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A REVIEW ON EVALUATION OF CAFFEINE CONTENT FROM DIFFERENT BRANDS OF MARKETED TEA POWDER

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Abstract:
Caffeine is a drug that stimulates the brain and nervous system. Caffeine is used to restore mental alertness during fatigue, also found in some headache and migraine medications, in dietary supplements used for weight loss. Quantitative analysis of caffeine content was done with UV-Spectrophotometric method. Chloroform was used as the solvent and concentrations of caffeine were measured at the wavelength of 274nm.Here we going to study and compare the evaluation of caffeine content from different brands of marketed tea products. The study is conducted or done by using UV-Spectrophotometer.

Keywords: *Caffeine, Chloroform, UV Spectrophotometer, Tea powder, Concentration, Absorbance, Range*

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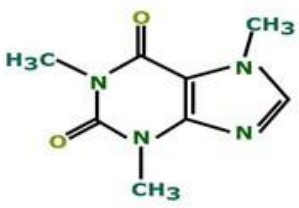
INTRODUCTION:

Caffeine is a central nervous system (CNS) stimulant of the methyl xanthine class[1]. It is mainly used as related to a cognitive enhancer, increasing alertness and attentional performance[2,3] caffeine acts by blocking binding of adenosine to the adenosine A_1 receptor, which enhances release of the neurotransmitter acetylcholine[4]. Caffeine has a three-dimensional structure similar to that of adenosine, which allows it to bind and block its receptor[5]. Caffeine also increases cyclic AMP levels through non-selective inhibition of phosphodiesters[6]. Its chemical formula is $C_8H_{10}N_8O_2$, its systematic name is 1,3,5-trimethylxanthine. Its structural formula is as shown below



CAFFEINE

Caffeine is a bitter, white crystalline purine, a methyl xanthine alkaloid, and is chemically related to the adenine and guanine bases of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). It is found in the seeds, fruits, nuts, or leaves of a number of plants native to Africa, East Asia and South America,[7] and helps to protect them against herbivorous and from competition by preventing the germination of nearby seeds,[8] as well as encouraging consumption by select animals such as honeybees[9]. The best-known source of caffeine is coffee bean, the seed of coffea plant. People may drink beverages containing caffeine to relieve or prevent drowsiness and to improve cognitive performance. To make these drinks, caffeine is extracted by steeping the plant product in water, a process called infusion. Caffeine containing drinks, such as coffee, tea and cola, are consumed globally in high volumes. In 2020, almost 10 million tonnes of coffee beans were consumed globally.[10] Caffeine is the world's most widely consumed psychoactive drug[11,12]. Unlike most other psychoactive substances, caffeine remains largely unregulated and legal in nearly all parts of the world. Caffeine is also an outlier as its use is seen as socially acceptable in most cultures and even encouraged in others.

Caffeine has both positive and negative health effects. It can treat and prevent the premature infant breathing disorders Bronchopulmonary dysplasia of prematurity and apnea of prematurity. Caffeine citrate is on the WHO model list of essential medicines.[13]. It may confer a modest protective effect against some diseases,[14] including Parkinson's disease[15]. Some people experience sleep disruption or anxiety if they consume caffeine,[16] but others show little disturbance. Evidence of a risk during pregnancy women limit caffeine to the equivalent of two cups of coffee per day or less[17,18]. Caffeine can produce a mild form of drug dependence–associated with withdrawal symptoms such as sleepiness, headache, and irritability–when an individual stops using caffeine after repeated daily intake[19,20,21]. Tolerance to the automatic effects of increased blood pressure and heart rate, and increased urine output, develops with chronic use (i.e., these symptoms become less pronounced or do not occur following consistent use).[22]

Caffeine is classified by the US food and drug administration as generally recognized as safe. Toxic doses, over 10 grams per day for an adult, are much higher than the typical dose of under 500 mg per day[23]. The European Food Safety Authority reported that up to 400 mg of caffeine per day (around 5.7mg/kg of body mass per day) does not raise safety concerns for nonpregnant adults, while intakes up to 200 mg per day for pregnant and lactating women do not raise safety concerns for the fetus or the breast-fed infants[24]. A cup of coffee contains 80-175 mg of caffeine, depending on what "bean" (seed) is used, how it is roasted (darker roasts have less caffeine), and how it is prepared (e.g., drip, percolation, or espresso)[25]. Thus, it requires roughly 50-100 ordinary cups of coffee to reach the toxic dose. However, pure powdered caffeine, which is available as a dietary supplement, can be lethal in tablespoon-sized amounts.

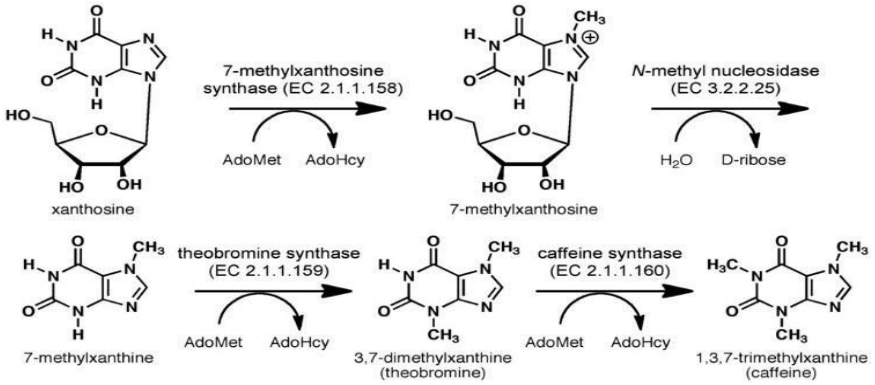
CHEMISTRY:

Pure anhydrous caffeine is a bitter-tasting, white, odorless powder with a melting point of 235-238 °C[26,27]. Caffeine is moderately soluble in water at room temperature (2 g/100 mL), but very soluble in boiling water (66 g/100 mL)[28]. It is also moderately soluble in ethanol (1.5 g/ 100 mL)[28]. It is weakly basic (pK_a of conjugate acid = ~0.6) requiring strong acid to protonate it[29]. Caffeine doesn't contain any stereo genic centers[30] and hence is classified as an achiral molecule[31].

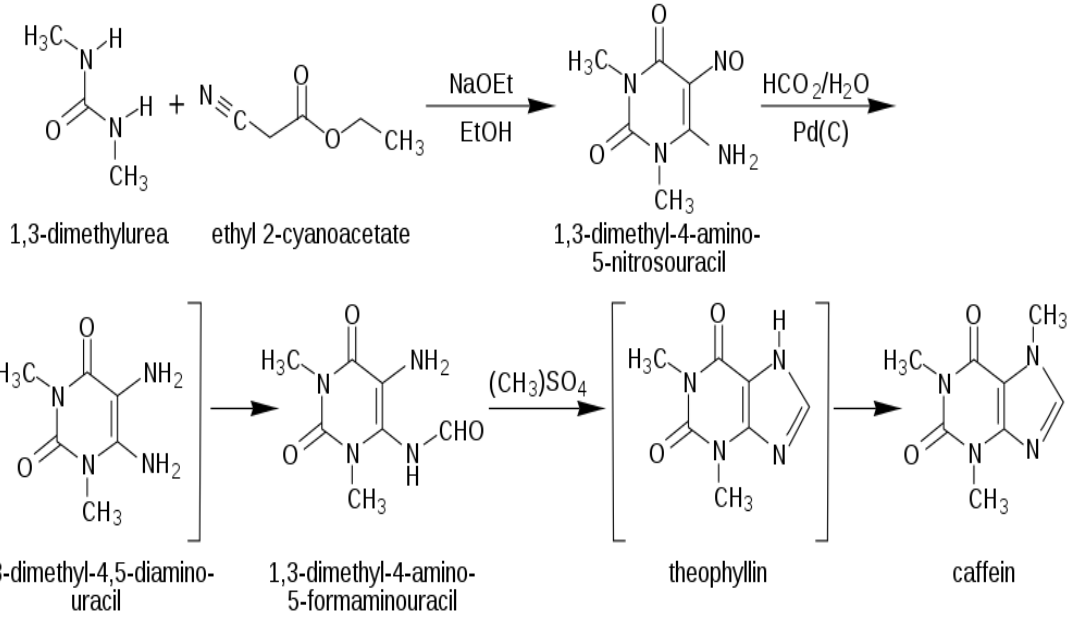
The xanthine core of caffeine contains two fused rings, a pyrimidinedione and imidazole. The pyrimidinedione in turn contains two amide functional groups that exist predominantly in a zwitterionic resonance the location from which the nitrogen atoms are double bonded to their adjacent amide carbons atoms. Hence all six of the atoms within the pyrimidinedione ring system are sp^2 hybridized and

planar. The imidazole ring also has a resonance. Therefore, the fused 5,6 ring core of caffeine contains a total of ten pi electrons and hence according to huckel's rule is aromatic.[32]

SYNTHESIS:



One biosynthesis route of caffeine, as performed by camellia and coffea species[33,34]



One laboratory synthesis of caffeine[35,36]

The biosynthesis of caffeine is an example of convergent evolution among different species[37,38,39].

Caffeine may be synthesized in the lab starting with dimethylurea and malonic acid[35,36,40.]

Commercial supplies of caffeine are not usually manufactured synthetically because the chemical is readily available as a by-product of decaffeination[41].

DECAFFEINATION:

Extraction of caffeine from coffee, to produce caffeine and decaffeinated coffee, can be performed using a number of solvents. Following are main methods:

❖ **WATER EXTRACTION:**

Coffee beans are soaked in water. The water, which contains many other compounds in addition to caffeine and contributes to the flavor of coffee, is then passed through activated charcoal, which removes the caffeine. The water can then be put back with the beans and evaporated dry, leaving decaffeinated coffee with its original flavor. Coffee manufacturers recover the caffeine and resell it for use in soft drinks and over-the-counter caffeine tablets[42]

❖ **SUPERCRITICAL CARBONDIOXIDE EXTRACTION:**

Supercritical carbon dioxide is an excellent nonpolar solvent for caffeine, and is safer than the organic solvents that are otherwise used. The extraction process is simple: CO_2 is forced through the green

coffee beans at temperature above 31.1 ºC and pressures above 73 atm. Under these conditions, CO_2 is in a "supercritical" state: it has gas like properties that allow it to penetrate deep into the beans but also liquid-like properties that dissolve 97-99% of the caffeine. The caffeine-laden CO_2 is then sprayed with high pressure water to remove the caffeine. The caffeine can then be isolated by charcoal adsorption (as above) or by distillation, recrystallization, or reverse osmosis.[42]

❖ **EXTRACTION BY ORGANIC SOLVENTS:**

Certain organic solvents such as ethyl acetate present much less health and environment hazard than chlorinated and aromatic organic solvents used formerly. Another method is to use triglyceride oils obtained from spent coffee grounds[42]

"Decaffeinated" coffees do in fact contain caffeine in many cases – some commercially available decaffeinated coffee products contain considerable levels. One study found that decaffeinated coffee contained 10 mg of caffeine per cup compared to approximately 85 mg of caffeine per cup for regular coffee[43].

METHODOLOGY

AIM OF THE STUDY

To compare the percentage purity of different brands of marketed tea products.

REAGENTS REQUIRED

- 0.01 g recrystallized
- Caffeine
- Distilled water
- Tea powder

APPARATUS REQUIRED

1. UV spectrophotometer
2. Analytical balance
3. Separating funnel
4. Standard flask

SAMPLES:

- Sample 1: Red Label
- Sample 2: Three Roses
- Sample 3: Sabari
- Sample 4: Eastea
- Sample 5: AVT

MATERIALS AND METHODS:

CHEMICALS

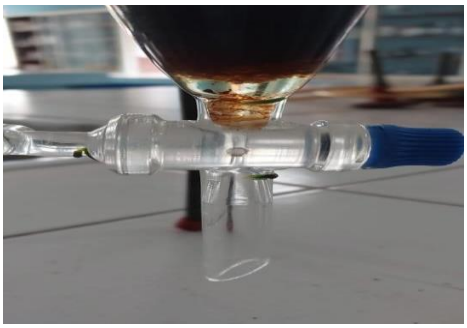
The chemicals used in this study include caffeine standard ($C_8H_{10}N_4O_2$) from Sigma Aldrich (Sigma-Aldrich Chemic GmbH, Munich, Germany), chloroform ($CHCl_3$) and sodium carbonate (Na_2CO_3) obtained from Merck (Merck, Darmstadt, Germany). All reagents used in this study were of analytical grade and all solutions were prepared by using distilled water. Five different brands of tea powder were purchased from chemical market. Caffeine is obtained by evaporation of the solvent from the washing. Caffeine occurs naturally in the tea plants it is more soluble in chloroform to the extent of 1g/10ml therefore caffeine can be extracted by chloroform from the aqueous mixture leaving behind the tannin salts [1]

PREPARATION OF CAFFEINE STOCK SOLUTION:

Dissolving 0.01g of recrystallized caffeine in 100ml chloroform in a volumetric flask. Dilution factor of 1ppm, 5ppm, 10ppm, 15ppm, 20ppm, 25ppm should be carried out. Measure the absorbance at 274nm.[2]

EXTRACTION OF CAFFEINE CONTENT FROM TEA POWDER

Exactly 2 g of each tea sample was weighed. 20 mL of distilled water was added to the sample and the content was heated and then boiled for 10 mins. A total of 2 g of sodium carbonate was added to each sample for precipitating tannins.[3] Samples were filtered and filtrates were concentrated to 5 mL by heating. From the given volume caffeine was extracted by adding 5 mL of chloroform in the separatory funnel. Caffeine was extracted by stirring in the separatory funnel for a few minutes. The lower caffeine-containing layer was separated and analysed for caffeine content with UV/V is spectrophotometer. 0.1 mL of each tea extract was mixed with 10 mL of chloroform and placed in a quartz cuvette. Absorbance was measured at 274 nm. Five samples of each brand of tea were analysed for caffeine content.[4]



INSTRUMENTATION

Ultraviolet spectroscopy is concerned with the study of absorption of UV radiation which ranges from 200nm to 400nm. Compounds which are coloured, absorb radiation from 400nm-800nm. But compounds which are colourless absorb radiation in the UV region. In both UV as well as visible spectroscopy, only the valence electrons absorb the energy, thereby the molecule undergoes transition from Ground state to excited state.

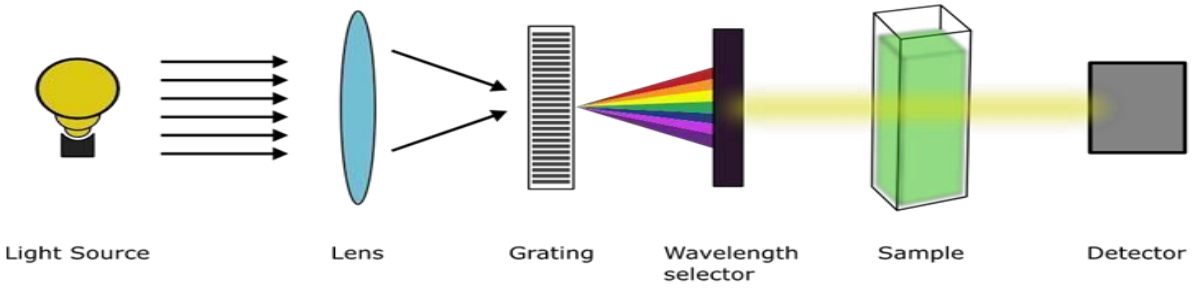
Spectroscopy is the measurement and interpretation of Electro Magnetic Radiation (EMR) absorbed or emitted. when the molecules or atoms or ions of a sample move from one energy state to another energy state. This change may be from Ground state to excited state or excited state to Ground state. At ground state, the energy of a molecule is the sum total of rotational and electronic energies. In other words, spectroscopy measures the changes in rotational, vibrational or electronic energies. Electromagnetic radiation is made up of discrete particles called photons.

Different parts of instruments are used. They are:

Colorimeters- which are usually inexpensive and less accurate. They measure either absorbance or transmittance or both and have filters for use with different coloured solutions. The range of wavelength used is usually small.e.g.,400nm to 700nm.

Spectrophotometers- which are little more expensive than colorimeters. They can be used for a wide wavelength region e.g., 360-900nm.

The different components are,



A. Source of light
B. Filters and Monochromators
C. Sample cells
D. Detectors

A. SOURCE OF LIGHT

The visible spectrum ranges from 400nm to 800nm. Hence any lamp source which gives adequate intensity of radiation over the entire wavelength region can be used. The requirements of a source of light for colorimeter are

- It should provide continuous radiation from 400nm-800nm.
- It should provide adequate intensity
- It should be stable and free from fluctuations.

The following are the sources of light used commonly.

1.Tungsten lamp:

As it satisfies the above criteria, this lamp finds its place in most of colorimeter and spectrophotometer. The lamp consists of a tungsten filament in a vacuum bulb similar to the ones used domestically. But it offers sufficient intensity.

2.Carbon arc lamp:

For a source of very high intensity, carbon arc lamp can be used. It
also provides an entire range of visible spectrum.

B. FILTERS AND MONOCHROMATORS

The source of light gives radiations from 400nm to 800nm. This is polychromatic (heterochromatic) in nature (light of several wavelength). In a colorimeter/ spectrophotometer, we require only monochromatic light. Hence a filter or monochromator is used which converts polychromatic light into monochromatic light though the efficiency of each differs considerably.

Filters are two kinds. They are:

1. Absorption filters
2. Interference filters

Monochromators are two kinds.

1. Prism type (Dispersive type & Littrow type)
2. Grating type (Diffraction grating & Transmission grating)

FILTERS:

1. Absorption filters :

These filters are made up of glass, coated with pigments or they are made up of dyed gelatin. They absorb the unwanted radiation and transmit the rest of the radiation which is required for colorimetry.

These filters can be selected according to the procedure:

- Draw a filter wheel (circle with 6 parts)
- Write the colours (VIBGYOR) in clockwise or anticlockwise manner, omitting indigo.

- If the colour of the solution is Red, we have to use green filter and if the colour of the solution is Green, we have to use red filter. (The colour of the filter is opposite to the colour of the solution. i.e., Complimentary in nature).
- Similarly, we can select the required filter in a colorimeter, based upon the colour of the solution.

Merits

- Simple in construction
- Cheaper Selection of filter is easy

Demerits

- Less accurate since band pass is more
- Intensity of radiation becomes less due to absorption by filters.

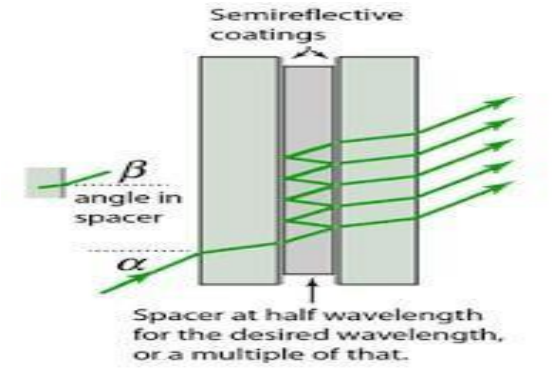
2.Interference filters

This filter is otherwise known as Fabry-Perot filter. The features are

- It has dielectric spacer film made up of CaF_2, MgF_2 or SiO between two parallel reflecting silver films.
- The thickness of dielectric spacer film can be 1/2λ (1st order), 2 λ/2 (2nd order), 3 λ/2 (3rd order), etc.
- The mechanism is that, the radiation reflected by the 2nd film and the incoming radiation undergoes constructive interference to give a monochromatic radiation, which is governed by the following equation.

$$\lambda = 2\eta b/m$$

where, λ = wavelength of light obtained
η = dielectric constant of layer material
b = layer thickness
m = order no. (0,1,2,3 etc.)



- Band pass is 10-15 nm. (i.e., if we select 500nm, the obtained radiation ranges from 490nm to 510nm)
- Maximum transmission is 40%.

Merits

- Inexpensive
- Lower band pass when compared to absorption filters and hence more accurate.
- Use of additional filter cuts off undesired wavelengths.

Demerits

- Peak transmission is low and becomes so when additional filters are used to cut off undesired wavelength.
- The band pass is only 10-15nm and hence higher resolution obtained with monochromators or gratings cannot be achieved.

MONOCHROMATORS:

Monochromators are better and more efficient than filters in converting a polychromatic light or heterochromatic light into monochromatic light. A monochromator has the following units: o Entrance slit (to get narrow source). o Collimator (to render light parallel). o Grating or prism (to disperse radiation). o Collimator (to reform the images of entrance slit). o Exit slit (to fall on sample cell).

1.Prisms

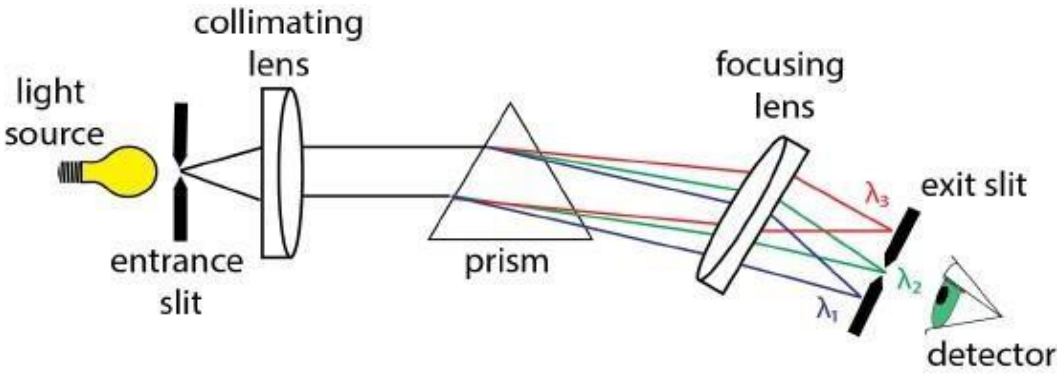
The prisms disperse the light radiation into individual colours or wavelengths. These are found in inexpensive instruments. The band pass is lower than that of filters and hence it has better resolution. The resolution depends upon the size and refractive index of the prism. The material of the prism is normally glass.

The two types of prisms available are:

a. Refractive type:

The following figure shows a prism, where the source of light, through entrance slit falls on a collimator. The parallel radiations from collimator are dispersed into different colours or wavelengths,

and by using another collimator, the images of entrance slit are reformed. The reformed ones will be either Violet, Indigo, Blue, Green, Yellow, Orange or Red. The required radiation on exit slit can be selected by rotating the prism or by keeping the prism stationary and moving the exit slit.



b. Reflective type (Littrow type mounting)

The principle of working is similar to the refractive type except that, a reflective surface is present on one side of the prism. Hence the dispersed radiation gets reflected and can be collected on the same side as the source of light.

2.Gratings

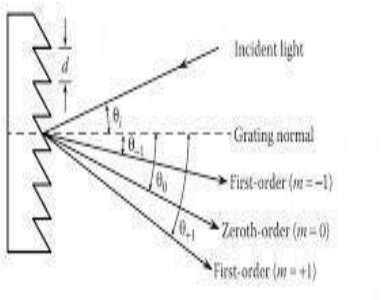
Gratings are the most efficient ones in converting a polychromatic to monochromatic light in the real sense.

Gratings are two types:

a) Diffraction grating
b) Transmission grating

a) Diffraction grating

Gratings are nothing but rulings made on some material like glass, quartz or alkyl halides depending upon the instrument, whether it is visible/ UV/ IR spectrophotometer. The number of rulings per mm also ranges from 20 grooves or lines per mm for IR spectrophotometer to 3600 grooves or more per mm for UV/visible spectrophotometer.



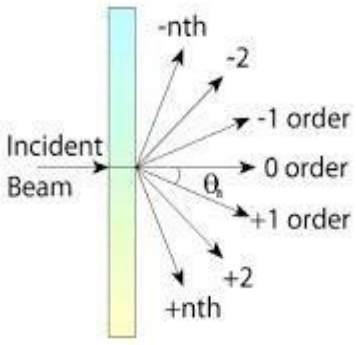
b) Transmission grating

Transmission grating is similar to diffraction grating, but refraction takes place instead of reflection. Refraction produces reinforcement. This occurs when radiation transmitted through grating reinforces with the partially refracted radiation.

The wavelength of radiation produced by transmission grating can be expressed by the equation

$$\lambda = \frac{d \sin\theta}{m}$$

Where, λ = wavelength of radiation produced
d = 1/ lines per cm
m = order no. (0,1,2,3, etc)
θ = angle of deflection / diffraction



C. Sample cells:

Sample cells or cuvettes are used to hold a sample solution. Their geometry as well as material varies with the instrument and nature of sample handled. The material of sample cell should not absorb at the wavelength being observed.

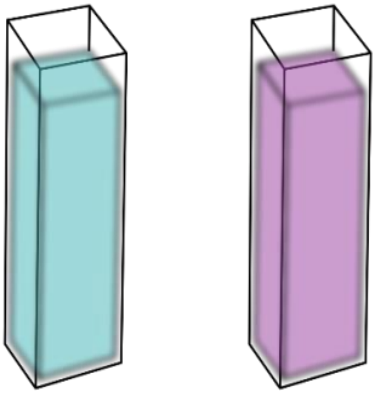
Cells are available which change with the following parameters.

Sample volume - Sample volume cells (0.5 ml or less) and large volume cells(5-10ml)

Shape of cell - Cylindrical (like test tube) or rectangular

Path length - 1cm(normally), upto 10cm (long pathlength), 1mm or 2mm (short pathlength) cells are available.

Material - Colour corrected fused glass for visible region. Polystyrene cells are available for use with aqueous solvents but cannot be used with organic solvents. For VU region, these cells must be made up of quartz since, glass absorbs UV radiation.



sample containers or cuvettes

D. Detectors:

Detectors used in UV/visible spectrophotometers can be called as photometric detectors.

When a radiation is passed through a sample cell, part of it is being absorbed by the sample solution and the rest is being transmitted. This transmitted radiation falls on the detector and the intensity of absorbed radiation can be determined or displayed. In these detectors, the light energy is converted to electrical signal which can be read or recorded.

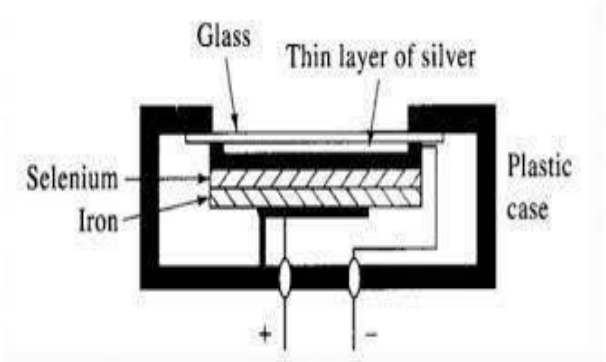
The most commonly used detectors are;

1. Barrier layer cell or Photo voltaic cell
2. Photo tubes or Photo emissive cells
3. Photo multiplier tubes

1)Barrier layer cell or Photo voltaic cell

These cells are the cheapest and are used in inexpensive instruments, like filter type colorimeters, fluorimeters and nepheloturbidimeters.

Photo voltaic cell



The detector has a thin metallic layer coated with silver or gold and acts as an electrode. It also has a metal base plate which acts as another electrode. These two layers are separated by a semiconductor layer of selenium. Selenium has extremely low electrical conductivity and hence the electrons are not mobile.

When light radiation falls on the selenium layer, these electrons become mobile and are taken up by the transparent metal layer. This creates a potential difference between the two electrodes and causes the flow of current, when the resistance in the external circuit is small. This flow of current causes deflection of the galvanometer needle, which depends on the wavelength and intensity of radiation. The sensitivity of the instrument is similar to that of human eye.

Disadvantages:

The amplification of the signal is not possible, because the resistance of the external circuit has to be low, fatigue effects and the lesser response of the detector with light other than blue and red light.

2)Photo tubes or Photo emissive cells

This detector is composed of an evacuated glass tube, which consist of a photo cathode and a collector anode. The photo cathode is coated with elements of high atomic volume like Caesium, Potassium or silver oxide which can liberate electrons, when light radiation falls on it. This flow of electrons towards anode produces a current proportional to the intensity of light radiation. Composite coatings like Caesium / Caesium oxide/ Silver oxide can also be used, which increases the sensitivity and range of wavelength in which the detector can be used (UV/ visible region). The signal from the detector can also be amplified using an amplifier circuit.

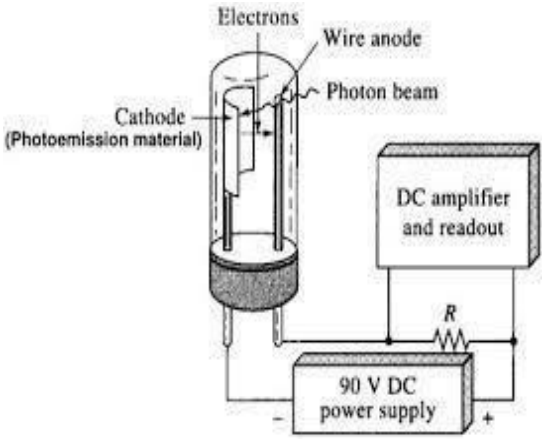
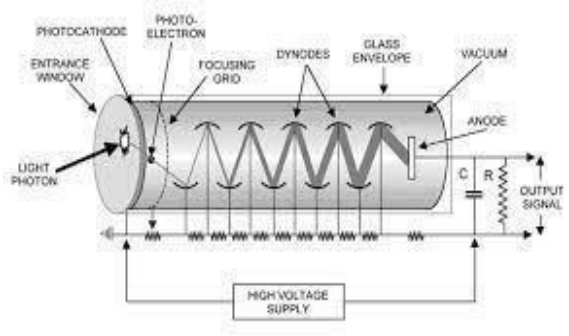


Photo tubes

Photo tubes have better sensitivity when compared to photo voltaic cell and hence are more widely used.

3)Photo multiplier tubes (PMT)

This type of detector is the most sensitive of all the detectors, expensive and used in sophisticated instruments. The principle employed in this detector is that, multiplication of photoelectrons by secondary emission of electrons. This is achieved by using a photo cathode and a series of anode (dyanodes). Upto 10 dyanodes are used. Each dyanode is maintained at 75-100V higher than the preceding one. At each stage, the electron emission is multiplied by a factor of 4 or 5 due to secondary emission of electrons and hence an overall factor of 10^6 is achieved.



PMT can detect very weak signals, even 200 times weaker than that could be done using photovoltaic cell. Hence it is useful in fluorescence measurements. PMT should be shielded from stray light in order to have accurate results.

CONCLUSION:

UV/Vis spectrophotometric method applied in this study for the quantitative analysis of the caffeine concentrations in tea. Additional advantages of this method are that it is inexpensive as well as easy to perform. Despite the relatively small number of the analysed samples, the results of this study gave a preliminary information about the caffeine content often consumed in tea products. Different brands of tea powder were taken and purity of samples were identified. Thus, we concluded that as the various tea powder shows different caffeine concentrations.

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***Melting Point** 238 DEG C (ANHYD)*
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234–236 °C Alfa Aesar 237°C Oxford University Chemical Safety Data 238°C LKT Labs [C0221]
237°C Jean-Claude Bradley Open Melting Point Dataset 14937
238°C Jean-Claude Bradley Open Melting Point Dataset 17008, 17229, 22105, 27892, 27893, 27894, 27895 235.25 °C Jean-Claude

Bradley Open Melting Point Dataset 27892, 27893, 27894, 27895
236 °C Jean-Claude Bradley Open Melting Point Dataset 27892, 27893, 27894, 27895
235 °C Jean-Claude Bradley Open Melting Point Dataset 6603 234–236 °C Alfa Aesar A10431, 39214 Experimental Boiling Point:
178 °C (Sublimes) Alfa Aesar 178 °C (Sublimes) Alfa Aesar 39214

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