CHAPTER ONE

Introduction

Food dyes are known to absorb and emit Light with different wavelength depending on the structure of the dye under study. Sunlight may cause thermal or photochemical effects in dyes.

1-1 Solar Radiation

Solar radiation is the total frequency spectrum of electromagnetic radiation produced by the sun. This spectrum covers visible light, near-visible radiation, ultraviolet radiation, infrared radiation, and radio waves. In terms of energy, sunlight at the earth's surface is around 52- 55%, infrared above 700 nm 43- 42% visible 400 to 700.5 - 3% ultraviolet below 400 nm. At the top of the atmosphere sun light is about 30% more intense, having about 8% ultraviolet (UV), with most of the extra UV consisting of biologically-damaging shortwave ultraviolet.*(Qiang, F u, 2003, Iqbal, M, 1983)*

The spectrum of the sun's solar radiation is close to that of a black body with a temperature of about 5.800 K. The sun emits electromagnetic radiation across most of the electromagnetic spectrum. Although the sun produces Gamma rays as a result of the nuclear fusion process, these super-high-energy photons are converted by internal absorption and the realization to lower-energy photons before they reach the sun's surface and are emitted out into space. The sun does not emit gamma rays. The sun does however emit X-rays, ultraviolet, visible light, infrared and even radio waves. The only direct signature of the nuclear process is the emission of neutrinos.

Although the solar corona is a source of extreme ultraviolet and X-ray radiation, these rays make up only a very small amount of the power output of the sun. The spectrum of nearly all solar electromagnetic radiation striking the earth's atmosphere spans arrange of 100nm to about 1mm (1000000 nm).

This band of significant radiation power can be divided into five regions in increasing order of wavelengths.

Ultraviolet C (UVC) range, which spans a range of 100 to 180 nm. The term ultraviolet refers to the fact that the radiation is at higher frequency than violet light (hence also invisible to the human eye). Owing to absorption by the atmosphere very little reaches the Earth's surface. This spectrum of radiation has germicidal properties, and is used in germicidal lamps.

Ultraviolet B (UVB) range spans 280 to 315 nm. It is also greatly absorbed by the atmosphere, and along with UVC is responsible for the photochemical reaction leading to the production of the ozone layer. It directly damages DNA and causes sun burn.

Ultraviolet A (UVA) spans 315 to 400 nm. This band was once held to be less damaging to DNA, and hence is used in cosmetic artificial sun tanning (tanning booths and tanning beds) and PUVA therapy for psoriasis. However, UVA is now known to cause significant damage to DNA via indirect routes (formation of free radicals and reactive oxygen species), and is able to cause cancer.*(Jessup, E, 1993)*

Visible range or light spans 380 to 780 nm. As the name suggests, it is this range that is visible to the eye. It is also the strongest output range of the sun's total irradiance spectrum.

Infrared range that spans 700 nm to 1.000.000 nm (1 mm). It is responsible for an important part of the electromagnetic radiation that reaches the Earth. It is also divided into three types on the basis of wavelength:

- Infrared –A: 700 nm to 1.400 nm.

-Infrared –B:1.400 nm to 300 nm.

-Infrared –C: 3.000 nm to 1 mm.

1-2 Light Interaction with Matter

It is the electrons in atoms and molecules that typically absorb and emit photons of light. it is worth noting that gamma rays are energetic enough

to interact with atomic nuclei and generate photons of light, but we will leave that to the physicists to pursue. When an electron absorbs low energy photons, like radio frequencies the "spin" of the electron is flipped. This effect is used in nuclear magnetic resonance imaging (NMR) and can also be used to generate the images from magnetic resonance imaging (MRI). When an electron absorbs infrared, visible and ultraviolet photons they change energy level. All electrons have a series of energy levels they can occupy. The lowest energy level is referred to as the "ground state". The highest level is the "ionization energy" or the energy required to completely remove the electron from the influence of the nucleus. In order for an electron to move from one level to a higher level it must absorb energy equal to the difference in the levels. Likewise , to move to a lower level the electron must give up energy equal to the difference (not for all cases).

There are a number of ways an electron can gain or lose energy. The ones of interest here are the absorption or emission of light. An electron can absorb a photon of light that strikes it only if that photon has the exact energy to change the electron to a higher allowed energy level. An electron already at a higher level can emit a photon of light having exactly enough energy to change that electron to one of its lower allowed levels. Note that an electron in the ground state cannot emit any photons as it already has the lowest possible energy.*(Skoog et al, 2007)*

1-2-1 Type of Spectroscopy

Spectroscopy is the study of the interaction of light with matter. There is two distinct aspects of this interaction that can be used to learn about atoms and molecules. One is the identification of the specific wave lengths of light that interact with the atoms and molecules. The other is the measurement of the amount of light absorbed or emitted at specific wavelengths. Both determinations require separating a light source into its component wavelengths. Thus, a critical component of any spectroscopic measurement

is breaking up of light into a spectrum showing the interaction of light with the sample at each wavelength.

Light interacts with matter in many ways. Two of the most common interactions are light that is absorbed by the atoms and molecules in the sample, Light that is emitted after interacting with the atoms and molecules in the sample.*(Pavia D L, Lampman G M, Kriz G S, 1998)*

1-2-1-1 Absorption Spectroscopy

Absorption spectroscopy is the study of light absorbed by molecules. For absorbance measurements, white light is passed through a sample and then through a device (such as a prism) that breaks the light up into its component parts or a spectrum. White light is a mixture of all the wavelengths of visible light. When white light is passed through a sample, under the right conditions, the electrons so the light coming out of the sample will be missing those wavelengths corresponding to the energy levels of the electrons in the sample. The result is a spectrum with black lines at the wavelengths where the absorbed light would have been if it had not been removed by the sample.

1-2-1-2 Emission Spectroscopy

Emission spectroscopy is the opposite of absorption spectroscopy. The electrons of the sample are promoted to very high energy levels by any one of a variety of methods (such as electric discharge, heat and laser light). As these electrons return to lower levels they emit light. By collecting this light and passing it through a prism, it is separated into spectrum. In this case, we will see a dark field with colored lines that correspond to the electron transitions resulting in light emission.

Spectroscopy finds wide application in the identification of chemical species.

1-2-1-3 Qualitative Spectroscopy

One of the most useful aspects of spectroscopy derives from the fact that the spectrum of a chemical species is unique to that species. Identical atoms and molecules will always have the same spectra. Different species will have different spectra. For this reason, the spectrum of a species can be thought of as a fingerprint for that species. Qualitative spectroscopy is used to identify chemical species to find a match.

1-4-1-4 Quantitative Spectroscopy

Quantitative spectroscopy is one of the quickest and easiest ways to determine how many atoms or molecules are present in a sample. This is because the interaction of light with matter is a stoichiometric interaction. At any given temperature. the same number of photons will always be absorbed or emitted by the same number of atoms or molecules in a given period of time. This makes spectroscopy one of the few techniques that can provide a direct measure of the number of atoms or molecules present in a sample.*(Hage D S, Carr J D,2011)*

Absorption spectroscopy is performed by passing light of all wavelengths through a sample and measuring how much of the light at each wavelength is absorbed. The interaction of atoms and molecules with water molecules make the absorbance of light in solutions a very complex phenomenon. By making absorbance measurement at various wavelengths and then plotting the result , one can create what is known as an absorbance spectrum .

1-2-2 Ultraviolet-Visible Spectroscopy

Ultraviolet-Visible Spectroscopy (Uv-Vis) refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region.

Ultraviolet spectrum are recorded by irradiating a sample with UV light of continuously changing wavelength. When the wavelength of light corresponds to the amount of energy required to promote a π- electron in an

unsaturated molecule to a higher level, energy is absorbed. The absorption is detected and displayed on a chart that plots wavelength versus percent radiation absorbed, molecules undergo electronic transition. This technique is complementary to fluorescence spectroscopy, in that fluorescence deals with transitions from the excited state to the ground state, while absorption measures transitions from the ground state to the excited state.

1-2-2-1 Principle of Uv-Vis Absorption

Molecules containing π-electron or non-bonding electrons (n-electron) can absorb the energy in the form of ultraviolet or visible light to excite these electrons to higher anti-bonding molecular orbital. The more easily excited the electrons (lower energy gap between the HOMO and the LUMO), the longer wavelength of light it can absorb.

1-2-2-2 Applications of Uv-Vis Spectroscopy

Uv-Vis Spectroscopy is routinely used in analytical chemistry for the quantitative determination of different analysis, such as transition metal ions, highly conjugated organic compounds, and biological macromolecules. Spectroscopic analysis is commonly carried out in solutions but solids and gases may also be studied.

Solutions of transition metal ions can be colored (such as absorb visible light) because d electrons within the metal atoms can be excited from one electronic state to another. The color of metal ion solution is strongly affected by the presence of other species, such as certain anions or ligands. For instance, the color of a dilute solution of copper sulfate is a very light blue; adding ammonia intensifies the color and changes the wave length of maximum absorption (λ_{max}).

Organic compounds, especially those with a high degree of conjugation, also absorb light in the UV or visible regions of the electromagnetic spectrum. The solvents for these determinations are often water for water-soluble compounds, or ethanol for organic soluble compounds.

While charge transfer complexes also give rise to colors, the colors are often too intense to be used for quantitative measurements. The Beer-Lambert law states that "the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and the path length". Thus, for a fixed path length, Uv-Vis Spectroscopy can be used to determine the concentration of the absorber in a solution.

The basic parts of a spectrophotometer are a light source, a sample holder, diffraction grating in monochromator or a prism to separate the different wavelengths of light and detector. The radiation source is often a tungsten filament (300-2500 nm), a deuterium arc lamp which is continuous over the ultraviolet region (190-400 nm), Xenon arc lamp which is continuous from 160-2000 nm, or more recently light emitting diodes (LED) for the visible wavelengths. The detector is typically a photomultiplier tube, a photodiode, a photodiode array or a charge-coupled device (CCD). Single photodiode detectors and photomultiplier tubes are used with scanning monochromators, which filter the light so that only light of a single wavelength reaches the detector at one time. The scanning monochromator moves the diffraction grating to "step through" each wavelength so that its intensity may be measured as a function of wavelength. Fixed monochromators are used with a charge coupled devices (CCDs) and photodiodes arrays. As both of these devices consist of many detectors grouped into one or two dimensional arrays, they are able to collect light of different wavelengths on different pixels or groups of pixels simultaneously.

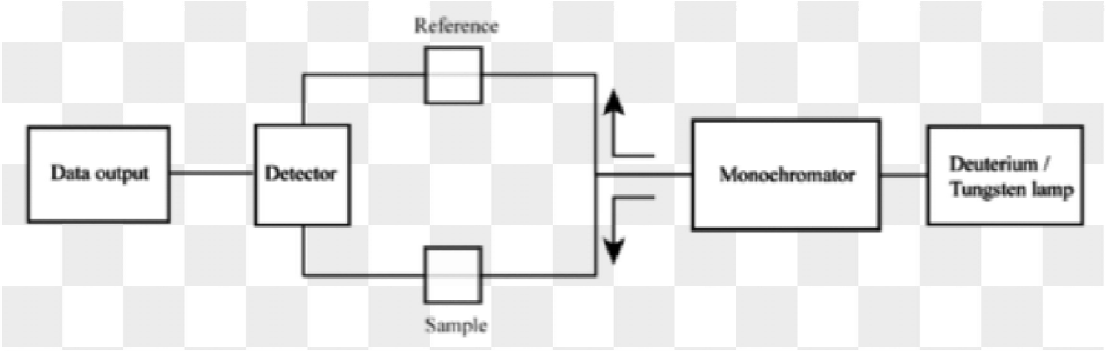

Figure 1-1. Schematic of Uv -Vis spectroscopy.

A spectrophotometer can be either single beam or double beam. In a single beam instrument, all of the light passes through the sample cell.

In a double beam instrument, the light is split into two beams before it reaches the sample. One beam is used as a reference; the other beam passes through the sample. The reference beam intensity is taken as 100% Transmission. and the measurement of absorbance is the ratio of the two beam intensities. Some double beam instruments have two detectors (photodiodes), the sample beam and reference beam are measured at the same time. In other instruments, the two beams pass through a beam chopper, which blocks one beam at a time. The detector alternates between measuring the sample beam and the reference beam is synchronism with the chopper. There may also be one or more dark intervals in the copper cycle. In this case, the measured beam intensities may be corrected by subtracting the intensity measured in the dark interval before the ratio is taken.

Sample for Uv-Vis Spectrophotometer are most often liquids, although the absorbance of gases and even of solids can also be measured. Samples are typically placed in a transparent cell, known as a cuvette. Cuvettes are typically rectangular in shape, commonly with an internal width of 1 cm (The width becomes the path length L in the Beer-Lambert Law). Test tubes can also be used as cuvettes in some instruments. The type of sample

container used must allow radiation to pass over the spectral region of interest. The most widely applicable cuvettes are made of high quality fused silica or quartz glass because these are transparent throughout the Ultraviolet, Visible and near infrared regions. Glass and plastic cuvettes are also common, although glass and most plastics absorb in the Ultraviolet which limits their usefulness to visible wavelength.*(Mark, P D, 2002)*

1-3 Beer-Lambert Law

The method is most often used in a quantitative way to determine concentrations of an absorbing species in solution, using the Beer-Lambert law:

$$A=log_{10}(I_0/I) = \varepsilon\, c\, L \qquad 1\text{-}1$$

Where A is the measured absorbance, in absorbance Units (AU), I_0 is the of the incident light at a given wavelength, is the transmitted intensity, L the path length through the sample and C the concentration of the absorbing species .

ε is molar absorptivity (extinction coefficient) with units of ($M^{-1}cm^{-1}$).

The Beer-Lambert Law is useful for characterizing many compounds but does not hold as a universal relationship for the concentration and absorption of all substances. A 2^{nd} order polynomial relationship between absorption and concentration is sometimes encountered for very large, complex molecules such as organic dyes (X ylenol Orange or Neutral Red, for example).

1-3-1 Spectral Bandwidth

Monochromaticity of light incident on the sample cell, which is the width of the triangle at one half of the peak intensity. A given spectrometer has a spectral bandwidth that characterizes how monochromatic the light is. It is important to have monochromatic source of radiation for analysis of the

sample. If this bandwidth is comparable to the width of the absorption features, then the measured extinction coefficient will be altered. In most reference measurements, the instrument bandwidth is kept below the width of the spectral lines. When a new material is being measured, it may be necessary to test and verify if the bandwidth is sufficiently narrow. Reducing the spectral bandwidth will reduce the energy passed to the detector and will; therefore, require a longer measurement time to achieve the same signal to noise ratio.

1-3-2 Stray Light

Another important factor is the purity of the light used. The most important factor affecting this is the stray light level of the monochromatic.

The detector used is broadband; it responds to all the light that reaches it. If a significant amount of the light passed through the sample contains wavelengths that have much lower extinction coefficients than the nominal one, the instrument will report an incorrectly low absorbance. Any instrument will reach a point where an increase in sample concentration will not result in an increase in the reported absorbance, because the detector is simply responding to the stray light. In practice the concentration of the sample or the optical path length must be adjusted to locate the unknown absorbance within a range that is valid for the instrument. Sometimes an empirical calibration function is developed, using known concentrations of the sample, to allow measurements into the region where the instrument is becoming non-linear.

As a rough guide, an instrument with a single monochromatic would typically have a stray light level corresponding to about 3 Absorbance Units (AU), which would make measurements above about 2 AU problematic. Amore complex instrument with a double monochromatic would have a

stray light level corresponding to about 6AU, which would therefore allow measuring a much wider absorbance range.

1-3-3 Deviations from the Beer-Lambert Law

At sufficiently high concentrations, the absorption bands will saturate and show absorption flattening. The absorption peak appears to flatten because close to 100% of the light is already being absorbed. The concentration at which this occurs depends on the particular compound being measured. One test that can be used to test for this effect is to vary the path length of the measurement. In the Beer–Lambert law, varying concentration and path length has an equivalent effect diluting a solution by a factor of 10 has the same effect as shortening the path length by a factor of 10. If cells of different path lengths are available, testing if this relationship holds true is one way to judge if absorption flattening is occurring.

Solutions that are not homogeneous can show deviations from the Beer-Lambert law because of the phenomenon of absorption flattening. This can happen, for instance some solution like copper (II) chloride in water Changes color at a certain concentration because of changed conditions around the colored ion (the divalent copper ion). For copper (II) chloride it means a shift from blue to green, which would mean that monochromatic measurements would deviate from the Beer-Lambert law.

1-3-4 Measurement Uncertainty Sources

The above factors contribute to the measurement uncertainty of the results obtained with Uv-Vis Spectrophotometry. If Uv-Vis Spectrophometry is used in quantitative chemical analysis then the results are additionally affected by uncertainty sources arising from the nature of the compounds and /or solutions that are measured. These include spectral interference caused by absorption band overlap, fading the color of the absorbing species (caused by decomposition or reaction) and possible composition mismatch between the sample and the calibration solution.

(Soovali, L E, 2006)

1-4 Infrared Spectroscopy

1-4-1 General Principle of Infrared Spectroscopy

In Uv-Vis Spectroscopy the absorption of visible or ultraviolet light can lead to an increase in the energy of the absorbing molecules due to electronic transitions. Molecules can also absorb other types of radiation, but these processes may involve different types of transitions than those that make use of a change in an electronic state. If a molecule absorbs infrared light (which has a lower energy than visible or ultraviolet light), this absorption is based on a change in the energy due to vibrations or rotations that are occurring in the molecules. A spectroscopic method that uses infrared light to study or measure chemicals is called Infrared spectroscopy (IR spectroscopy).

The simplest kind of bond vibration involves stretching of a bond between two atoms, For example, water is often depicted as Aromatic, bent molecule with a bond angle of about 105^0 and bond lengths of roughly 96 pm. This model implies that a water molecule is a static object, but in reality a water molecular is always undergoing some changes in it is bond angles and bond occur more energetically, and the energy levels that describe these vibrations occur at distinct values (quantized). The differences in energy present for electronic transitions. As a result, the energy for a photon that is needed to cause excitation in a vibration is also much smaller and is in the same range as is found for infrared light even smaller amounts of energy are needed to cause molecules to rotate more rapidly, corresponding to the energy present in microwave radiation. These small changes in rotational energies are often superimposed on the changes in vibrational energies that are seen in IR spectroscopy and lead to broadening of the observed absorption bands. This broadening occurs in a similar manner to the way in which vibrational and rotational transitions plus electronic transitions lead to broad absorption

bands for molecules in Uv-Vis spectroscopy. Most Uv-Vis absorption spectra exhibit one or two broad peaks, where as IR spectra might have dozens of very narrow peaks. This is because only one or two electronic transitions often dominate a Uv-Vis spectrum, but each molecule can have many ways of undergoing vibrational transitions to produce an IR spectrum.

IR spectra are most commonly represented as a plot of T% versus wave number (in units of micrometers). This way of plotting a spectrum is in contrast to Uv-Vis spectra, which are usually plotting in terms of absorbance versus wavelength (in units of nanometers). The result is that an IR spectrum has a very different appearance from a typical Uv-Vis spectrum. The former typically show a large number of peaks, each of which is quite narrow compared to the entire range of wavelengths in the spectrum.*(Shriner R L, Hermann C K F, Morrill T C 1997)*

1-4-2 Instrumentation for Infrared Spectroscopy

Typical system components IR spectroscopy is similar to Uv-Vis spectroscopy in that it requires a source of light, a mean for separating this light into different wavelengths, sample holder, and detector. However, the specific instrument components in IR spectroscopy are made of different materials and often operate on different principles than the devices that are used for Uv-Vis spectroscopy.

The source of light in IR spectroscopy is usually an inert rod that is heated to a much lower temperature than is used for light sources in visible spectroscopy. As are a result, the maximum absorbance that is now obtained through blackbody radiation will occur in the infrared region. Glass and fused silica are opaque at wavelengths greater than 2.5μm, so the glowing source must not be in a glass bulb or in a casing that is made of these substances. The heated material is either silicon carbide (is

called a globar), or is a mixture of rare-earth oxides (producing a device known as a Nernst glower).

Scanning Instruments and Fourier Transform Infrared Spectroscopy one difficulty in using IR spectroscopy is that CO_2 and H_2O in air both absorb IR radiation considerably and obscure the spectrum of the desired sample. For that reason, IR spectrophotometers are often double-beam devices in which the spectrum of air is subtracted from the spectrum of the sample, leaving only the spectrum of the desired sample.

Until recently, most IR instruments were double-beam scanning instruments. The wavelength was changed gradually as T% was measured and the resulting spectrum is plot. Amore common instrument found in modern laboratories is one that makes use of Fourier transform infrared (FTIR) spectroscopy. An FTIR instrument allows all wavelengths of IR radiation to fall on the sample simultaneously. Instead of separating the wavelengths in time or space, the wavelength dependence of T% is gained by use of a device called an interferometer, which causes positive and negative interference to occur at sequential wavelengths as a moving mirror changes the path length of the light beam. The initial output of the detector does not look anything like a spectrum, but this direct output is transformed into a spectrum by application of the mathematical process called a "Fourier transform". The major advantage of FTIR is in the speed with which a spectrum can be obtained, typically just a few seconds. This means a large number of spectra can be gathered in a short time. This high rate of data acquisition also makes it possible to combine a large number of spectra to help to remove random fluctuations in the signal "noise". The more spectra that are averaged, the better the signal-to- noise ratio will become. This approach, means that a good spectrum can be achieved for a small concentration of analyze and that a lower limit of detection for measurement of the analyte can be obtained.*(Hage D S, Carr J D, 2011)*

1-4-3 Applications of Infrared Spectroscopy

IR spectroscopy is most frequently employed for qualitative identification of nearly pure compounds. Because each compound gives several peaks, an IR spectrum of a mixture is very difficult to interpret. The groups of atoms is call "functional groups" have characteristic vibrational energies and characteristic IR absorption wavelengths that can be used in the process.

One can be even more certain as to the identity of a compound if its spectrum has been included in a library of IR spectra. A match between a measured spectrum of an unknown and a library spectrum is regarded as good evidence that the unknown substance is the same as the identity of the library spectrum. Modern IR instruments frequently come with a computer that contains a library of several hundred or thousand compounds that can be searched rapidly for agreement with a measured spectrum.*(Hardt P C, Schore N E, 2011)*

1-5 Food Coloring

It is said that "eat with our eyes as much as with our mouth". Food color is any substance that is added to food or drink to change its color; food coloring is used both in commercial food production and in domestic cooking.

In the European Union (EU) all food additives are given labeling codes commonly referred to as "E – number". With in food additive, 43 are food colors approved by EU which 9 of them are azo dyes.

Azo dyes are food additives that are added to food stuff mainly to make up for colors losses during food processing , to enhance natural colors, Azo dyes are widely used , not just food stuff , but also used in pharmaceutical products or cosmetics to be more stable than natural colors.

1-5-1 Purpose of Food Coloring

People associate certain colors with certain flavors, and the color of food can influence the perceived flavor in anything from candy to wine. For this reason, food manufacturers add dyes to their products. Sometimes the aim is to simulate a color that is perceived by the consumer as natural, such as adding red coloring to glace cherries (which would otherwise be beige), but sometimes it is for effect, like the green ketchup that Heinz launched in 2000.

While most consumers are aware that food with bright or unnatural colors (such as the green ketchup, or children's cereals such as Fruit Loops) likely contain food coloring, far fewer people know that seemingly "natural" foods such as oranges and salmon are sometimes also dyed to mask natural variations in color. Color variation in foods throughout the seasons and the effects of processing and storage often make color addition commercially advantageous to maintain the color expected or preferred by the consumer. Some of the primary reasons include

- Offsetting color loss due to light, air, extremes of temperature, moisture and storage condition.
- Masking natural variations in color.
- Enhancing naturally occurring colors.
- Providing identity to foods.
- Protecting flavors and vitamins from damage by light.
- Decorative or artistic purposes such as cake icing.

Food coloring are tested for safety by various bodies around the world and sometimes different bodies have different views on food color safety. In the United States, "FD&C" number (which generally indicates that the Food and Drug Administration (FDA) has approved the colorant for use in foods, drugs and cosmetics) are given to approve synthetic food dyes that do not

exist in nature, while in the European Union, "E numbers" are used for all additives, both synthetic and natural, that are approved in food applications.

Most other countries have their own regulations and list of food colors which can be used in various applications, including maximum daily intake limits.

1-6 Synthetic Food Colors

Also known as Artificial Food Colors are manufactured chemically and are the most commonly used dyes in the food, pharmaceutical and cosmetic industries.

Seven dyes were initially approved under the pure food and Drug Act of 1906, but several have been delisted and replacements have been found.*(Wise, G B,2013)*

Current seven

In the USA, the following seven artificial colorings are permitted in food under act of 2007:

- FD&C Blue No.1_Brilliant Blue FCF, E133 (Blue shade).
- FD&C Blue No.2_Indigotine, E132 (Dark Blue shade).
- FD&C Green No.3_Fast Green FCF, E129 (red shade).
- FD&C Red No.4_Allura Red AC, E129 (Red shade).
- FD&C RedNo.3_Erythrosine, E127 (Pink shade).
- FD&C Yellow No.5_Tartrazine, E102 (Yellow shade).
- FD&C Yellow No .6 _Sunset yellow FCF, E110 (orange shade).

In this research I will discuss the following types:

1-6-1 Tartrazine E102

Is a synthetic lemon yellow azo dye primarily used as a food coloring It is also known as E number E102, FD&C Yellow5,Acid yellow23,Food Yellow4 and trisodium1-(4-sulfonatopheny)-4-(4-sulfonatophenylazo)-(5pyrazolone)- (3- carboxylate).

Tartrazine is a commonly used color all over the world, mainly for Yellow.

products containing Tartrazine like ice cream, hard candy cake mixes, corn chips, soft drink like energy and sports drinks, jam, jelly, also it used in personal care and cosmetics products like soaps , moisturizers, lotions and perfumes.

1-6-1-1 Health Effects

Tartrazine appears to cause the most allergic and intolerance reactions of all the azo dye, particularly among asthmatics and those with an aspirin intolerance.

Symptoms from Tartrazine sensitivity can occur by either ingestion or cutaneous exposure to a substance containing Tartrazine. Symptoms appear after period of time ranging from 6 to 14 minutes with Tartrazine sensitivity, but progress has been made in reducing people's Tartrazine sensitivity in study of people who are simultaneously sensitive to both a spirin and Tartrazine.14 hours, Total avoidance is the most common way to deal.*(Abbey, J. et at 2013)*

1-6-2 Sunset Yellow E110

Is also known as Orange yellow S, is a petroleum-derived orange (azo dye). sunset is used in food, cosmetics and drugs. For example, it's used in candy, desserts and preserved fruits.

1-6-2-1 Health Effect

The acceptable daily intake is 0-4 mg/kg under both European Union (EU). Sunset yellow has no carcinogenicity, genotoxicity or developmental toxicity in the amounts at which it is used. It has been claimed since late1970s under the advocacy of Benjamin Feingold that sunset yellow causes food intolerance and ADHD (Attention Deficit Hyperactivity Disorder) like behavior in children but there is no scientific evidence to support these broad claims. It is possible that certain food coloring may act

as atrigger in those who are genetically predisposed, but the evidence is weak. *(Feingold, B F. 1973)*

1-6-3 Allura Red E129

Is a red azo dye, it is used as a food dye and has the E number E129. The compound is a derivative of naphthalene, it is dark red powder. It usually comes as a sodium salt, but can also be used as both calcium and potassium salts. These salts are soluble in water, .Allura red is a very popular dye throughout the world. Annual production in 1980 was greater than 2.3 milion kilograms. *(Meggos H, 1995)*

1-6-3-1 Health Effect

There is some evidence to support claims that certain food coloring agents may cause food intolerance and ADHD-like behavior in some childern; others say that certain food coloring may act as an ADHD trigger in those who are genetically predisposed, but the evidence is weak and not supported by the United States Food and Drug Administration (FDA).

In the United States, Allura Red is approved by the FDA for use in cosmetics, drugs and food. When prepared as a lake it is disclosed as red 40 lakes or red 40 Aluminum lakes. It is used in some tattoo inks and is used in many products, such as soft drinks, children's medications, and cotton candy. It is by far the most commonly used red dye in the United States. *(Feingold, B F 1973)*

1-6-4 Green E142

As a food dye, it has E number (E142). It can be used in mint sauce, desserts, gravy granules, sweets, ice creams and tinned peas. Green is prohibited as a food additive in Canada, United States, Japan and Norway. It is approved for use as a food additive in the Europe, Australia and New Zealand.

Green is a vital dye, meaning it can be used to stain living cells. It is used in disorders of the eye's surface.

1-6-4-1 Health Effect

Green may cause allergic reactions and is one of the colorants that the Hyperactive Children's Supports group recommends to be eliminated from the diet of children.*(Abbey J. et at. 2013)*

1-7 Literature Review

-Toxicological effects of food additives Azo dyes, studies (Tartrazine (E102), Sunset yellow (E110) and Allura red (E129)).

It has been suggested exposure to azo dyes are associated with increased risk for hyperactivity effects on child behavior or increase ADHD (Attention deficit Hyperactivity Disorder). Commissioned by Britain food standards Agency (FSA) tested the effects of azo dyes mixture with Sodium benzoate, A common preservative in 3 –years old and 9 –years old children. Department of biomedical sciences and veterinary public health 2014.

-Spectrophotometric analysis of food dyes, since the early 1900s the food and Drug Administration (FDA) has narrowed the list of approved artificial dyes for foods, drugs and cosmetic (formerly many dyes and other food additives contained toxic substances such as heavy metals, arsenic and formaldehyde. These were banned as too dangerous for human consumption).

-Effects of sunlight exposure on some foods organic dyes using spectral analysis absorption ,different food dyes (Tartrazine) divided into two groups , first group was exposed to sun light for ten days while the second group was not exposed to the sun the absorbability of each groups have been investigated using Uv-Vis devise.

1-8 The Study Objective

The main objective of this study is to obtain the compound of artificial food dyes and the changes will occurs of it's when exposed to sunlight to fourteen days.

1-9 OutLines

Thesis composed of four chapters, chapter two covers the experimental part of this work and The material used. In addition to setup used.

Chapter three presents the results and calculations of Molar absorbitivity.

Chapter four presents the discussions, followed by conclusions and recommendations.

CHAPTER TWO

Experimental Techniques

This chapter reviews the experimental techniques and equipment that were used in this study. Samples were prepared and put in the different devices Fourier Transformation Infrared Spectroscopy (FTIR), Ultra violet- Visible Spectroscopy (UV-VIS).

2-1 Materials

Food Dyes (Sunset yellow E110, Tartrazine E102, Allura red E129 and Fast green E142) which are brought from Ahmed Adel Trading Est.

2-2 Absorption spectroscopy (UV-VIS Spectroscopy)

UV -VIS spectroscopy is one of the simplest and yet most useful optical techniques for studding optical and electronic properties of nano materials. Most of the organic molecules and functional groups are transparent in the portion of the electromagnetic spectrum which call the ultraviolet and visible regions. That is the region, where wavelength ranges from 190nm to1100nm.

Measurement of light absorption due to electronic transition in the sample. The atoms and molecules absorb energy and the electrons is travel from ground state to a higher state (excited state).

The electromagnetic radiation that is absorbed has energy equal to the energy difference between the excited and ground state. The operating principle is based on beer's law, the absorbance or optical density as a function of wavelength, is related to the incident light intensity and transmitted light intensity, concentration of a solution sample, path length of the sample, absorption coefficient and molar absorptivity (formerly

known as molar extinction coefficient). Used UV mini, UV-VIS spectrophotometer from Shimadzu, SED-SPEC-48 (Japan).

2-3 Fourier Transforms Infrared Spectroscopy (FTIR)

Infrared is a common vibrational spectroscopy technique useful for determination of compounds functional groups. Compounds having covalent bond, whether organic or inorganic absorbs various frequencies of electromagnetic radiation in the infrared region of the electromagnetic spectrum. In terms of wave numbers, the vibrational infrared extends from about 4000 to 400 cm^{-1}.

In Infrared absorption process molecules are excited to a higher energy state when they absorb infrared radiation. Radiation in this energy range corresponds to the range encompassing the stretching and bending vibrational frequencies of the bonds in most covalent bonds. As for many harmonic oscillator, when a bond vibrates, it's energy of vibration continuously and periodically changing from kinetic to potential energy and back again. The total amount of energy is proportional to the frequency of vibration. Used Shimadzu- IR Affinity from Japan.

2-4 Methods

The food dyes (Sunset yellow E110, Tartrazine E102, Allura red E129, Fast Green E142) in the form of coarse powder were crushed by a hand mortar, and divided into three groups A, B and C.

Group A that was not exposed to sun light (control), group B was exposed to sun light for seven days and group C was exposed to sun light for fourteen days.

These dyes were weight about 0.02g with a sensitive balance and mixed well with 10 ml of distilled water to obtain a homogenous solution for each type of these dyes, took a small sample from the solution and placed in the UV glass holder and studied the absorbency of these dyes and

painted her curves by computer, spectra treated using origin 8.6 computer program .

The samples were prepared for FTIR by added little powder from the dyes, were compressed to make the cylindrical shape which can be analyzed. The sample prepared then placed in the device and studies the chemical structures of these dyes.

CHAPTER THREE

Results and Analysis

This chapter summarizes the data obtained during this work. Absorption spectra using UV-VIS Spectrometer are shown in figures 3-1 to 3-4 and transmission spectra using FTIR Spectrometer are shown in figure 3-5 to 3-8. Data analyzed using Gaussian fitting spectra, collected information (peak number, peak position, wavelength shift, functional group and band position) are taboulated.

3-1 UV –VIS Results

UV-VIS Spectroscopy results for four types of dyes E102, E110, E129, E142, control before exposure to sun light, seven days and fourteen days after exposure to the sunlight.

Figure 3-1, shows the absorption spectra of Sunset Yellow E110 before exposure to sun light (called control) and after exposure to sun light for seven days and fourteen days, found that the absorbency of Sunset yellow dye before sun exposure is 2.15 a.u, after seven days of exposure to sun's rays became 2.28 a.u and after fourteen days of exposure to sun's rays became 2.72 a.u, in another peak the absorbency before sun exposure is 1.4 a.u, after seven days of exposure to sun's rays became 1.5 a.u and after fourteen days of exposure to sun's rays became 2.05 a.u, it absorbed at 480nm and293nm. In the following table shows shift in the range of absorption (wavelengths) this is mean occurs a change in the band gap of these compounds.

3-1-1 Sunset yellow E110

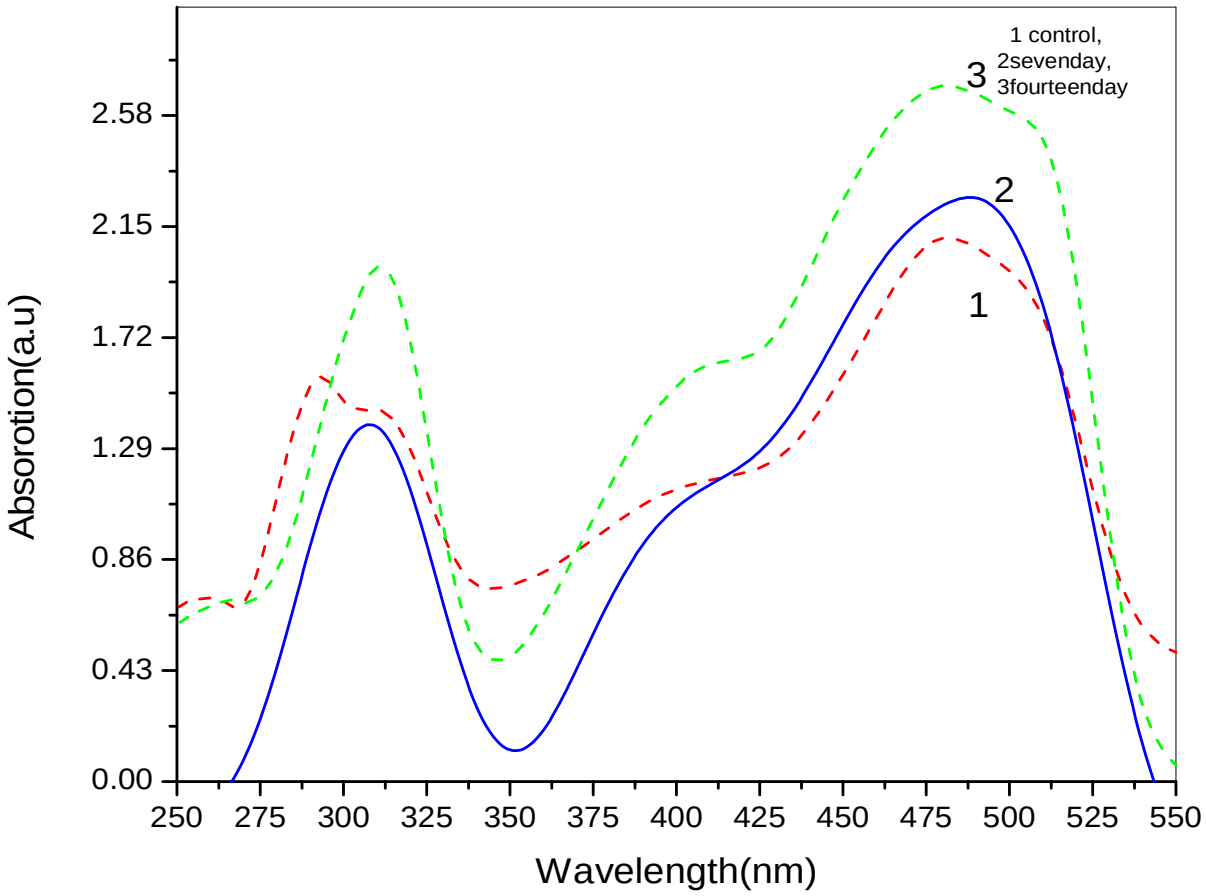

Figure 3-1 Absorption spectra of Sunset Yellow E110 before and after sunlight exposure.

3-1-2 Sunset yellow E110

Parameter obtained from figure 3-1

λ (nm)	Peak 1	shift	λ (nm)	Peak 2	shift	λ (nm)	Peak3	Shift
Control	480.94	-	control	407.1	-	control	293.5	-
7 days	488.88	Red shift	7 days	397	Blue shift	7 days	3078	Red shift
14days	478.10	Blue shift	14days	405	Blue shift	14days	310.48	Red shift

3-1-3 Tartrazine E102

In figure 3-2 shows the absorption spectra of Tartrazine E102 before exposure to sun light (called control) and after exposure to sunlight for seven days and fourteen days. found the absorbency of Tartrazine dye before sun exposure is 2.87 a.u, after seven days of exposure to sun's rays became 3.12 a.u and after fourteen days of exposure to sun's rays became 3.88 a.u, in another peak the absorbency before sun exposure is 0.61 a.u, after seven days of exposure to sun's rays became 0.73 a.u and after fourteen days of exposure to sun's rays became 0.87 a.u, it absorbed at 408nm and 288nm.

In the following table shows shift in the range of absorption wavelengths this is mean occurs a change in the band gap of these compounds.

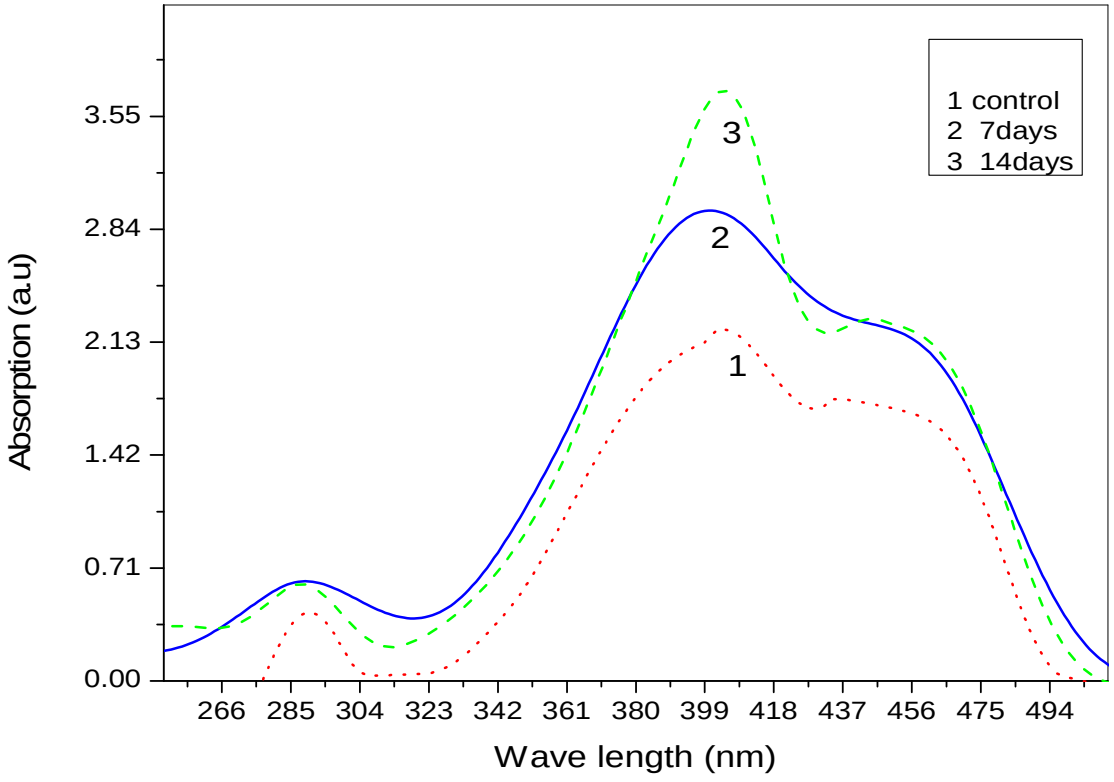

Figure 3-2 Absorption spectra of Tartrazine E102 before and after sunlight exposure.

3-1-4 Tartrazine E102

Parameter obtained from figure 3-2.

λ (nm)	Peak 1	shift	λ (nm)	Peak 2	shift	λ (nm)	Peak 3	shift
control	428.8	-	control	409.84	-	control	288.24	-
7days	449.6	Red shift	7 days	398.64	Blue shift	7 days	290.32	Red shift
14days	436.4	Red shift	14days	404.24	Blue shift	14days	286.8	Blue shift

3-1-5 Allura Red E129

In figure 3-3 shows the absorption spectra of Allura Red E129 before exposure to sun light(called control) and after exposure to sun light for seven days and fourteen days. found the absorbency of Allura Red dye before sun exposure is 2.02 a.u, after seven days of exposure to sun's rays became 2.06a.u and after fourteen days of exposure to sun's rays became 2.46 a.u, in another peak the absorbency before sun exposure is 0.92 a.u, after seven days of exposure to sun's rays became 1.39 a.u and after fourteen days of exposure to sun's rays became 1.42 a.u, it is absorbed at 506 nm, 329 nm and 293 nm. In the following table shows shift in the range of absorption (wavelengths) this is mean occurs a change in the band gap of these compounds of this dye.

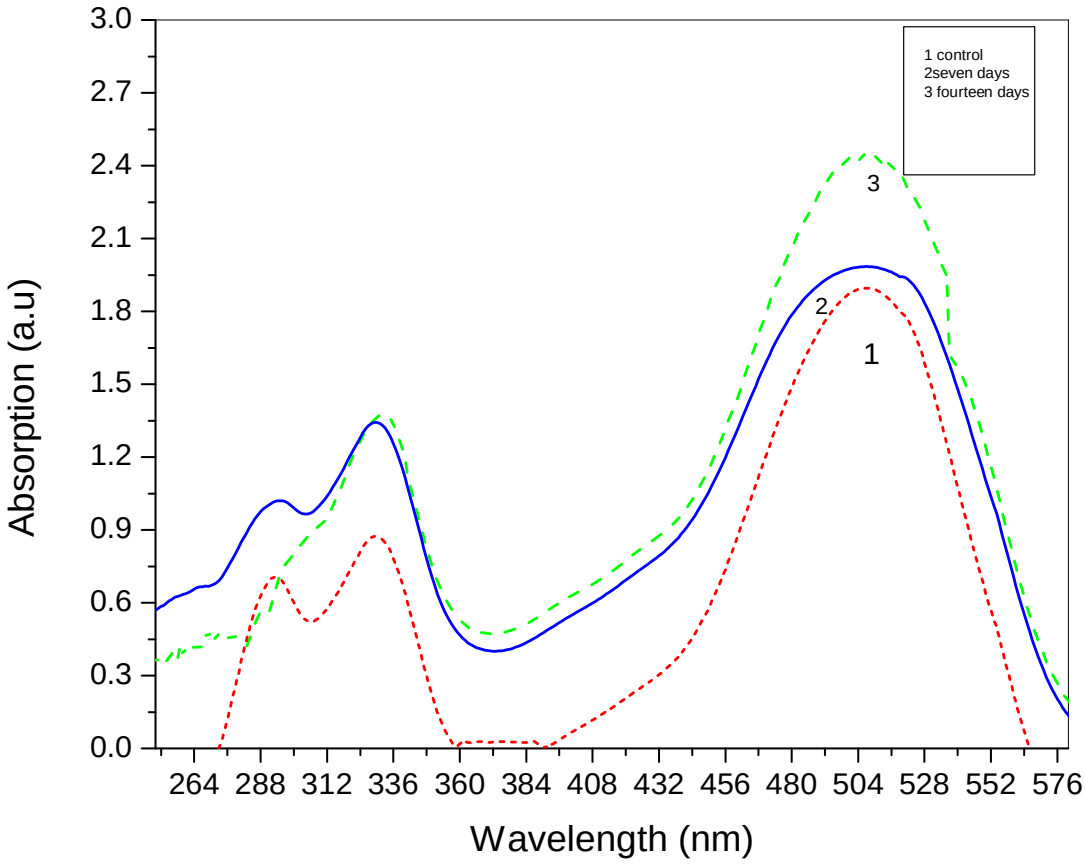

Figure 3-3 Absorption spectra of Allura Red E129 before and after sunlight exposure.

3-1-6 Allura Red E129

Parameter obtained from figure 3-3 above.

λ (nm)	Peak1	Shift	λ (nm)	Peak 2	shift	λ (nm)	Peak 3	shift
control	506.50	-	control	329.4	-	control	293.5	-
7days	508.08	Red shift	7days	331.05	Red shift	7days	294.7	Red shift
14 days	504.92	Blue shift	14 days	332.31	Red shift	14 days	288	Blue shift

3-2-7 Fast Green E142

In figure 3-4 shows the absorption spectra of Fast Green E142 before exposure to sun light (called control) and after exposure to sun light for seven days and fourteen days, found the absorbency of Fast Green dye before sun exposure is 1.21 a.u, after seven days of exposure to sun's rays became 1.6 a.u and after fourteen days of exposure to sun's rays became 1.87a.u, in another peak the absorbency before sun exposure is 0.98 a.u, after seven dyes of exposure to sun's rays became 1.36 a.u and after fourteen days of exposure to sun's rays became 1.53 a.u, in the third peaks the absorbency before sun exposure is 0.1 a.u, after seven days of exposure to sun's rays became 0.5 a.u and after fourteen days of exposure to sun's rays became 0.65, it's absorbed at 627 nm , 417 nm and 291 nm

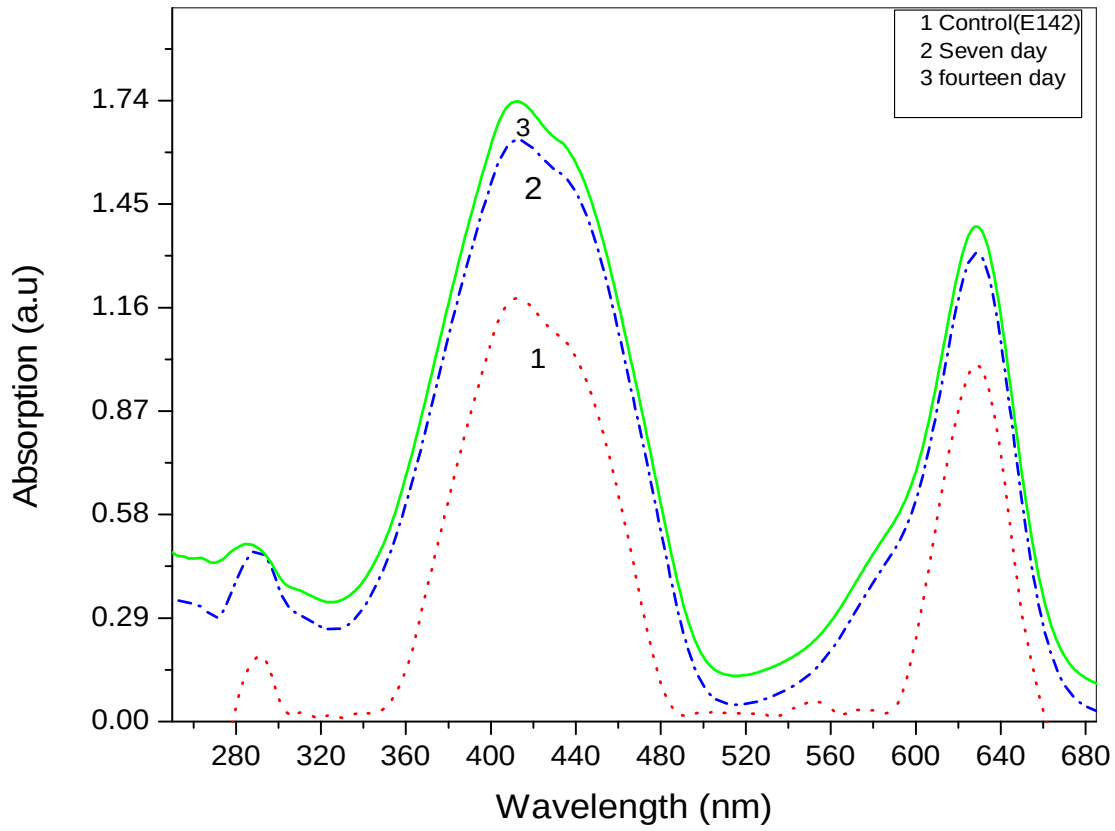

Figure 3-4 Absorption spectra of Fast Green E142 before and after sunlight exposure.

3-1-8 Fast Green

Parameter obtained from figure 3-4 avove

λ (nm)	Peak 1	shift	λ (nm)	Peak 2	shift	λ (nm)	Peak 3	shift
control	417.88	-	control	291	-	control	627.99	-
7 days	413.41	Blue shift	7 days	288.76	Blue shift	7 days	629.56	Red shift
14 days	411.83	Blue shift	14 days	285.92	Blue shift	14 days	626.41	Blue shift

3-2 FTIR Results

3-2-1Sunset Yellow E110

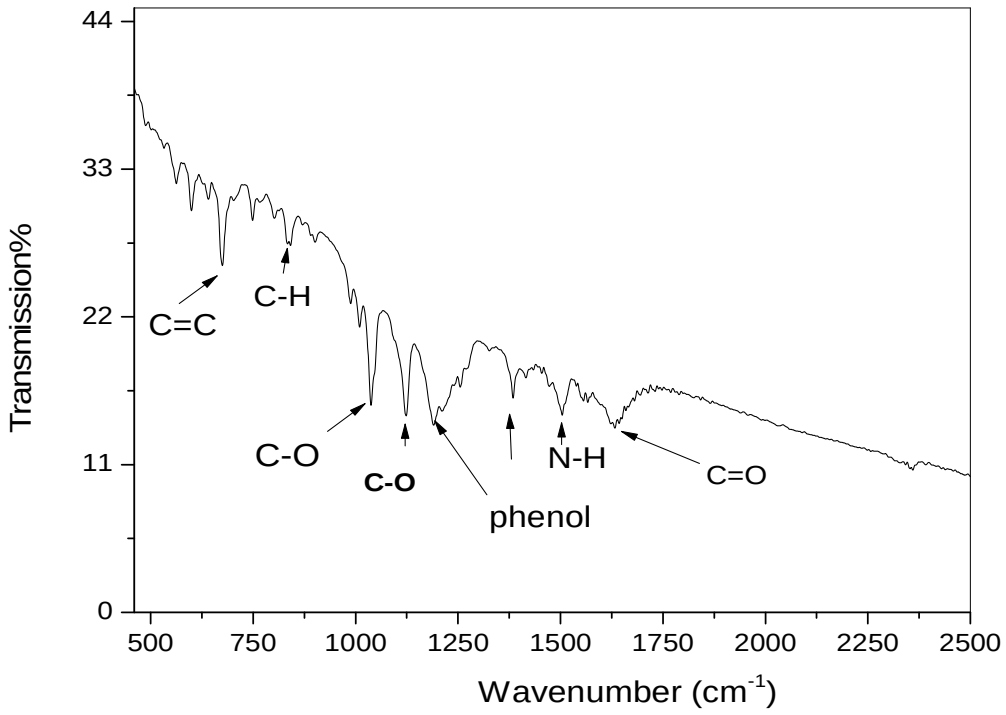

Figure 2-1 FTIR. spectroscopy result for sunset yellow E110.

3-2-2 Sunset Yellow E110

Table obtained functional group to Sunset yellow E110,band position, type of bond and intensity appearance of any group.

Functional group	Band position (cm^{-1})	Type of bond	Relative Intensity
Aromatic	560, 608, 748, 896	C-H	Strong
Cis-alkens	677.33	C=C	Medium
Alcohols	1036.44 ,1130	C-O	Strong
Phenol	1185 ,1255	C-O	Strong
Carboxylic acid	1388.4	C-O	Weak
Aromatic	1505, 1568	C-H	Medium
Aromatic	1630	C=O	Medium

3-2-3Tartrazine Yellow E102

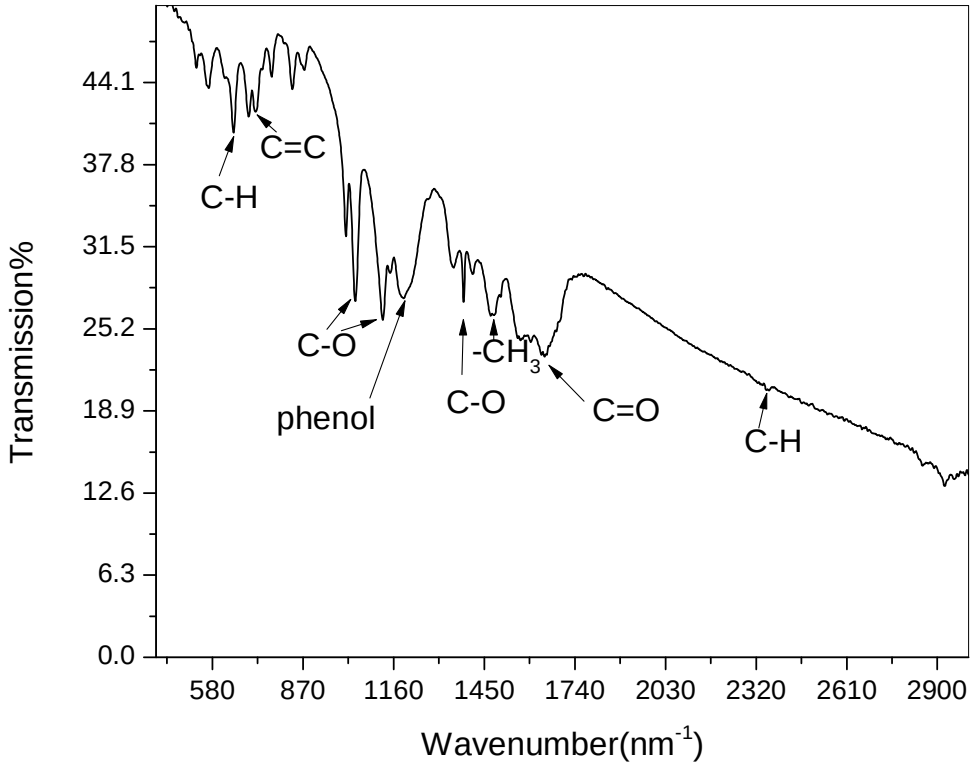

Figure 2-2 FTIR spectroscopy result for Tartrazine yellow E102.

3-2-3 TartrazineE102

Table obtained functional group to Tartrazine E102,band position, type of bond and intensity appearance of any group.

Functional group	Band position (cm^{-1})	Type of bond	Relative Intensity
Aromatic	568, 647, 771, 1404	C-H	Strong to medium
Cis-alkene	709.33	C=C	Strong
Alcohols	1045, 1123	C-O (1^0,2^0)	Strong and Broad
Phenol	1192.88	C-O	strong
Carboxylic acid	1356	C-O	Strong
Aromatic	1473.77	$-CH_3$	Medium
Amides	1639.11	C=O	Strong

3-2-5Allura red (E129)

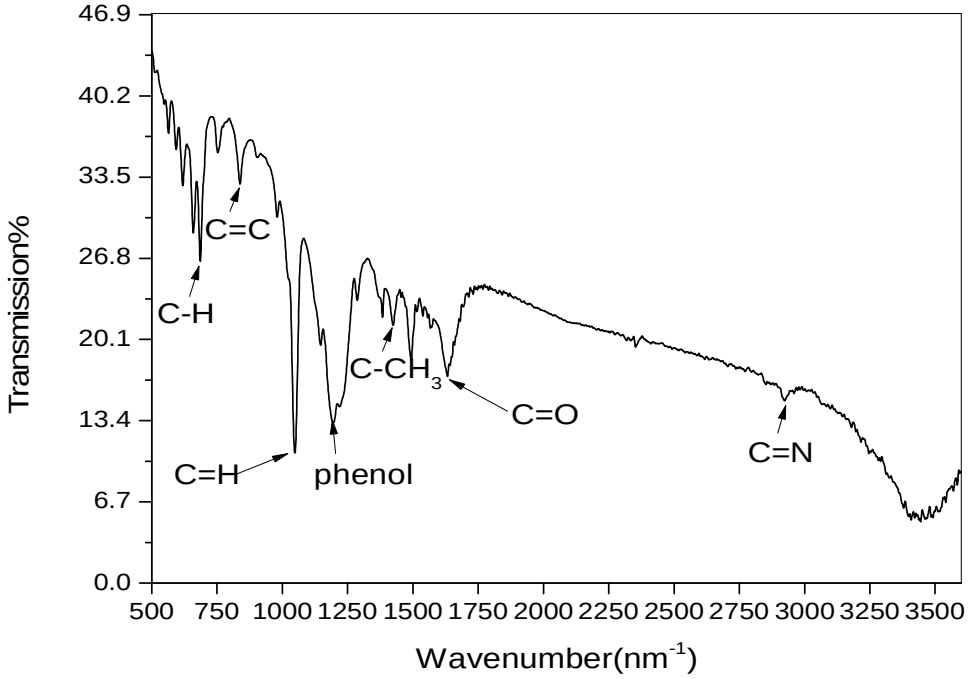

Figure 2-3 FTIR spectroscopy result for Allura Red E129.

3-2-6 Allura Red E129

Table obtained functional group to Allura red E129,band position, type of bond and intensity appearance of any group.

Functional group	Band position (cm^{-1})	Type of bond	Relative Intensity
Aromatic	480, 568, 656, 755, 832,1044	C-H	Strong
Trans-alkene	974	C=C	Medium
Phenol	1280 , 1378	C-O	Strong
Amines	1562	N-H	Medium
Aromatic	2924	C=N	Weak

3-2-7 Fast Green E142

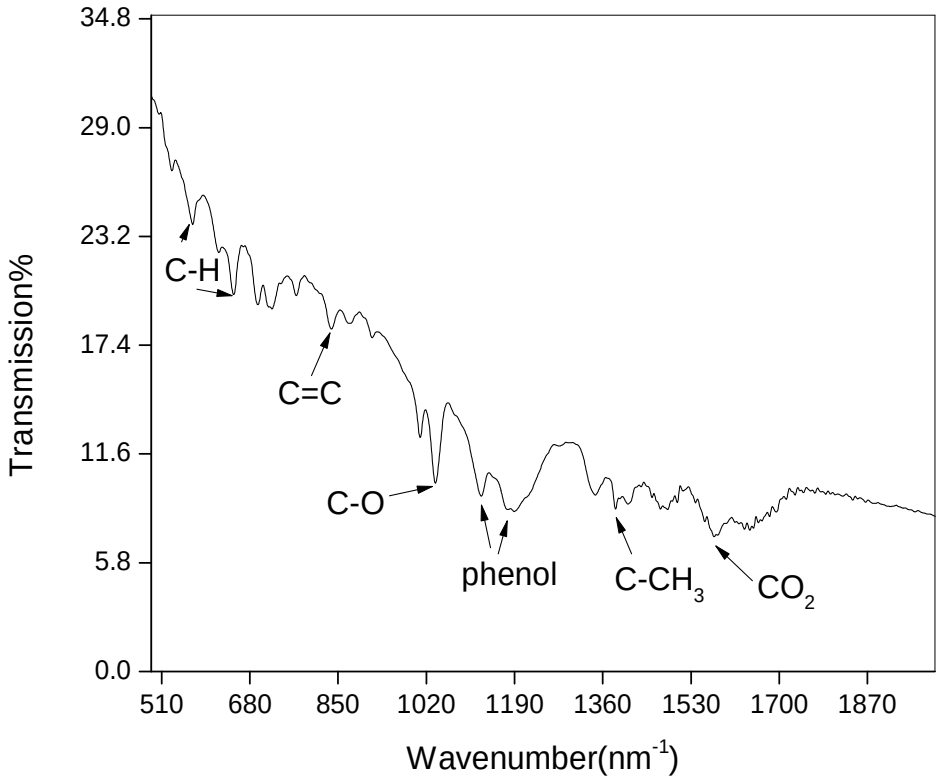

Figure 2-4 FTIR spectroscopy result for the Fast Green E142.

3-2-8 Fast Green E142

Table obtained functional group to Fast Green E142,band position, type of bond and intensity appearance of any group.

Functional group	Band position (cm^{-1})	Type of bond	Relative intensity
Aromatic	568, 648, 718, 770	C-H	Strong
Aromatic	842 ,868	C=C	Strong
Alcohols	1034, 1122	C-O	Medium
Phenol	1192, 1334	C-O	Strong and broad
Alkanes	1378, 1474	$C-CH_3$	Weak

Alkenes	1642	C=O	Medium

3-3 Analysis

3-3-1 the Ultraviolet-Visible Spectroscopy (Uv-Vis)

The UV -Vis optical absorption spectra of four samples for any one type of food dyes (Sunset yellow E110 ,Tartrazine E102 ,Allura Red E129 and Fast Green E142) before and after exposures of the sunlight .

Observed increased of absorbance rate, this increase is due to increase the concentration of dyes solution because the sun's rays is evaporated the water from the dyes solution, increase of concentration mean increase of the effects of human body. when the duration of exposure to sun light is increase is expected a larger increased in absorbability and occurs a changed in the geometry structure, that is mean changed in the band gap of these compounds , resulting in to a shifted in the range of absorption.

3-3-2 Calculation the Molar Absorptivity(extinction coefficient)

Calculated the Molar Absorptivity to measured the concentrations of dyes soluation after seven days and fourteen days under exposure to sun light.

3-3-2-1 Sunset yellow E110

The weight of this dye is 0.02 g, The Molar mass of it is 452.36 g/mol, so the weight of this dye by mol is:-

$C = 0.02/452.36 = 4.4217 \times 10^{-6} mol$

To measure Molar Absorptivity use the Beer Lambert Law:-

$A = \varepsilon C L$

$\varepsilon = A / C L$

Where:

ε is Molar absorpitivity ($mol^{-1}cm^{-1}$),

A = absorbance (a .u),

C = concentration (mol)and L = path length (cm).

λ_{max} (nm)	Molar absorptivity ($mol^{-1}cm^{-1}$)×10^4
480.94	4.86
293.50	3.17

3-3-2-2TartrazinE102

The weight of this dye is 0.02 g, The Molar mass of it is 534.36 g/mol, so the weight of dye by mol is:-

C = 0.02/534.36 = 3.7427×10^{-6} (mol)

λ_{max} (nm)	Molar absorptivity ($mol^{-1}cm^{-1}$)×10^4
409.84	7.67
288.24	1.629

3-3-2-3Allura Red E129

The weight of this dye is 0.02 g, The Molar mass of it is 496.42 g/mol, so the weight of this dyes by mol is:-

C =0.02/496.42 =4.0288×10^{-6}(mol).

λ_{max} (nm)	Molar absorptivity ($mol^{-1}cm^{-1}$)×10^4
506	5.01
329	2.28
293	1.22

3-3-2-4 Green E142

The weight of this dye is 0.02 g, The Molar mass of it is 576.62 g/mol, the weight of this dyes by mol is:-

C = 0.02/576.62 =3.4684×10^{-6}(mol).

λ_{max} (nm)	Molar absorptivity (mol^{-1} cm^{-1})$\times10^4$
627.99	2.83
417.83	3.49
291	2.88

3-3-3 The Fourier Transform Infrared (FTIR)

An infrared spectrum represents a fingerprint of a sample with absorption peaks which correspond to the frequencies of vibrations between the bonds of the atoms making up the material. Because each different is unique combination of atoms, no two compounds produce the exact same infrared spectrum, the amount of transmission of the peaks in the spectrum is a direct indication of the amount of material present.

We found in all food dyes in their compound PHENOL, ELCOHOLS that is mean the cumulative effect of this food dyes is toxic.

CHAPTER FOUR

Discussion and Recommendations

Four types of artificial food dyes had been exposed to the sunlight. The samples were studied using Fourier Transformation Infrared spectroscopy and UV-VIS spectroscopy techniques.

Sunset yellow E110 in UV-VIS spectrometer shows the peak at 293.5 nm this peak is conjugation of benzene atoms (phenol), simple substituted benzenes absorb between 250 – 300 nm, the absorbency of peak is higher that is mean the concentration of phenol in this dye is high.

Tartrazine E102 in UV-VIS spectrometer shows the absorbency of this peak of phenol at 288.24 nm is lower, that's mean the concentration of it in this dye is low.

Allura Red E129 in UV-VIS spectrometer shows the absorbency of this peak of phenol at 293nm is higher, that's mean the concentration of it in this dye is high.

Fast Green E142 in UV-VIS spectrometer shows the of absorbency of this peak of phenol at 291nm is lower, that is mean the concentration of it in this dye is low.

In FTIR results in all samples were observed functional groups (Aromatic, Alcohols and Phenol (chemical benzene)). This Artificial food dyes the cumulative effect of it is toxic.

This is food dyes is dangers because:

- Contain the chemical benzene.
- Possible carcinogen.

Estrogen Enhancers, Sunset yellow and Tartrazine have been shown to behave like estrogen in the human body. This bad Because high level of

estrogen, regardless of the source, can contribute to breast cancer, among other highly undesirable effects.

Conclusions

- Study showed that the spectra of food dyes tested change during sunlight exposure. This change indicates to increase of concentration of dye probably due to thermal effects.
- Concentration increases with the increase of exposure time.
- FTIR showed Alcohol and Phenol molecules, probably due to photochemical interactions between the dyes and sunlight.
- More studies are needed in this area.

REFERENCES:

Abbey, J et at (2003), Encyclopedia of food safety, Hazards and Diseases.

Feingold, B. F, (1973),Introduction to clinical allergy charlesc Thomas ISBNO398-02797-8

Hage, D. S, Carr J. D,(2011), Aalytical Chemistry and Quantitative analysis (University of Nebraska Lincoln, 2011 Upper Saddle River New Jersey 07548).

Iqbal, M. (1983), Introduction to Solar Radiation.

Jessup, E.(1993), A Brief History of the Solar Radiation Program.

Leo, J.B, (1972), Direct Solar Radiation on Various Slopes from 0 to 60 Degree(North latitude USA Retrieved 15 Jan 2014).

Liu, B.Y. H, (1960), The Interrelation Ship and Characteristic Distribution of Direct, Diffuse, and total Solar Radiation.

Wise, G. B,(2013), What is Food Coloring Made of.

Pavia, D.L, Lampman, G.M, Kriz, G. S, (1998), Introduction to Spectroscopy.(Department of Chemistry Western Washington University Bellingham).

Qiang, F U, James R, (2003), Radiation. Encycopedia of Atomospheric sciences. In Holton 5[Rad-s]. Amsterdam; Academic Press.

Rajeev, J B M, Sharma Nidhi, (2013). Electrochemical Studies on A Pharmaceutical Azo Dye.

Roger, W M, (2004). Analytical Methods for Food Additives.

Shhriner, R.L, Hermann, C.K.F , Morrill, T. C,(1997), The Systematic Identification of Organic Compound (7thed, Newyork).

Skoog et al, (2007). Principles of Instrumental Analysis (6^{th}Ed).

Soovali, L E, (2006). Uncertainty Sources in Ultra-violet Spectrophotometric Measurment.