caffeine, mass of caffeine and percentage of caffeine are derived from these measured values by applying Beer-Lambert's law.

3.5 Data Analysis

The absorbance versus wavelength data in these experimental activities found from computerized recorder interfaced with the spectrophotometer. In this section property of pure caffeine in water, qualitative investigation of property of coffee as a function of different storage conditions and time, property of caffeine in coffee as a function of storage conditions and time and finally half life time of raw ground coffee were determined .

3.5.1 Investigations of Property of Pure Caffeine in Water

Molar decadic absorption coefficient of caffeine in water was calculated. A characterization of pure caffeine as a function of time was investigated. Concentration of caffeine versus time was plotted after calculating concentration from measured absorbance applying Beer –Lambert's law



3.5.2 Property of Coffee as Function of Storage Conditions and Time

Coffee sample combined with water at 60/25 (mg/ml) ratio stored at 8 °C in closed solution form, at 23 °C in solution form open and stored at 23 °C in powder form open; absorbance versus wave length spectra normalized and overlapped to observe qualitatively. In addition, for above three coffee samples wavelength versus absorbance spectra of caffeine extracted was observed by overlapping and normalizing, further caffeine concentration versus time graph was also plotted

3.5.3 Investigations of half-life of caffeine in coffee

Using concentration versus time graph after mono exponential decay fitting, half-life of raw ground coffee was extrapolated.

4. Results and Discussion

In this section properties of caffeine and coffee in water as function of temperature, water activity, oxygen intake and time are presented. The characterization of caffeine content in coffee as function of time and half-life of coffee are also presented.

4.1 Absorbance of Pure Caffeine

For the characterization of caffeine in water solution, four samples of caffeine with different concentrations were prepared and absorption spectra were taken(see fig.4.1).

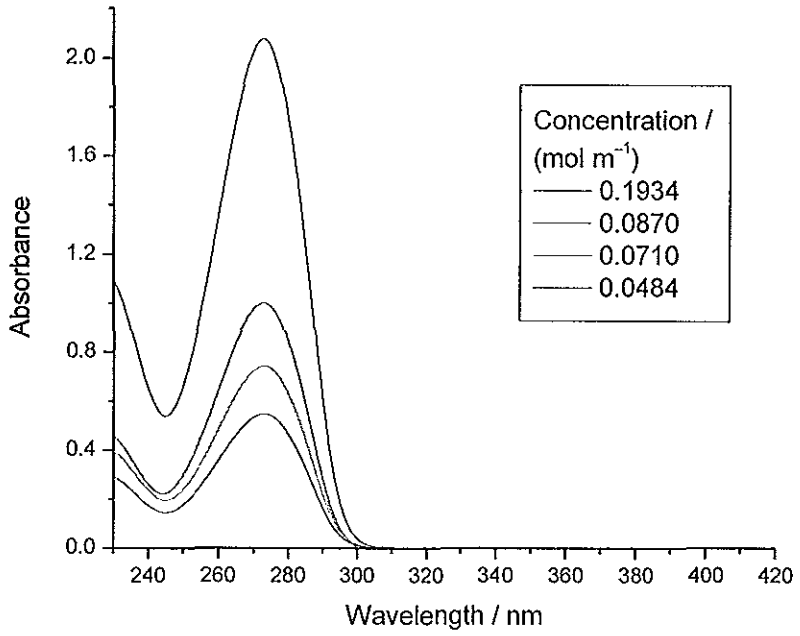


Figure 4.1. Absorbance versus wavelength of pure caffeine with different concentrations.

The concentration of caffeine along with the absorbance values of wavelength of maximum absorption λ_{max}, and the calculated integral absorption I_A and the molar decadic absorption coefficient ε are summarized in Table 4.1.

Table 4.1. Summarized form of feature of pure caffeine.

λ_{max} (nm)	A_{max}	Concentration (mol/m^3)	I_A (m^2/mol)	ε (m^2/mol.)
273	1.00	0.0877	124.4	1140
273	0.547	0.0484	129.4	1120
273	2.070	0.1934	124.0	1075
273	0.742	0.0710	120.6	1045

Concentration versus absorbance graph for above pure caffeine experiment was drawn as follow to check whether Beer-Lambert's law is valid or not.

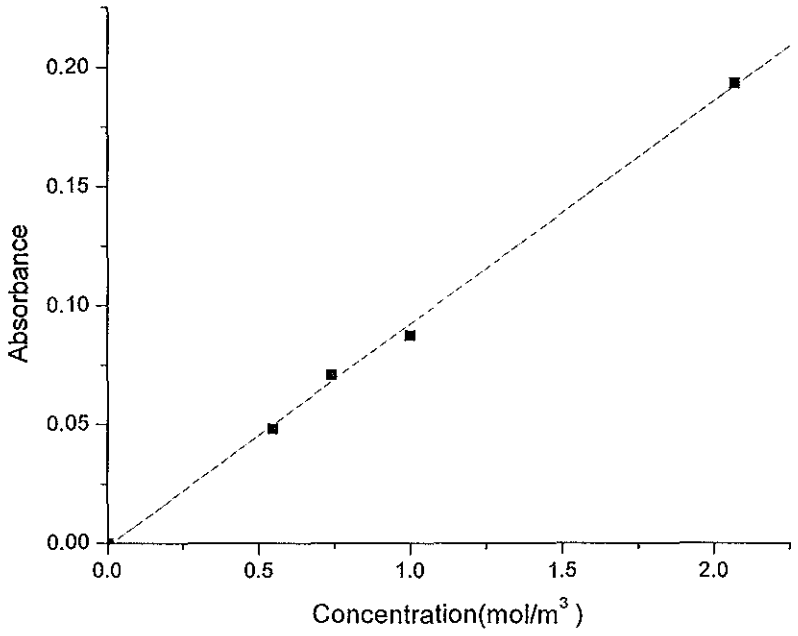


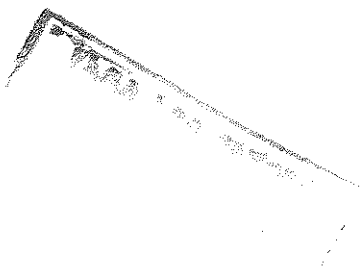
Figure 4.2. Verification of Beer's Law for above pure caffeine experiment.

From the fig.4.2.above absorbance versus concentration graph was linear. Which indicates absorbance is directly proportional to concentration (Beer-Lambert's law is valid). This means that the current studies were carried out according to the Beer's law ranges.From the spectra it can be observed that caffeine absorbs in the spectral range between 245 nm to 300 nm. The λ_{max} of caffeine in water is found to be at 273 nm. There is no pronounced shift in λ_{max} indicating the electronic transition occurring is local transition without significant charge transfer.

In water solution there was no shift in different concentration and a linear relationship between concentration and absorbance was found. Further, molar decadic absorption coefficient and transitional dipole moment were computed by applying Beer Lambert's law. The mean value of molar decadic absorption coefficient of caffeine in water was 1095 ± 37 m^2 mol^{-1}. Similarly, the mean value of transitional dipole moment of pure caffeine in water was calculated to be (11.3± 0.2) X 10^{-30} C m. The two values in comparison with accepted values the percentage error were 1.5% for transitional dipole moment of caffeine in water and 1.7% for molar decadic absorption coefficient of caffeine in water; which are negligible error margins.

4.2 Investigation of Properties of Coffee as Function of Storage Conditions and Time

For this specific purpose, definite types of systems interconnected with its surrounding by certain microscopic properties were considered. The coffee samples were prepared based on the experimental procedures described in the previous section. The samples were stored separately under three different storage conditions and temperatures.



4.2.1 Properties of Coffee Stored Under Closed Condition at Temperature of 8 °C

600 mg of coffee was dissolved in 250 ml of water and stored at 8 °C in incubator. Each time the sample was taken for a very short time and spectra were taken. The time span between successive sampling was 3 days. The results of the measurements are displayed in figure 4.3.

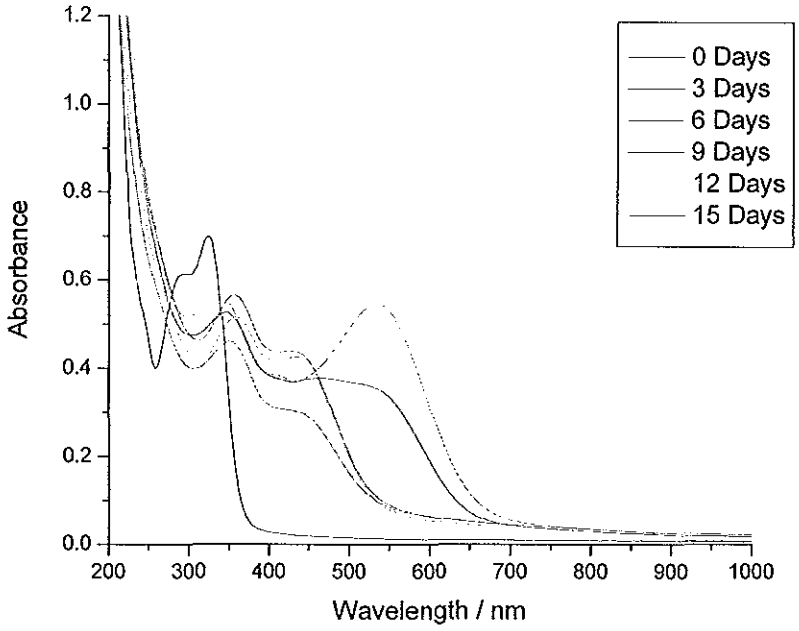


Figure 4.3. Spectra of Coffee in water solution kept open at 8 °C as a function of time.

The spectra measured after 3, 12 and 15 days showed a decrease in intensity at 273 nm. At the same time there appeared new bands at 357 nm and at 445 nm. The intensities of the two bands decreased as a function of time. The spectrum taken after 6 days showed relatively distinct absorption features, with the color of the solution became orange.

This phenomenon might be due to chemical reaction in the solution used in the experiment. The coffee solution stored under closed condition may exchange heat and work but not mass with the surroundings. Thus the factors contributing may have aroused from the possible transformation of coffee in water as a function of temperature.

Table 4.2. Summary of feature of coffee stored under closed system at 8^0c

Time (Days)	λ_{max} (nm)	Absorbance at λ_{max}	No of peaks
0	324.0	0.699	2
3	278.8	0.566	2
6	367.2	0.546	2
9	272.0	0.527	2
12	278.8	0.516	2
15	275.2	0.461	2

From the observation of the spectra of fig 4.3. number of peaks through out experimental time was the same. The position at which the maximum peak absorbance obtained for coffee solution decreased with time (see fig 4.8. and fig 4.13.). Moreover slight change in wavelength at intense absorption observed, which implies change in structure of coffee due to heat effect during stirring which may cause loss of some constituents of coffee as well as synthesization of some others. There was again slow reaction rate due to low temperature of storage as summarized in Table 4.2.

4.2.2 Properties of coffee under open solution system stored at temperature of 23 °C

Temperature plays a major role in facilitating reaction kinetics. It is long known that the reaction rate of a reaction depends exponentially on temperature. In this part the

results of the measurement made on coffee solution kept and stored under open condition at temperature of 23 °C are presented. Absorption spectra were taken every 3 days for a total of 15 days.

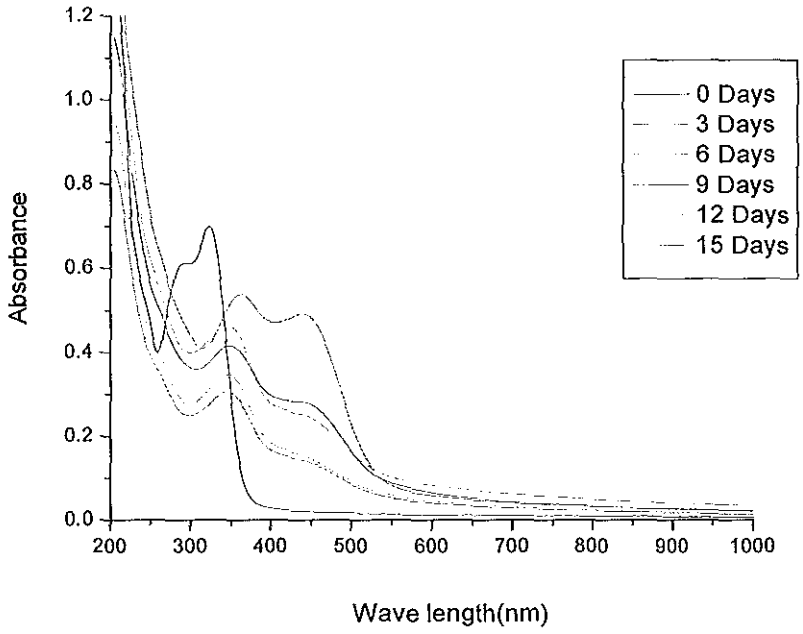


Figure 4. 4. Spectra of coffee in water solution kept open at 23 °C as a function of time.

Even though there is a small shift in λ_{max}, the band shape of the spectra taken after 3, 6, 9, 12 and 15 days are all similar. The intensities of the bands decreased as the number of days increased. The important parameters are given in Table 4.3.

Table 4.3. Features of coffee stored under open system in solution at 23 °C.

Time (days)	λ_{max} (nm)	Absorbance	No of peaks
0	324.0	0.6992	2
3	282.0	0.5391	2
6	273.6	0.4653	2
9	274.0	0.4164	2
12	274.0	0.3490	2
15	273.2	0.3053	2

The decrease observed at 23 °C is higher compared to the decease observed in the case the sample stored under the closed condition but at 8 °C. This qualitative information is in agreement with the expected temperature effect.

4.2.3 Properties of coffee stored under open system in powder form at 23 °C

The property of coffee as raw powder is expected to differ from that of the coffee solution. To investigate the property of raw coffee and to compare the relationship and difference of the spectra with that of the solution stored in open condition, raw coffee was ground and stored open at temperature of about 23 °C for about 15 days. Each sampling time, a given amount of ground and sieved raw coffee was weighed, dissolved in water, filtered and spectra were taken.

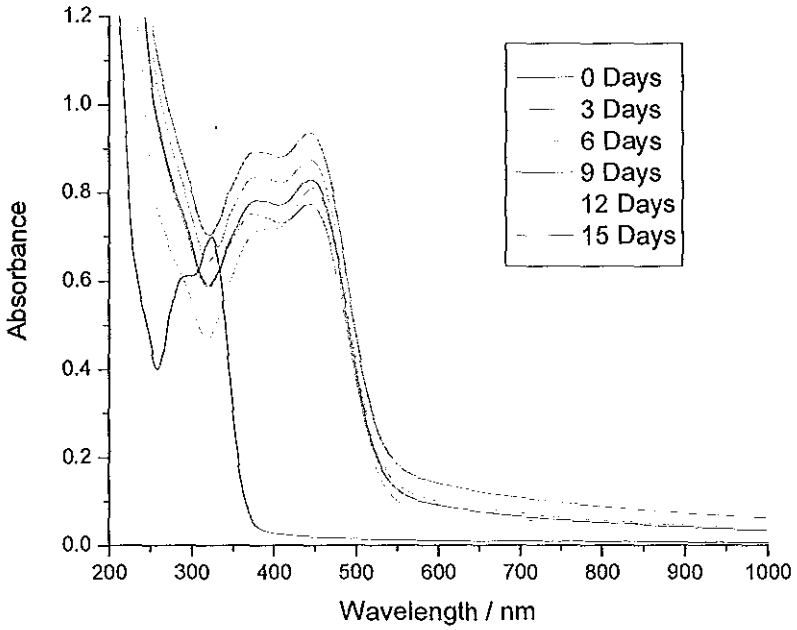


Figure 4.5. Spectra of coffee in water solution kept open at 23 °C.

All the spectra, except the original spectrum, showed a decrease in intensity. The band at 332 nm disappeared and two new bands at 378 nm and 445 nm appeared. The position of the lower energy peak is the same as that found for the coffee solution kept under the same condition. In the high energy peak there is an average deviation of 25.8 nm. Moreover, in the open powder case the intensity of the lower energy peak is higher.

Table 4.4. Spectral features of coffee stored open at 23 °C in powder form.

Time (days)	λ_{max} (nm)	Absorbance	No of peaks
0	324	0.6992	2
3	322.4	0.9372	2
6	322.4	0.8759	2
9	321.6	0.8302	2
12	323.6	0.8114	2
15	322	0.7249	2

For coffee stored in powder form the most influential environmental factor is oxygen.

4.3. Caffeine Content in Coffee as Function of Storage Conditions and Time

In this part the change in caffeine concentration is presented. Below absorbance spectra of caffeine spectra extracted using dichloromethane (DCM) are shown.

Water was used as solvent to dissolve coffee powder. Then the spectrum of coffee solution was recorded. The band shape spectrum of coffee and caffeine was quite different, indicating interference of bands of other constituents of coffee with the absorption band of caffeine. As a result, it was not convenient to study the characteristics of caffeine from the coffee spectrum. To over come this difficulty, the three steps (rounds) caffeine extraction procedures [55] was applied.

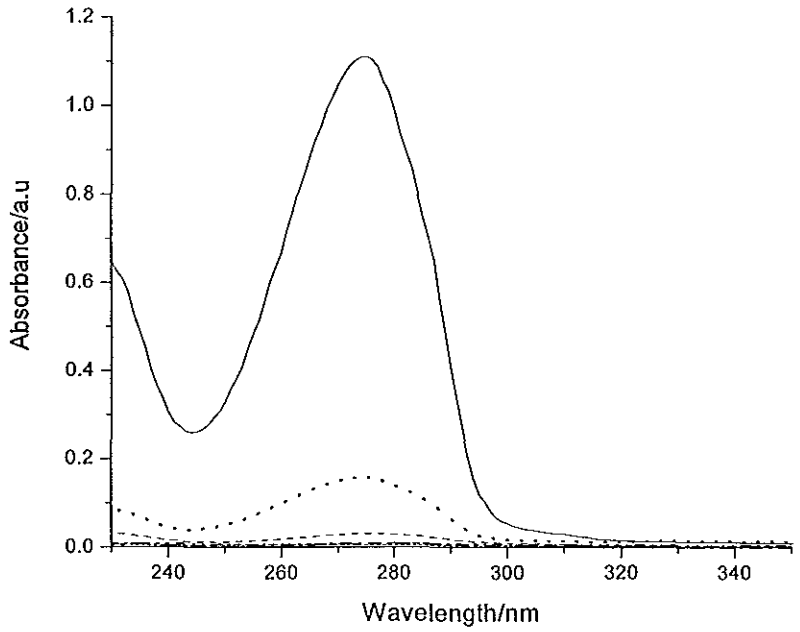


Figure 4.6. Absorbance spectra of caffeine extract after successive extraction steps.

In the procedure six successive extractions were recommended. However, as can be seen from the spectra above, applying only three extraction steps resulted with an error of 1-2 % in caffeine concentration. This error margin was taken to be negligible enough for our purpose and considered throughout in this work.

4.3.1 Time Dependence of Caffeine Concentration in Coffee Solution Stored at 8 °C

In order to study the time dependency, a given amount of coffee mentioned in section 3.4.1 was dissolved and kept closed at a fixed temperature of 8 °C. Then the concentration of caffeine was determined in 3 days interval.

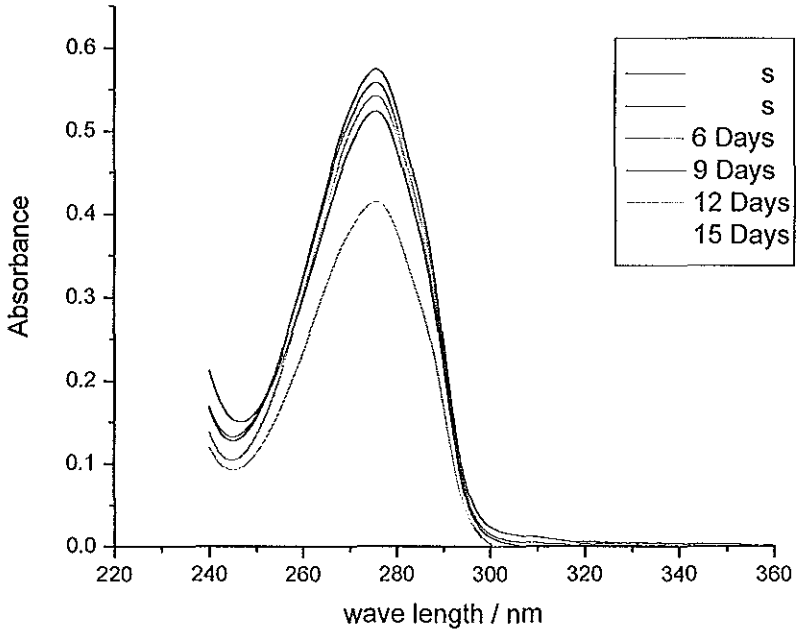
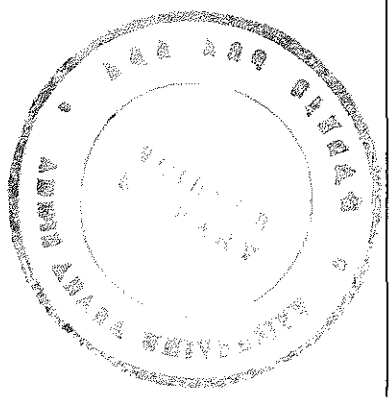


Figure 4.7. Spectra of caffeine extract from coffee solution stored under closed condition at 8 °C.

From the spectra one can observe a decrease in caffeine concentration as a function of time. Otherwise, the band shape of spectra is the same and similar to the band shape of caffeine. λ_{max} was found to be 275.6 nm. All the important results extracted from the spectra are summarized in table 4.5.

Important features of caffeine in coffee stored at 8 °C.

Time (hr)	Conc. of Caffeine (mol m^{-3})	% Loss	% of Caffeine
0	0.0569	0	1.24
72	0.0552	2.97	1.2
144	0.0535	5.92	1.15
216	0.0518	8.80	1.09
288	0.0499	12.15	1.05



Both loss rate and remaining percentage changes slowly, which means low rate of change of concentration.

The important parameter in describing the decrease in concentration of caffeine is rate constant. In order to determine the rate constant the concentration of caffeine was plotted as a function of time. The data points are fitted through a mono exponential fit, since the decomposition of caffeine is the first order rate of reaction. However, the fit resulted in a negative value for the concentration at infinite time ($c_{t\to\infty} = -0.0512 \pm 0.3377$). This result is unphysical. Thus, we were forced to consider a pseudo zero order rate of reaction.

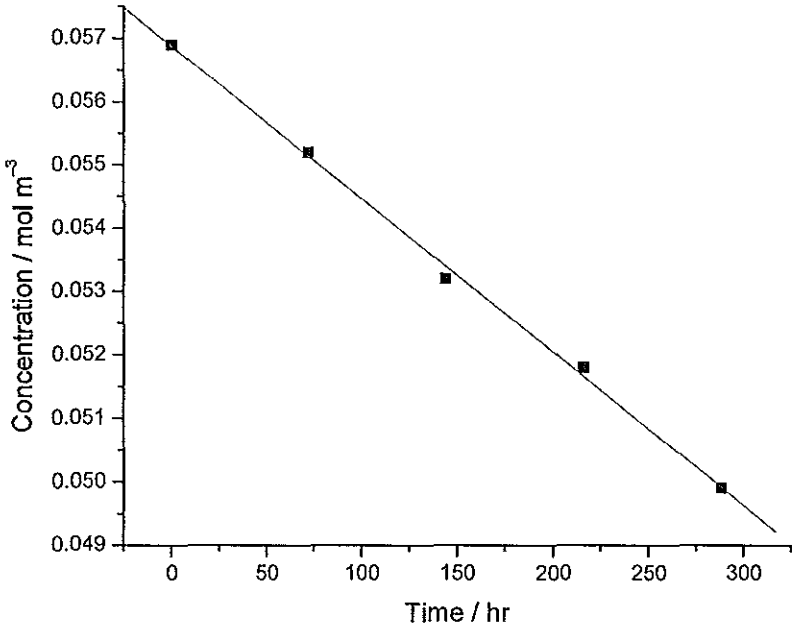


Figure4.8. Concentration of caffeine versus time graph for closed storage condition.

The pseudo zero order expression fits the data points with the linear regression coefficient of $R = 0.99894$. From the fit the rate constant of decomposition $k_d = 2.41667\text{x}10^{-5}$mol m^{-3} hr^{-1}.Half-life time of caffeine $t_{1/2} = [Co]/2kd = 1177$ hrs (50 days) implies the reaction is very slow over half-life.

The rate of decomposition of the active ingredient, caffeine, most possibly be inhibited by composition (water activity) and environmental factor (temperature). There was low decomposition. The observed decomposition maybe due to low temperature, over lapping bands, or heating effect on coffee (while stirring) there was considerable reaction rate as summarized in table 4.5.

4.3.2. Properties of Caffeine in Coffee Stored in Solution Form Under Open System at 23 °C

Coffee solution in open 1 liter amber bottle stored under open system at temperature of 23^0c.Every three days interval spectra were taken (see figure 4.7.)

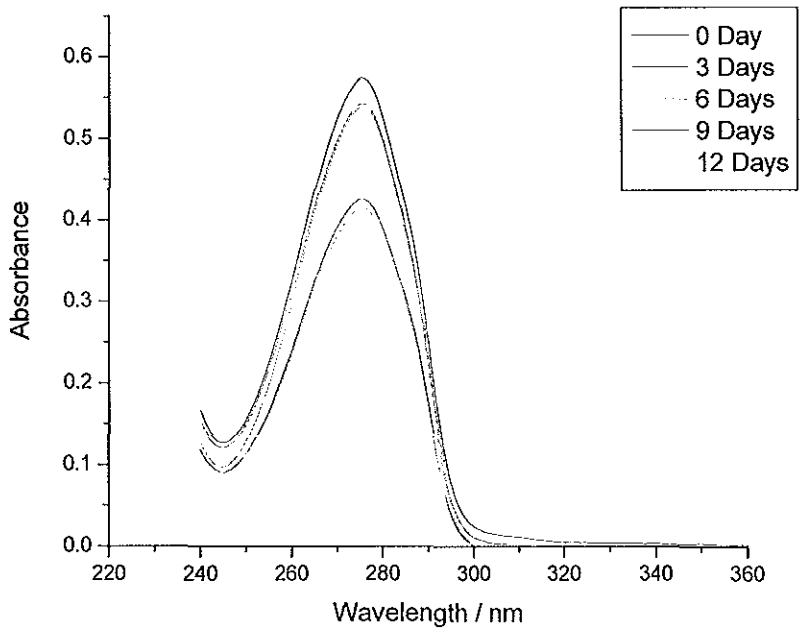


Figure 4.9. Spectra of caffeine extract from coffee solution kept under open condition at 23 °C.

In the same trend as observed for coffee solution kept under closed condition, the caffeine concentration decreased as a function of days. The absorption maximum wavelength observed was also the same (275.6nm through out). In this case however the decrease is much pronounced.

Table 4.6. Parameters taken from the spectra above (Figure 4.7.).

Time (hr)	λ_{max} (nm)	Conc. of Caffeine (mol/m^3)	% Remaining	% Caffeine in Coffee
0	275.6	0.05689	100.00	1.24
72	275.6	0.05298	93.13	1.11
144	275.6	0.04898	86.08	1.04
216	275.6	0.04499	79.08	0.93
288	275.6	0.04107	72.19	0.85

The caffeine concentration decreased as a function of time. Percentage of caffeine, remaining percentage and lose rate decrease very fast. The decrease is slowed down, as the storage time increased as well as the expected chemical reaction order of caffeine is first order. This behavior is expected for exponential decay decomposition. As a result concentration versus time graph fits again using the mono exponential function. However, the fit resulted in a negative value for the concentration at infinite time ($c_{t\to\infty} = -0.0446 \pm 0.2257$ mol m^{-3}). This result does not fit physical condition. As a result we were forced to consider a pseudo zero order rate of reaction again (see figure 4.10).

The pseudo zero order expression fits the data points with the linear regression coefficient of $R = 0.99999$. From the fit rate constant of decomposition of caffeine k_d=5.50417x10^{-5}molm^{-3}hr^{-1}.Half-life of caffeine $t_{1/2} = [Co]/2kd = 517$ hrs (22days) that implies the reaction was faster than closed storage condition.

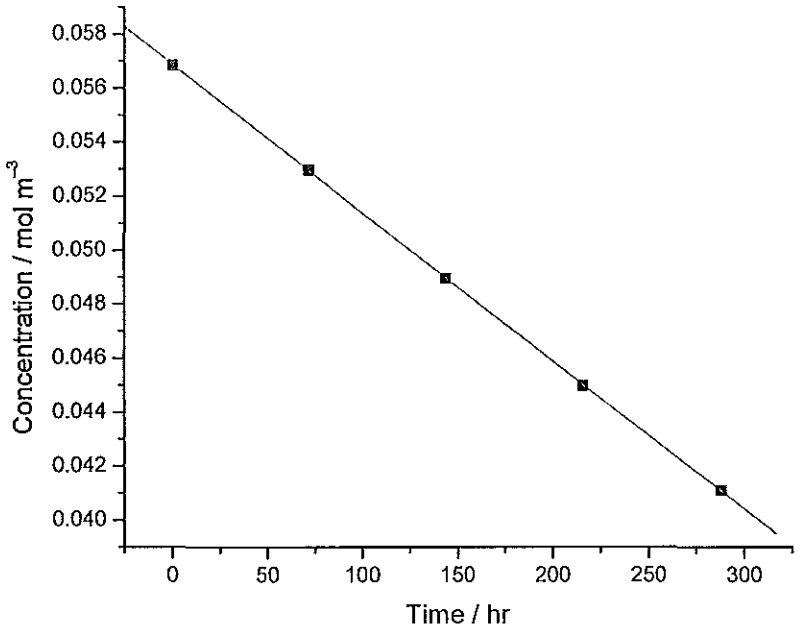


Figure 4.10. Concentration of Caffeine extract as a function of the storage time.

In general due to the possible oxidation under ambient atmosphere, caffeine decomposes as a function of exposure time. The effect of temperature is catalyzing the decomposition process. Thus the decomposition kinetics will be fastened. The important parameters from the spectra are summarized in table 4.6.

4.3.2 Properties of Caffeine in Coffee Stored in Powder Form Under Open Condition at 23 °C

Raw and ground coffee stored at almost constant oxygen (O_2), water activity (a_w) and temperature (T) were removed every three days for continues 15 days, weighed on a balance and caffeine extracted from it. Absorption spectra was taken using spectrophotometer (see figure 4.10.)

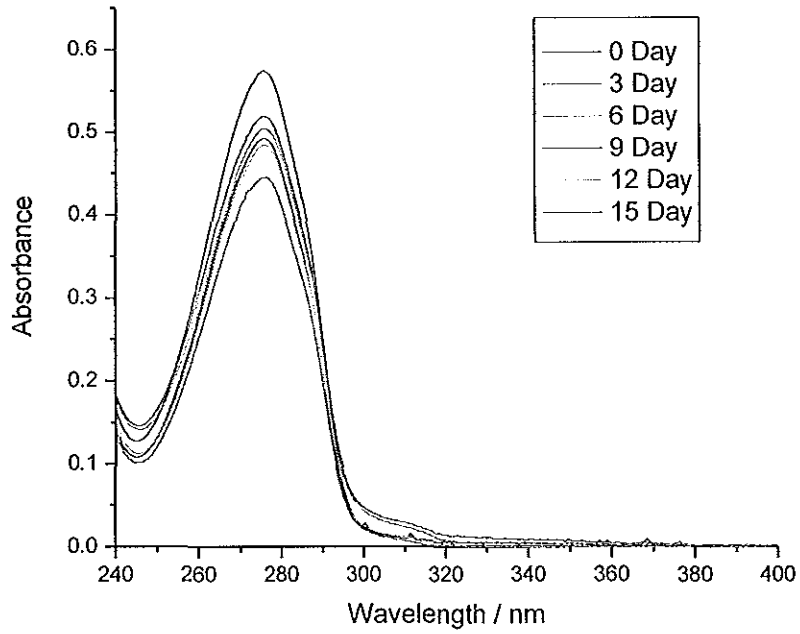


Figure 4.11. Spectra of caffeine extract from coffee powder stored open at 23 °C.

Similarly as observed for coffee solution storage condition, the caffeine concentration decreased as a function of time through out. The absorption maximum wavelength observed varies between 275.6 and 276nm. In this case however the decrease is little bit less than open solution condition over the first 360 hrs. Important parameters are summarized in table 4.7.

Table 4.7. Important features of caffeine in coffee stored in powder form at 23 °C.

Time (hr)	Conc. of Caffeine (mol/m^3)	% Remaining	% Caffeine in coffee
0	0.05689	100	1.24
72	0.05339	93.85	1.11
144	0.05018	88.2	1.04
216	0.04779	84	0.98
288	0.04568	80.3	0.93
360	0.04377	76.94	0.87

As observed from above quantitative summary; loss rate, remaining percentage and percentage of caffeine in coffee decreased with time and speed of decrease is very fast about the beginning for open air solution storage conditions than open air powder storage system. In case of open-air solution reaction takes place in water medium, whereas in open-air powder form the medium is rarefied air, which is more conducive for chemical reaction. Even though the resistance of powder form of coffee against chemical reaction (activation energy) is high at the beginning than solution form; while after speed of reaction increases with time more for powder form and as a result half life decay time is shortest for powder form storage than other conditions. Caffeine concentration decreased as a function of time. The decrease in concentration is slowed down as the storage time increased and directly proportional to initial concentration. This behavior is subjected to exponential decay decomposition. Thus the data, concentration versus time, was fitted using the mono exponential function.

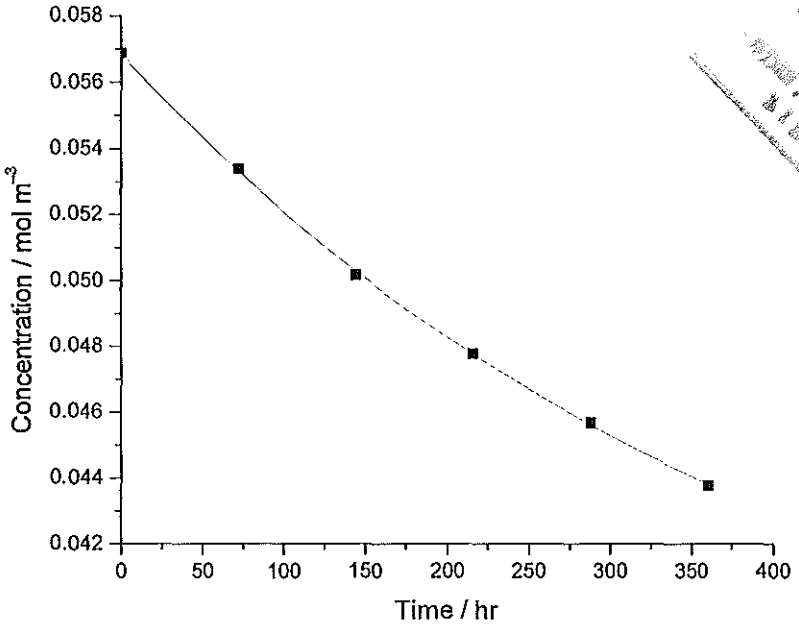


Figure 4.12. Concentration of caffeine extract versus storage time for powder form.

the magnitude of the factor that influence the rate of decomposition of coffee products; the content of active ingredient 'caffeine' also decisive factor. Thus, in this study caffeine was assumed as the most active coffee constituent responsible for effects of coffee like stimulating, bitter-tasting, mood altering etc. Most of our societies drink coffee in need of above-mentioned effects of coffee.

Data analysis involves plotting concentration of caffeine versus time and using appropriate algorithm derived from exponential decay and pseudo first order models to extrapolate the time at which certain percentage of caffeine lost from coffee so that coffee and coffee product after this point is ineffective (can not fit human consumption due to its low quality)

Because experiments concerned with raw coffee take an extended amount of time (products with inherently long shelf lives) extrapolation is often used (55). An interpolated value at a point out side the convex hull of an in put data set is reliable since the original data is consistent and the applied algorithm assumed to be appropriate.

4.5-5.1%. Thus, further investigation is necessary to unveil (reveal) thermal dependence of caffeine content in coffee of different origin; dependence of caffeine content in coffee as a function of length of roasting time; dependence of content of caffeine in coffee on its origin and shelf life determination of coffee using optical methods based on characteristics lose rate of other components of coffee in addition to caffeine.

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