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INDUSTRY DETAILS

Under the 12th Five-year plan (2012-2017), the Ministry of Human Resource Development (M.H.R.D), Government of India, has taken an initiative at national level to set up certain labs across the country which focuses on Industrial collaboration with the various Industries available out there and hence can develop products and do research works in various fields. National Design Innovation Network and Open Design School will link these DIC's and evolve a nationwide ecosystem of knowledge and resource sharing for imparting training and foster the innovative culture of designing products, processes and technologies of need to society. The MHRD has approved the establishment of DIC's at National level institutes and universities such as IIT's, NIT's and other Central Universities. The M.H.R.D. has approved the establishment of a DIC at Panjab University, Chandigarh to focus on innovations around engineering products, add values to the available engineering designs and promote early stage start-up companies.

Several ideas are being perused for developing a new pedagogy in teaching and training in design, new fabrications and innovations. A number of design technologies for smart cities, biomedical devices, advanced materials, navigational and tourism aids, green environment, energy & traffic management, communication etc. will be taken up at the DIC at PU. The following four technology areas are currently in progress.

a. Traffic Sensing & Information Technologies.
b. Medical Devices & Restorative Technologies.
c. Energy Harvesting and Management Technologies.
d. Transparent Ceramic Materials & Technologies.

The proposed Design Innovation Centre aims to carry out specialized class room courses and core laboratory training in the above technical areas of designing. Laboratory facilities are presently being developed to provide infrastructure for designing and development of new products and processes for traffic management, energy scavenging, ceramics and dental restorations.

- To enhance knowledge and training of students in innovative applied skills.
- To provide liberal laboratory facilities in specified areas of technical expertise and guide students to develop targeted prototypes.
- To take up collaborative research to give solutions to industry.

- To develop facilities for testing and optimization of prototypes.
- To evolve team work and ecosystem that would be driven by synergies required for incubating start-ups and thus, promote entrepreneurship.
- To enhance Business Incubation facilities in defined areas of technical expertise.

The M.H.R.D. has developed these DIC's under Hub and Spokes Model. In DIC Panjab University the Hub is at University Institute of Engineering & Technology, which was established by Panjab University as a constituent institute in 2002. The spokes under DIC Panjab University are at:

1) Dr. Harvansh Singh Judge Institute of Dental Sciences.
2) PEC University of technology.
3) Central Scientific Instrumentation Organization (CSIO), which is a constituent unit of CSIR.

INTRODUCTION

1.1 SPECTROPHOTOMETRY:

Spectrometry can be defined as the study of how light interacts with matter, by analysing the measurements and reactions of wavelength of light and its radiation intensity. It cannot be called a unique or specialized field but it is an integral component of the scientific processes under a variety of disciplines like physics, chemistry, material and chemical engineering, biochemistry, and clinical applications. Spectrophotometry is a technique to measure the amount of light that a chemical substance absorbs. It is done by measuring the power or intensity of light that passes through the sample solution in the form of a beam. The principle behind this technique is that a substance absorbs or transmits light over a particular range of wavelength spectrum. This method can also be employed to measure the amount or concentration of a known chemical in the sample.

It is known that every chemical compound or substance transmits, reflects or absorbs a certain range of wavelength in electromagnetic spectrum and this transmittance or absorbance by a particular compound is measured is the study of spectrophotometry. It is employed largely for quantitative analysis in various domains such as chemistry, biology, biochemistry, physics, chemical and material engineering, applications at clinical or industrial level, etc. An application that involves the chemical compounds or materials can use this technique. For example, in the field of biochemistry, it is applied for determining enzyme-catalysed reactions by studying the absorbance over a period of time at certain intervals. In case of clinical applications, it is employed for examining blood or tissues for the purpose of diagnosis. Also, there are many variations among spectrophotometry. Some examples are atomic absorption spectrophotometry or atomic emission spectrophotometry [1].

A spectrophotometer can be defined as an instrument that measures the rate of photons absorbed after the light passes through the sample solution. The number of photons is determined by measuring the intensity of light. Using this, the concentration of a known chemical compound can also be estimated. Depending on the range of wavelength of light source, it can be classified into two different types:

UV-visible spectrophotometer: It uses light over the range 185 - 400 nm, that is ultraviolet range, and 400 - 700 nm, that is visible range, of the electromagnetic radiation spectrum.

IR spectrophotometer: uses light over the range of range 700 - 15000 nm, that is Infrared, of the electromagnetic radiation spectrum.

Fig. 1.1 UV – Spectrophotometer

In visible spectrophotometry, certain substances can be analysed by observing the colour of the transmission or the absorption. Consider a case where a sample solution absorbs light over all visible ranges. Such solution appears to be black theoretically. In other case, if a sample solution transmits all visible wavelengths it appears to be white, that is transparent to all colours of light. In a case, if a solution absorbs red light it will transmit or reflect green light and thus appear to be green. This is because green is the complementary colour of red. Spectrophotometers practically use a prism to narrow down a certain range of wavelengths being transmitted and block other wavelengths so that only a particular range of light is passed through solution.

1.2 DEVICES AND MECHANISM:

A spectrophotometer comprises of two components; a photometer and a spectrometer. A spectrometer is the component which produces or disperses and measures the light. A photometer is a component that measures the intensity of light being transmitted [2].

Spectrometer: It generates the required range of light. First, a collimator in the form of lens transmits a beam of photons that passes from a prism, acting as monochromator, and further splits it to several component. Then a slit acting as wavelength selector transmits a particular wavelength, as shown in Figure 1.2.

Photometer: After passing a narrow range of wavelength through sample solution, number of photons being absorbed are detected by photometer and a signal is sent to a measuring device, as illustrated in Figure 1.2.

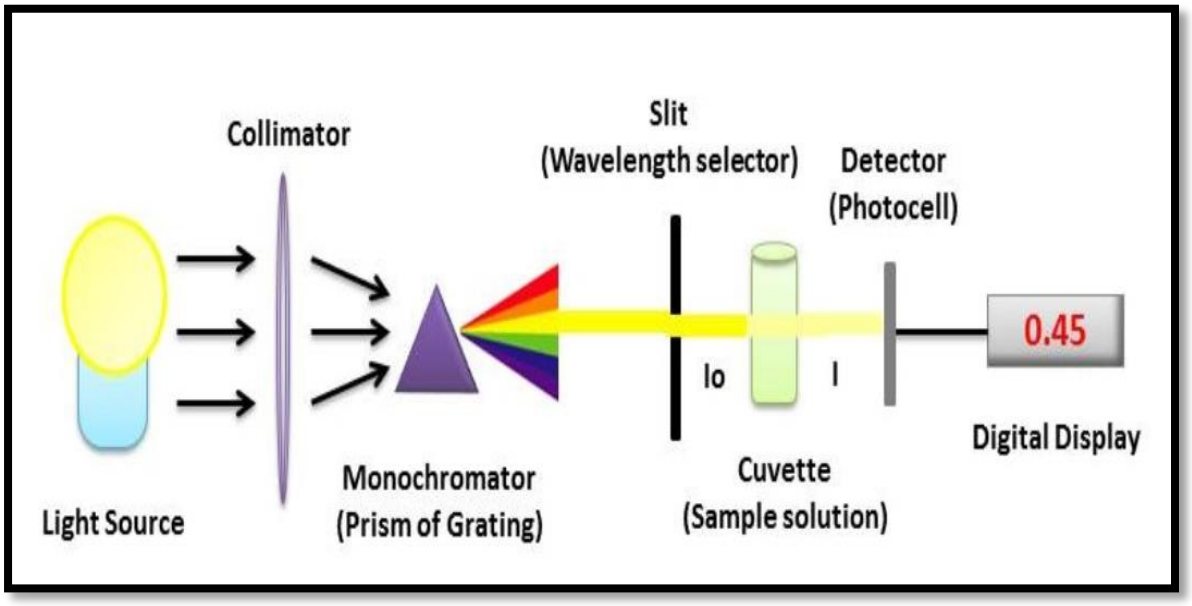


Fig. 1.2 Mechanism of a Spectrophotometer

1.3 INSTRUMENTATION:

The essential components of Spectrophotometer include:

1. Light Source
2. Monochromator
3. Sample Containers
4. Detectors
5. Display devices

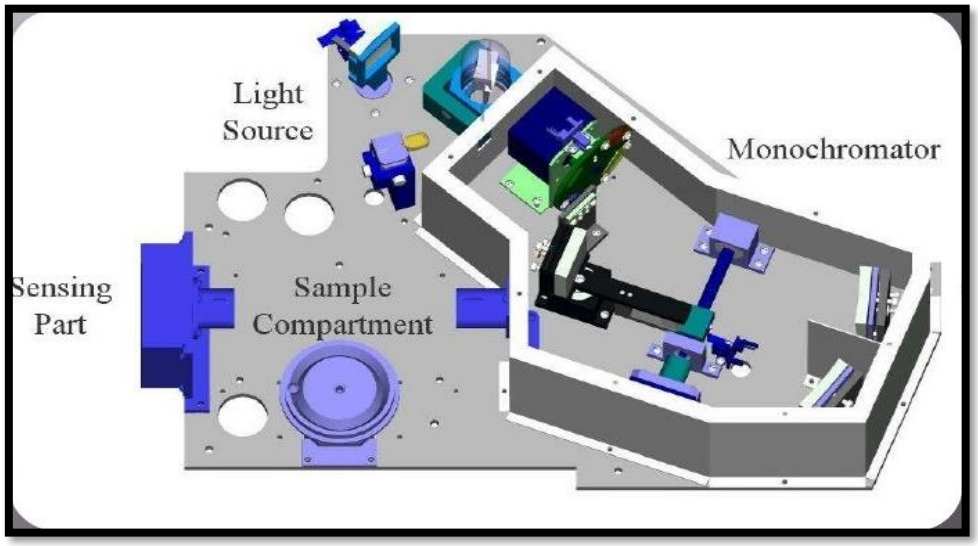


Fig. 1.3 Instrumentation of Spectrophotometer

1.3.1 Light Source:

The light source provides an amount of light which is powerful enough to make a detection. A light source generally generates a polychromatic light as output over a range of the spectrum. The most commonly used light sources are:

- Xenon Lamp
- Tungsten Lamp
- Deuterium/Hydrogen Lamp

1.3.2 Monochromator:

A monochromator takes polychromatic light as input from the lamp and yields monochromatic light as its output. The 3 parts of monochromator are:

1) Dispersion device
2) Entrance slit
3) Exit slit

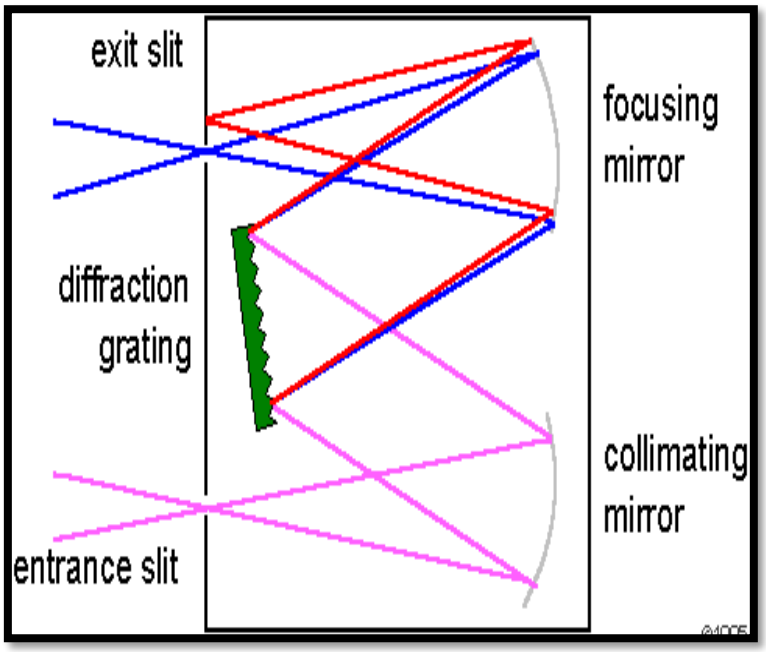


Fig. 1.4 Working of Monochromator

Fig. 1.4 depicts the working of a monochromator. A polychromatic light enters it through its entrance slit and is processed inside using mirrors and diffraction grating. From the exit slit, a monochromatic light is output.

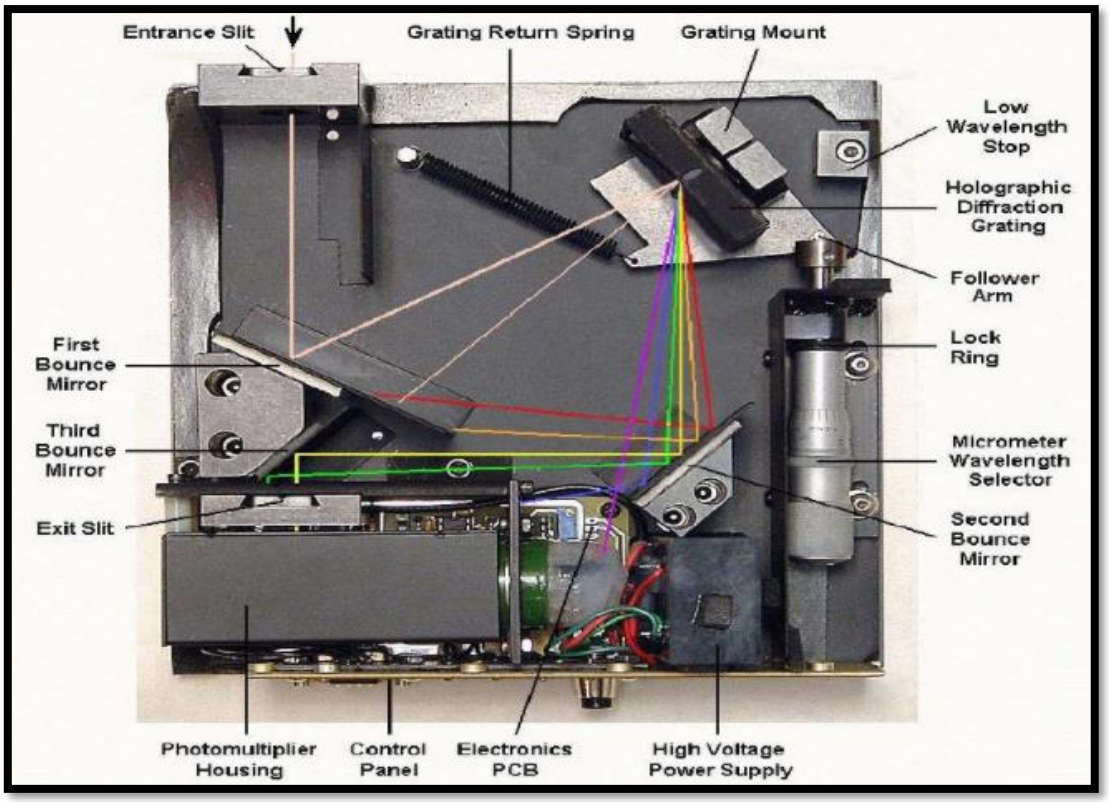


Fig. 1.5 A Monochromator

1.3.3 Sample Containers:

Cuvettes are used in a spectrophotometer to carry sample under analysis. A cuvette is generally a small tube sealed at one end and in the shape of square. It is either made of plastic or glass or quartz of optical grading. It is designed to hold samples to run experiments in spectroscopy. Cuvettes should be clear and without impurities as they might affect the readings. Cuvettes are chosen based on transparency through the spectral range under consideration.

Fig. 1.6 A Cuvette

1.3.4 Detectors:

A photosensitive device can detect radiant energy provided it is sensitive to light in the range of spectrum under consideration. The photocell and phototube are examples of photodetectors which produce current proportional to the number of photons striking the detector.

1.3.5 Display devices:

The data of detector after being processed by controller is displayed to user by a readout device, like an analog meter, an LCD or a digital display. This output can also be sent to a computer or printer for further reference.

Fig. 1.7 An LCD screen

1.4 PRINCIPLES OF SPECTROPHOTOMETRY:

The methods that employ the absorption of electromagnetic radiation as a parameter for analysis constitute an important part of chemical study. Spectrophotometry has a significantly high accuracy as compared to other techniques for analysis and hence is widely used among physicochemical methods. The ease and precision with which a spectrophotometer can operate vouches for its wide use [3].

In the spectrometry analysis, the intensity or the power of radiations being transmitted by a sample solution placed between the source of light and the detector is analysed. A relationship of the power being absorbed or transmitted and the wavelength is known as absorption spectrum. It characterises the elements or compounds of absorbing sample and is the base for qualitative analysis of solution.

1.5 LAWS OF PHOTOMETRY:

Several types of interactions occur between light and matter when a beam of radiant energy hits it. These might include reflection, diffraction, refraction or some other kind of interference. Among these, absorption is important for analysis as certain wavelengths are removed selectively by the matter. This energy is transferred to molecules and atoms in the matter. These particles then jump to excited states from their ground state [4].. The two fundamental laws determining the part of incident light being absorbed by the molecules and atoms are:
(1) Lambert's law

(2) Beer's law

1.5.1 Lambert's Law:

Lambert's law states that when a monochromatic light is passed through a transparent sample solution, there is an exponential decrease in the radiant power of the transmitted light when the thickness of the light absorbing substance rises arithmetically [5].

The law is given as a function:

$$Log(T) = Log (P_t / P_i) = - k_1(b) \quad \text{.....} \quad 1\text{-}1$$

where, P_i = Power of incident light

P_t = Power of transmitted light

T = Transmittance Observed

k_1 = Constant of proportionality

1.5.2 Beer's Law:

Beer's law states that the radiant energy of a unicolor beam of light decreases linearly as the concentration (c) of the compound that absorbs light increases. Thus,

$$Log(T)=Log(I_t/I_0) = - k_2(c) \quad \text{.....} \quad 1\text{-}2$$

Where, k_2 = constant of proportionality.

The laws defined in the equation 1-1 and 1-2 can be combined to generate a new proportionality constant 'K' which is defined as:

$$Log(P_0/P_t) = K(bc) \quad \text{.....} \quad 1.3$$

The ratio $Log(P_0/P_t)$ is transmittance (T), that is dimension-less entity. 'K(bc)' is a pure number as it is a logarithmic entity. Thus equation 1.3 can be given as:

$$A = K(bc) \quad \text{.....} \quad 1.4$$

where A is known as absorbance, $A= \log(1/T) = Log(P_0/P_t)$, 'K' is the absorptivity constant which depends upon the wavelength of the radiant energy and absorbing behaviour of the chemical compounds, whose concentration(c) is expressed in mg/ml. If the concentration is defined in moles/litre, the constant 'K' is replaced by ε, which represents molar absorptivity [6]. Thus,

$$A = \varepsilon(bc) \quad \text{.....} \quad 1.5$$

Equation 1.5 is the fundamental law that controls the absorption of electromagnetic radiations, which can be applied to solutions, gases and solids as well.

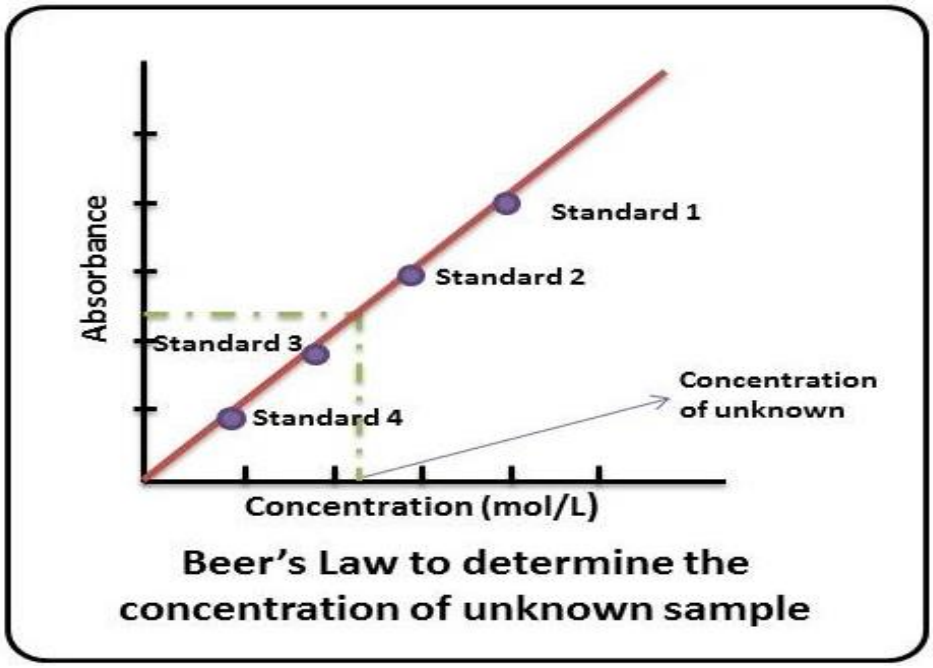


Fig. 1.8 A Beer's Law Demonstration

1.6 APPLICATIONS OF SPECTROPHOTOMETER:

- Determination of concentration of an unknown solution.
- Detection of organic compounds by analysing the maxima of absorption values.
- Determination of Color of the unknown compund based on the spectral range observed.
- Detection of impurities (UV absorption spectroscopy is one of the best methods for determination of impurities in organic molecules).
- Chemical Kinetics (The absorption changes are detected when the UV radiations are passed through reaction cells).
- Molecular weight determination.

1.7 STATE OF THE ART:

The common spectrophotometers available these days include those working ultraviolet range and infrared range. These spectrophotometers also operate in the visible range of the spectrum. The spectrophotometers generally use in the high schools and colleges work in the range of 200 nm – 1400 nm based on the versions available in the market. The price range of these spectrophotometers varies from 2,50,000 to 6,00,000. A high-cost of these spectrometers motivates the development of a low-cost product that can perform on the similar lines.

WORK DONE

2.1 REQUIREMENT OF LOW-COST SPECTROPHOTOMETER:

The cost of a laboratory scale spectrophotometer is high, and thus it is not feasible to use it for the routine experiments. Even in high schools and colleges, the spectrophotometer is not made available for use on daily basis. Owing to its wide range of applications, it can be put to extensive use if made available at low cost. The sensitivity and efficiency might be compromised to some extent in this process of cost reduction, but it shall be possible to perform basic experiments with the accuracy which is equivalent to a high-end spectrophotometer [7].

In addition to its application in schools and research, it can be made available in laboratories to conduct experiments in which it performs accurately. A few such examples include blood tests in which density and colour of blood changes after some kind of pre-processing. Another area where the results can be comparable to actual spectrophotometer include tests on different proteins. Owing to a low-cost this instrument can be used to perform tests which might actually require very sophisticated instruments in induatries.

2.2 HARDWARE USED:

The hardware for the prototype is selected keeping in mind the ultimate aim of cost-reduction. The basic elements in the instrumentation of the prototype are same as that of the high-end spectrophotometer. These instruments are replaced by the low-cost substitutes of actual components. Below are mentioned the hardware components used in the prototype.

2.2.1 RGB Led:

In place of the light source, an RGB LED is used. RGB LEDs combine three colours that can generate over 16 million hues of light. Being a cheap source of light with a potential to emit light over a wide spectral range (visible), it is an ideal fit for substituting the light source of a spectrophotometer [8]. When placed at a suitable distance, it emits a beam powerful enough to pass through the sample to perform the experiments. With different inputs to control red, green and blue light, it also provides the ability to control the wavelength of light emitted. This can be done by controlling the power of light emitted in the form of red, green and blue colours. The current, or indirectly the power sent through each pin of the LED can be controlled by regulating the duty-cycle. A wave with higher duty-cycle sends more current and hence more power to the LED. Higher power increases the intensity of light output by the LED. Different combinations of intensity of red, green and blue help generating light of different wavelengths.

Fig. 2.1 RGB LED

2.2.2 Intensity sensor:

An intensity sensor can work as a good detection device provided we need to measure only the intensity and not the wavelength of light that is being detected. In the prototype, a digital Intensity sensor is used as a detection device. The sensor being used in the prototype is XCULMA TSL-2561. The TSL2561 Intensity Sensor is a complex light sensor which gives a flat response over most of the visible spectrum. The sensor measures both infrared and visible light to render a response better than the human-eye. It is an integrating sensor which means that it sinks light for a certain time period. Hence, it has capacity to detect both small and large amounts of light. The sensor can communicate directly using I2C protocol and can conduct light ranges from 0.1 - 40k+ lux. Moreover, the sensor consists of 2 integrating ADCs that simultaneously integrate currents of 2 photodiodes. It requires a supply voltage of 3V and a current of 0.6mA. It is a low-cost sensor, which detects the intensity of radiation in both visible and infrared range of spectrum. The range of this sensor varies from 0 lux to 40,000 lux depending upon the number of photons that hit its diode.

Fig. 2.2 TSL 2561 sensor

2.2.3 Raspberry Pi:

Raspberry pi is a small sized controller that can be employed to drive the light source and take input from the digital sensor. With ability to store high-volumes of data on its SD-card, reasonably fast processing and send data online using Wi-fi, it becomes a perfect fit to be used in the prototype. It can either be connected to a monitor or to an LCD display for the purpose

of output of the data. A Raspberry Pi – 3 model B is used in the prototype. The availability of wi-fi module in the Pi gives the advantage of connecting to internet remotely. Using a 5V power-supply, it gives out enough current to drive the peripherals. Being an Open-source resource, it can be used in the product without legal restrictions. Moreover, it is readily available in the market which overcomes the limitation of its replacement in case of any fault.

Fig. 2.3 Raspberry-Pi 3 Model B

2.2.4 Test tubes and Cuvettes:

Test tubes are used for initial experiments instead of using optical grade quartz cuvettes to cut down cost factor. These are used to hold the sample and transmit light through them. The test tubes are made of borosil and are transparent to visible light. These test tubes can however not be used for other ranges of spectrum like UV (below 350nm) and IR range as they show absorption properties in these ranges. Being cylindrical in shape, the pose a limitation of spreading the light inconsistently over the surface. These test tubes come in different sizes and brands. However, optical grade quartz cuvettes can be used to avoid the limitations of borosil glass test tubes.

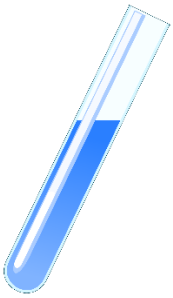


Fig. 2.4(a) A Test tube

Fig. 2.4(b) A cuvette

Cuvettes are used in a spectrophotometer to carry sample under analysis. A cuvette is generally a small tube sealed at one end and in the shape of square. It is either made of plastic or glass or quartz of optical grading. It is designed to hold samples to run experiments in spectroscopy. Cuvettes should be clear and without impurities as they might affect the readings. Cuvettes are chosen based on transparency through the spectral range under consideration.

2.2.5 Display devices:

Display devices are used to output the result and make it available to the user for analysis. In the prototype, the display device is either a monitor or raspberry pi is remotely accessed on laptop screen. An LCD display can also be used with raspberry pi to output the values and plots of experiment.

2.3 SOFTWARE USED:

2.3.1 Raspbian:

Raspbian is a computer OS for raspberry pi controller which is based on Debian. It is a free OS which is optimized for the hardware. Several raspbian images include jessie, stretch etc. The Operating System being used on Raspberry Pi is Raspbian (2017-11-29) release. It is a light OS, suitable to run all the basic functions of raspberry pi. It comprises of several packages pre-compiled to help run programs. Libraries that are required to take input from sensors and provide output to peripherals are easily available. I our case, we have compiled libraries required for TSL-2561 and RGB LED for the sake of availability of easy to use functions. The Raspbian OS provides an easy way to interface with display devices such as a monitor using HDMI port.

2.3.2 Python 3:

Python is used for the purpose of programming owing to its simplicity. The libraries included are compatible with python and provide ready to use functions. The programming to control LED and Intensity Sensor is done in Python 3. The collected data from the sensor is uploaded on spreadsheets or on excel sheets using python scripts. The plots can either be made using google's spreadsheet tools or excel sheets. Another way to plot these graphs is using python tools and these tools also provide a method to remove minor discrepancies from the plotted data.

2.3.3 Libre Office:

The data collected from sensors is stored in the form of excel sheets using Libre Office. This data collected in the form of tables is easy to be available for users to analyse. The results are plotted in the form of suitable graphs.

2.4 PROTOTYPE:

The prototype is designed as an alternative for actual spectrophotometer. The design is made such that the noise from external sources of light is minimum. The designed prototype is not as sensitive and efficient as an actual spectrophotometer but can be used to perform basic experiments with reasonable accuracy. The prototype costs around 10,000 INR.

In the designed spectrophotometer, the body is kept black to absorb redundant light. A stand cuvette is fixed so that there is no alteration between any two test runs. The light source is kept close to the cuvette containing sample solution to avoid any kind of dispersion. The light passing from the sides of cuvette is also blocked with black walls. A space is provided inside the box to place Raspberry Pi. The sides of the box were carved to leave its ports accessible from outside. The prototype can be connected to a monitor using either HDMI cable or ethernet cable.

Fig. 2.5 Designed Prototype

To run a test, first a baseline is set using only the solvent, in which the solute is dissolved, and running the source of light throughout the working range of spectrum [9]. The values received are in the form of intensity of visible light and infrared light. The value of Full spectrum is calculated as the sum of these two values. The absorbance of sample is calculated by taking the negative logarithm of the ratio of intensity of light transmitted through sample and intensity of light transmitted through solvent. These values are stored in a sheet for future reference. The range over which the data is to be collected can be customized. It is also possible to see the intensity values at one particular wavelength.

The code we have used during the training period are attached in APPENDIX.

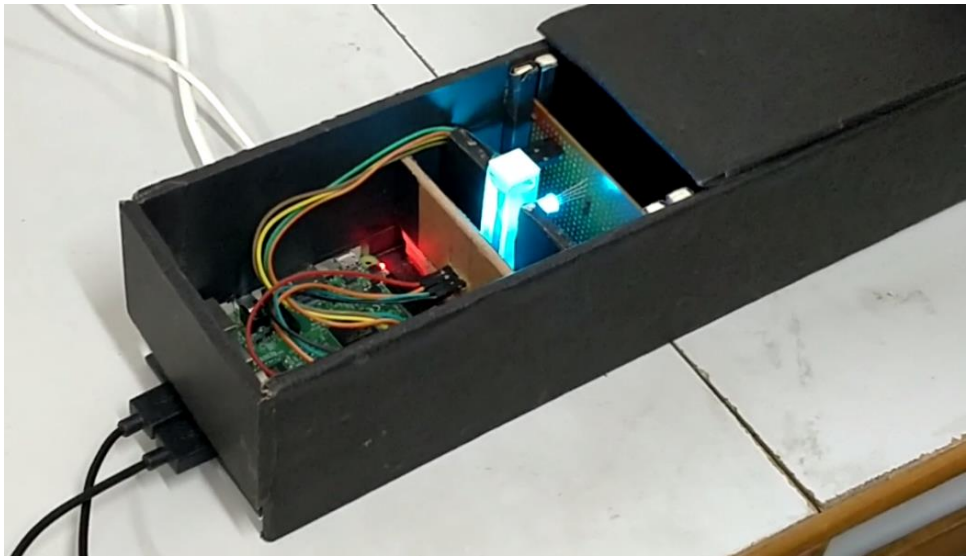


Fig. 2.6 A Demo of Working of Prototype

2.5 ABSORBANCE V/S CONCENTRATION:

2.5.1 For a Red colour dye:

The relationship between absorbance and concentration of sample solution is defined by Beer's Law. According to this law, amount of light absorbed by substance is directly proportional to its concentration under ideal conditions. A sample with high concentration absorbs more light and a sample with lower concentration absorbs less light. Because of this proportionality, this law makes it possible to determine the concentration of unknown sample based on the amount of light it absorbs under similar conditions.

Based on the hypothesis of linear relationship between concentration and absorbance, the first experiment conducted in the lab was to determine the absorbance of solution of colour dye at different concentrations. The materials used for conducting the experiment include Raspberry Pi, TSL 2561 sensor, Test tubes, RGB LED and a red colour dye. The ingredients of the synthetic food colour include:

- Sodium Chloride
- Sunset Yellow (FCF 15985)
- Carmoisine (14720)

The steps performed to conduct the experiment are discussed below.

- A solution with concentration of 15mg/ml in water is prepared using a red dye (food color).

- A white light is generated by emitting equal power of the red, blue and green colours from the RGB LED.
- The intensity of white light transmitted by this solution is noted.
- 1-millimeter water is added to this solution and the intensity of transmitted light is noted down.
- Step 3 is repeated until the intensity v/s concentration graph loses its linearity.
- Light of different colors (blue, cyan, green, magenta, yellow and green) is passed through this red solution.

Observations and Conclusion: As it can be seen in Fig. 2.5 the relationship between concentration of solution and its intensity of transmitted light is approximately linear [10]. The intensity of spectrum in the visible and IR range is analysed. The intensity values are measured in lux. Further, it can be concluded that the experiment shall be repeated by fixing the problems faced for the better analysis of situation.

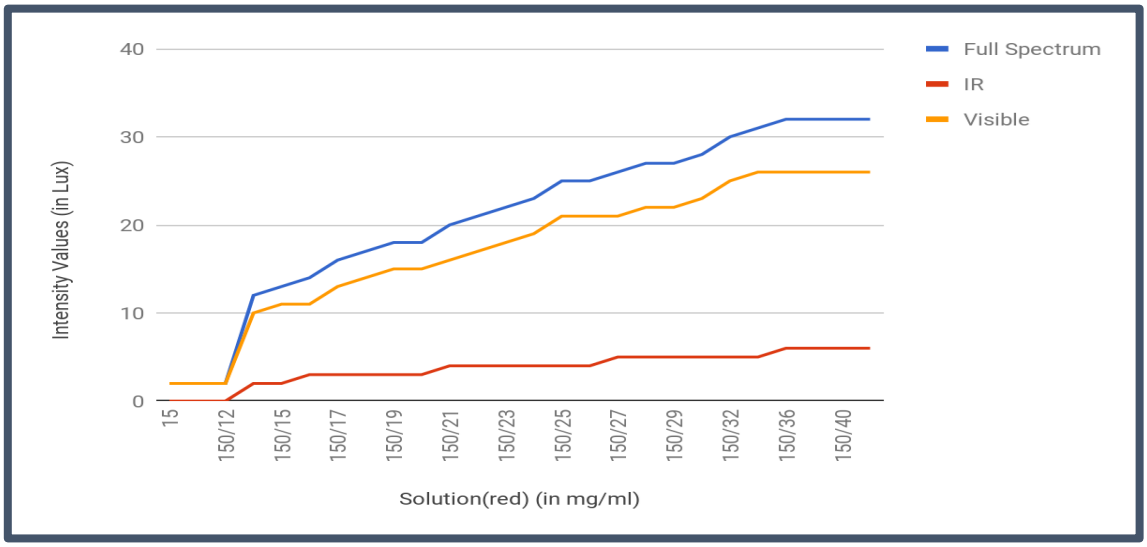


Fig. 2.7 Intensity of Transmitted light v/s Concentration of Red dye

The colour of solution can be determined based on the wavelength of light it absorbs. The sample solution will absorb all the colours except the colour of its own. In our case, the sample solution has a raspberry red colour. So, it absorbs all the colours that do not have any red component but transmits the light that has red component in it. As shown in Fig. 2.6, the light having red color as its component is transmitted but light having only green or blue components is not transmitted.

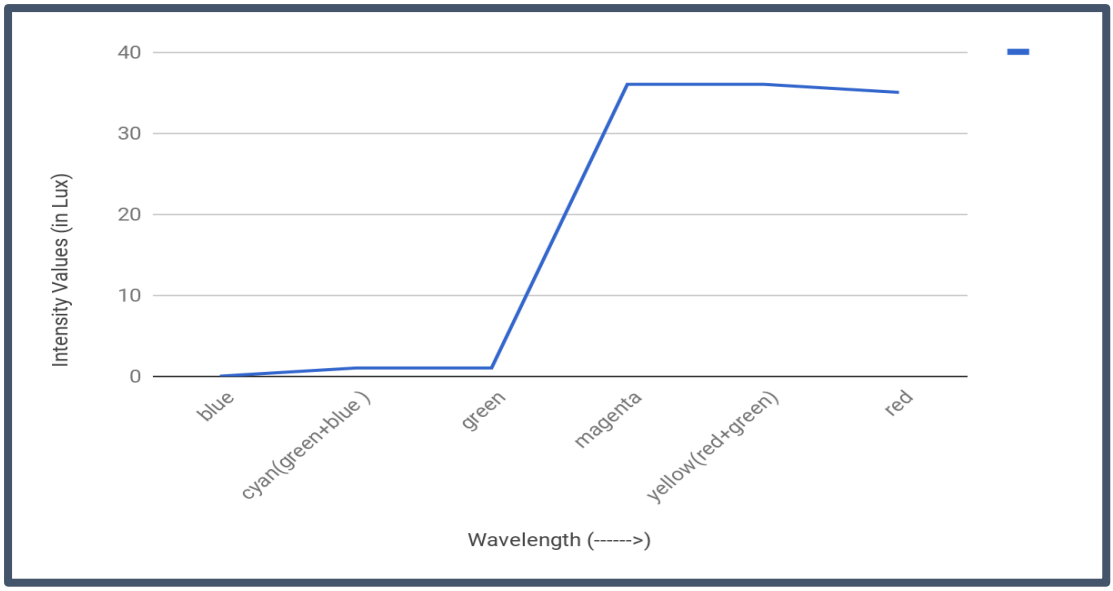


Fig. 2.8 Intensity of transmitted light v/s Colour of light

2.5.2 Problems faced:

- Distance between the test tube and LED was observed to be large that led to scattering of light.
- The cylindrical nature of test tube led to distortion of light being transmitted.
- Use of different test tubes for preparing different samples led to structural variations in the test setup giving inaccurate readings.
- The readings vary a lot with minor disturbance in the apparatus setup.
- The food colour was not easily miscible at higher concentrations of solutions.

2.5.3 For a yellow colour dye:

Based on past experience, the experimental setup was changed to rectify the problems that were faced earlier. In the new setup, the LED was moved closer to the test tube to reduce the scattering loss. Instead of using different test tubes while performing the experiment, same test tube was used for every iteration of the experiment. In this test tube, different sample solutions were transferred to perform the analysis. The setup was fixed to remove the minor disturbances such as small variations in the positioning of test tube. The test tube was revolved using vortex to mix the dye properly in water. The base for test tube was carved from a wooden piece to restrict its movement. Test tube was covered with a black paper to avoid the passage of light through its side walls.

The second experiment was performed using a yellow colour dye. The choice of colour is completely arbitrary and the selection was made on the availability of colour in the local

market. The materials used for conducting the experiment include Raspberry Pi, TSL 2561 sensor, Test tubes, RGB LED and a red colour dye. The ingredients of the synthetic food colour include:

- Sodium Chloride
- Tartrazine (19140)

The steps performed to conduct the experiment are discussed below.

- A solution with concentration of 10mg/ml is prepared using a yellow dye (food color).
- The intensity of white light transmitted by this solution is noted.
- The concentration of solution is reduced to half and the intensity of transmitted light is noted.
- Step 3 is repeated until the intensity v/s concentration graph loses its linearity.

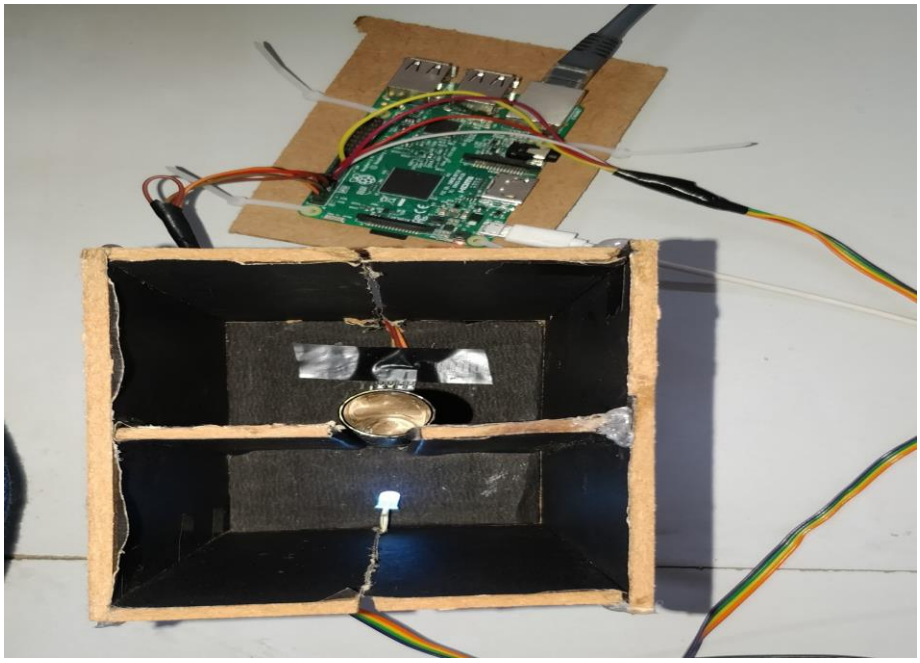


Fig. 2.9 A revised prototype after fixing the problems.

Observations and Conclusion: It can be seen in the graph that the intensity of light transmitted by the solution decreases as its concentration increases. Based on this graph, the concentration of an unknown solution was predicted with a high accuracy. The spectrum of light in visible and IR range is under consideration. As the concentration of solution decreases to the range of 10^{-11} mg/ml, the saturation is achieved and the solution becomes colourless to the naked eye and to the spectrophotometer.

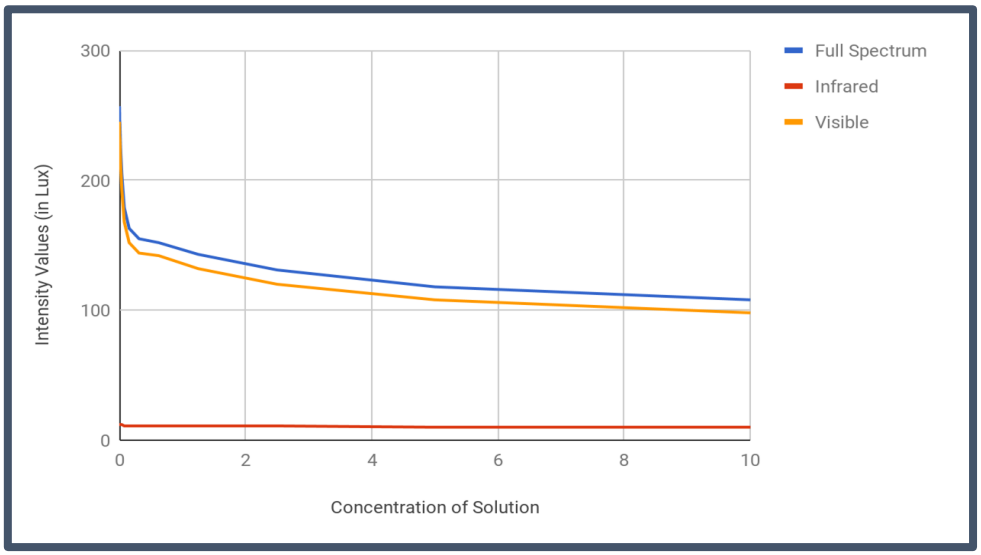


Fig. 2.10 Intensity of light v/s Concentration of Yellow dye

Setting a baseline of water, which is our solvent, the absorbance of sample solution can be calculated using the equation 2.1.

$A = -\log_{10} (I/I_o)$ …… 2.1

Where,

I = the intensity of light received by the intensity sensor.

Io = the intensity of light received by sensor in case of water.

The plot of these values is shown in Fig. 2.9.

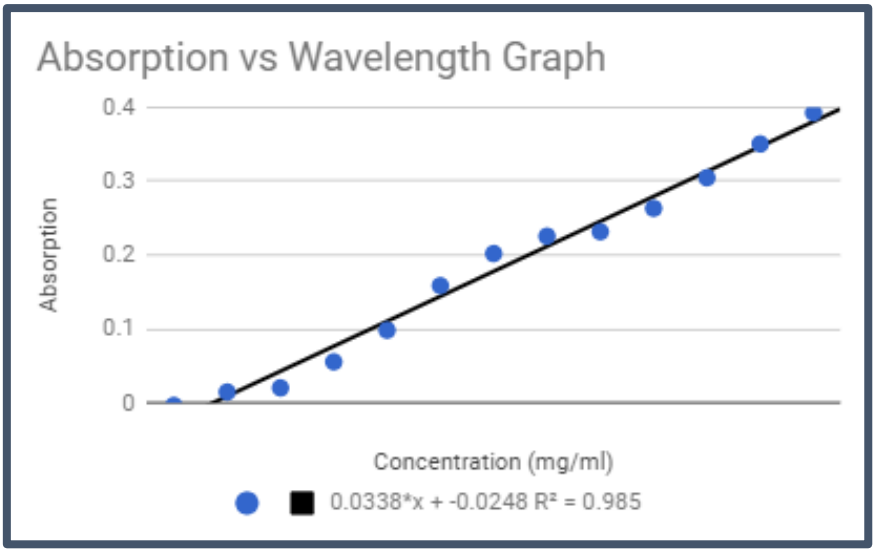


Fig. 2.11 Absorbance v/s Concentration of Yellow dye

Based on the equation generated from this line fitting model, we can calculate the concentration of unknown sample solution of yellow colour dye. The equation 2.2 can be used for calculating the concentration of solution based on above experiment.

$$C = \frac{A+0.0248}{0.0338} \quad \text{..... 2.2}$$

Where, C = Concentration of Yellow dye solution

A = Absorbance of light by solution

2.6 MAPPING WAVELENGTH TO RGB:

To vary the wavelength of light using an RGB LED, it is required to provide the value for each of red, green and blue component on the scale of 0 to 100. This value denotes the duty cycle of a wave that is input power for each of the red, green and blue components. The controller, that is raspberry pi, converts this value to a scale of 0 to 255 and the power of light of each component emitted by the LED is supplied accordingly.

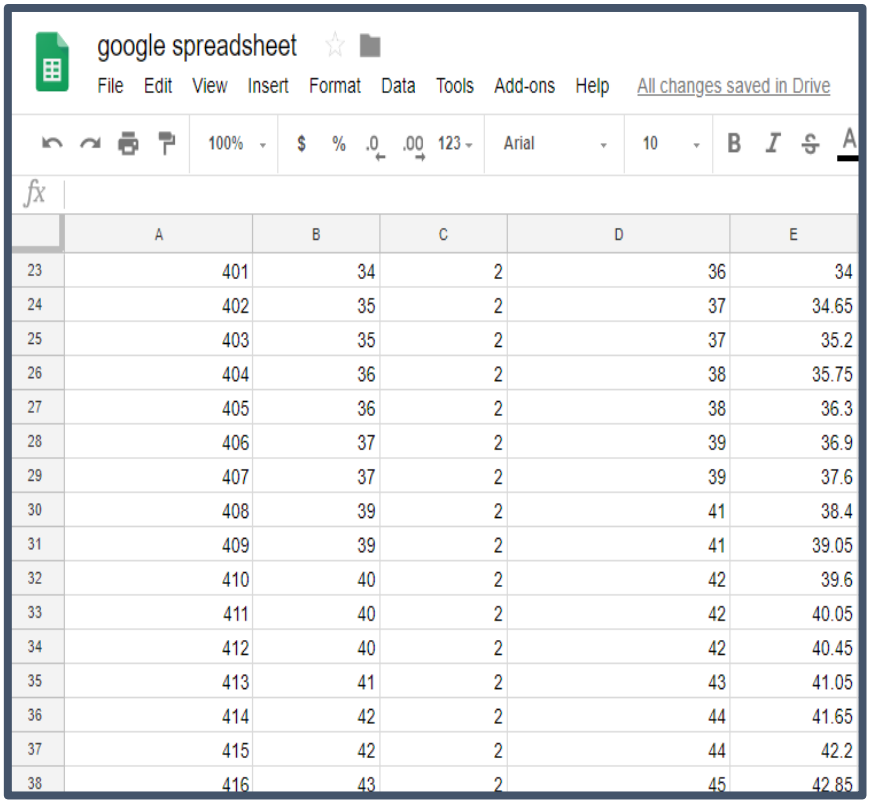
Wavelength Interval	RGB
380 – 440 nm	attenuation = 0.3 + 0.7*(wavelength - 380) R = ((-(wavelength - 440) / (440 - 380)) * attenuation) G = 0.0 B = (1.0 * attenuation)
440 – 490 nm	R = 0.0 G = ((wavelength - 440) / (490 - 440)) B = 1.0
490 – 510 nm	R = 0.0 G = 1.0 B = (-(wavelength - 510) / (510 - 490))
510 – 580 nm	R = ((wavelength - 510) / (580 - 510)) G = 1.0 B = 0.0
580 – 645 nm	R = 1.0 G = (-(wavelength - 645) / (645 - 580)) B = 0.0
645 – 750	attenuation = 0.3 + 0.7 * (750 - wavelength) / (750 - 645) R = (1.0 * attenuation) G = 0.0 B = 0.0

Table 2.1 Wavelength to RGB mapping

The variable “attenuation” is used for the purpose of correction of intensity through this range of visible spectrum.

2.7 UPLOADING VALUES TO GOOGLE SPREADSHEET OR EXCEL SHEETS:

An important part of the experiment is to store the intensity values at different points of time for the purpose of analysis and later comparison. The python library associated with the TSL 2561 sensor just displays the intensity values once to be noted down. To upload these values to the excel sheets or google spreadsheets different libraries are required. The task of uploading these values to the sheets not only consumes negligible time but also saves a lot of human effort. It makes it easy to analyse the data and save it for future comparison. Another feature that is provided by these sheets is of inbuilt functions that make it easy to manipulate data to make it more usable. The sheets also provide ready to use plotting functions that help to visualise data for its better understanding.



	A	B	C	D	E
23	401	34	2	36	34
24	402	35	2	37	34.65
25	403	35	2	37	35.2
26	404	36	2	38	35.75
27	405	36	2	38	36.3
28	406	37	2	39	36.9
29	407	37	2	39	37.6
30	408	39	2	41	38.4
31	409	39	2	41	39.05
32	410	40	2	42	39.6
33	411	40	2	42	40.05
34	412	40	2	42	40.45
35	413	41	2	43	41.05
36	414	42	2	44	41.65
37	415	42	2	44	42.2
38	416	43	2	45	42.85

Fig. 2.12 Uploading values to google spreadsheet

2.8 FAT CONTENT IN MILK:

The fat contents in milk differ not only between dairy products but also within. Raw farm milk, skimmed milk, semi-skimmed milk and full fat milk: each have their own percentage of fat. Raw milk or the milk directly obtained from cows or buffaloes has an average fat content of 4.4 grams per 100 grams. This can be skimmed to obtain milk with full fat or get lower fat

varieties. The standard set for a full-fat milk is 3.5% of fat. For semi-skimmed milk, it is 1.5% of fat according to standard. Buttermilk and skimmed milk are very low on fat i.e. contain 0.2% and 0.1% fat respectively but these percentages may vary due to international differences in standards.

From application point of view, it thus beneficial to have an easy to use, low-cost equipment that can predict the fat content in milk given a sample. Such an equipment can be used at home to determine the fat contents, but more importantly, it can be used at an industry level to determine the aft content. Looking at a wide market of societies dealing with milk and milk products, this spectrophotometer as a product has a wide scope. Based on previously fed data, an equation can be generated that can predict the fat content in a given sample based on the amount of light transmitted through the sample.

An experiment was performed to study the response of the prototype to the variations of fat content in the milk. The steps performed for the experiment are discussed below.

- Milk with different fat percentage was collected from Verka booth.
- Two variants that were used in the experiment had 0.5% and 6% fat content respectively.
- The samples were taken in cuvette one by one and analysed throughout the range of visible spectrum.
- The readings were noted and plotted for the study.

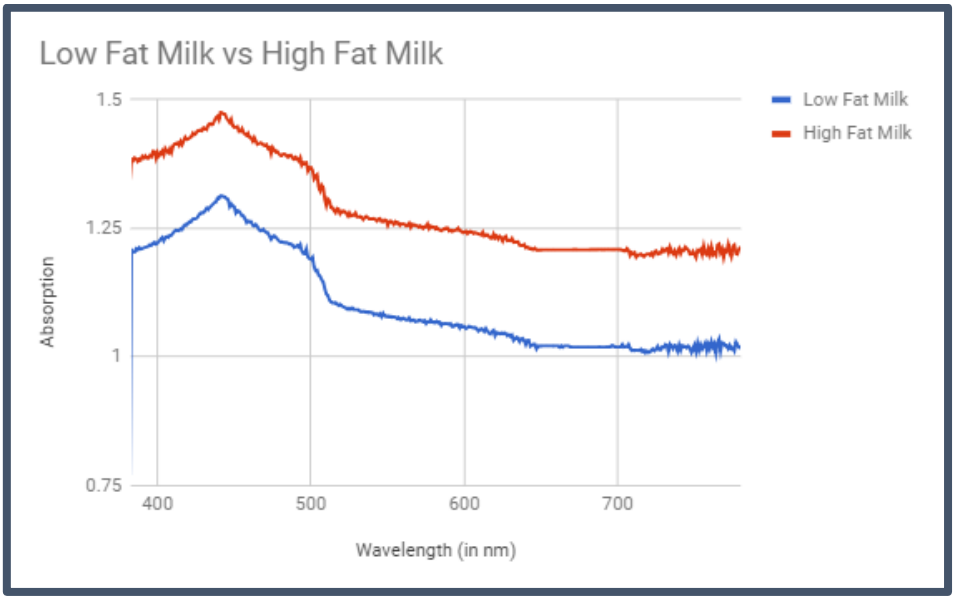


Fig. 2.13 Absorbance v/s Wavelength for milk with different fat percentages.

Observations and Conclusion: It can be seen from the graph in Fig. 2.10 that the absorption of high fat milk is more that absorption of low fat milk. The difference in the two absorption spectra are almost constant. The peak of the spectra falls around 445nm. It is observed that we can use a similar model to determine fat content at an industrial level. Using a small volume of sample, we can determine the amount of fat in the milk that is available. With proper calibration and repetition f experiments, the error in the measured values of fat content can be reduced significantly and the percentage of fat can be determined with reasonable accuracy.

2.9 SUGAR SOLUTION IN WATER:

Dissolving sugar in water is a physical change as no new substances are produced during this process. However, it is useful to have an instrument that can detect the sugar contents in water. When table sugar is added to water, it brings a yellow tone to the solution. This yields a solution that is no more colourless and hence can be detected by our spectrophotometer. The solution show peak of absorbance in the yellow-green range of spectrum. The amplitude of this absorbance peak depends upon the concentration of sugar solute in water. If the concentration is high, the light absorbed by the solution is more.

Steps involved to analyse the relationship between concentration of sugar solution and absorbance of light are discussed below.

- Different concentrations of sugar solutions in 10 ml water were prepared by adding 30g, 22.50g, 15g, and 7.5g of refined sugar in different test tubes.
- It was ensured that sugar is completely dissolved in the solvent leaving no residue behind.
- 3.5ml of solution from 1 test tube is transferred to the cuvette.
- A spectrum of visible light from 380nm to 750nm is passed through the cuvette and analysed through intensity sensor.
- Step 3 and 4 are repeated for all the samples available.

Observations and Conclusion: Absorbance for different concentrations of solution was observed over the range of visible spectrum. It can be seen that the sample with highest concentration of sugar shows highest absorbance and the one with lowest concentration shows lowest absorbance. The absorption spectra are similar for all the samples throughout the range under consideration.

From the plot, it can be concluded that we can determine the concentration of an unknown sample of sugar solution provided we have analysed some samples first. It is however not a robust test for measuring the concentration of an unknown sample as the quality of sugar may vary from one set of samples to other. The impurities in sugar might include sulphur which add to colour in the solution. It is also not possible to analyse a solution of pure glucose with the designed prototype without any kind of pre-processing as the solution of pure glucose is colourless.

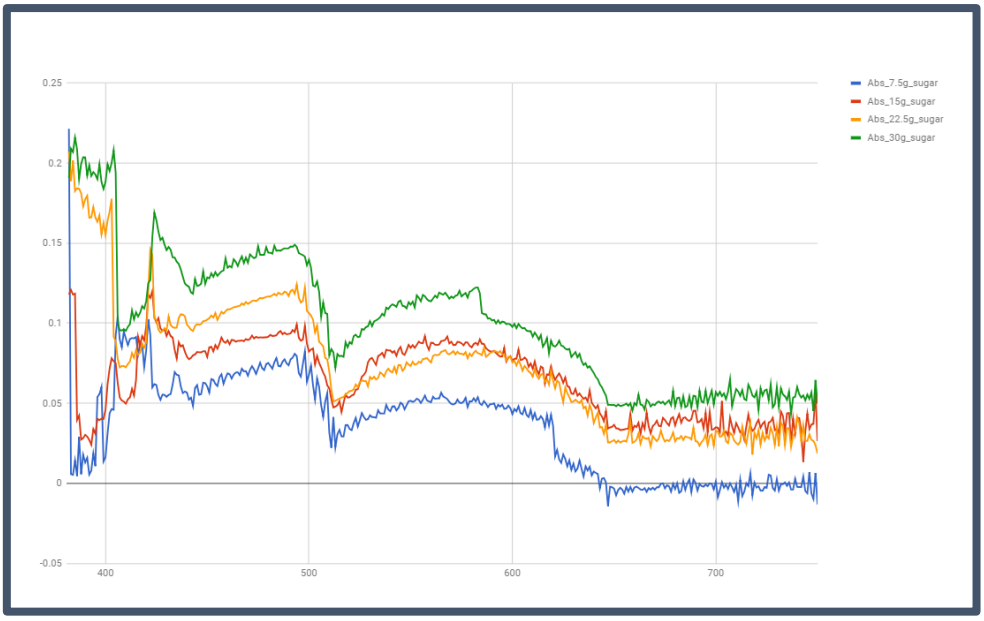


Fig. 2.14 Absorbance v/s Wavelength for different sugar concentrations

2.10 DETECTION OF IMPURITIES IN WATER SAMPLES:

A spectrophotometer can also be used to determine the presence of impurities in water. The water from different sources: distilled water, RO water, ground water, tap water have different impurities and hence different hardness. These properties lead to the possibility of classification of different water samples. But since there is no colour to detect, the designed prototype can not be used for this detection. When experiment is performed, the samples show minor differences in their absorption spectra but this can not be used for the desired purpose with confidence yet.

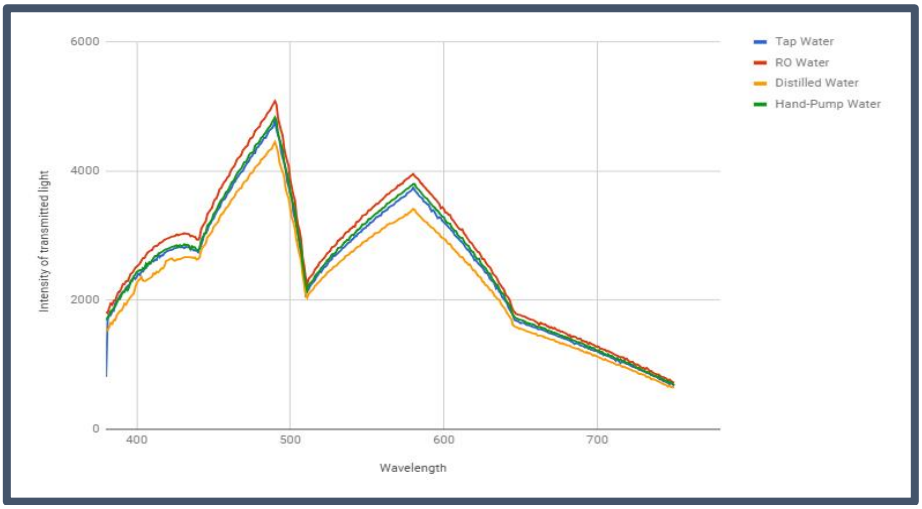


Fig. 2.15 Intensity v/s Wavelength for different water samples

2.11 COMPARISON BETWEEN THE PROTOTYPE AND ACTUAL SPECTROPHOTOMETER:

It is important process of designing product to compare the designed prototype with some standard to know how well the prototype is performing in comparison to the standard. In this case, the designed prototype was compared to the actual spectrophotometer by running a basic experiment. The difference in the two meters was hence analysed. The experiment performed for this comparison is discussed below.

- A yellow colour dye was used to prepare a sample with some arbitrary concentration.
- The sample was analysed in the range of 380nm to 750nm using a UV-Vis laboratory graded spectrophotometer.
- The same sample was analysed over the same spectral range using the designed spectrophotometer.
- The two results were compared and analysed.

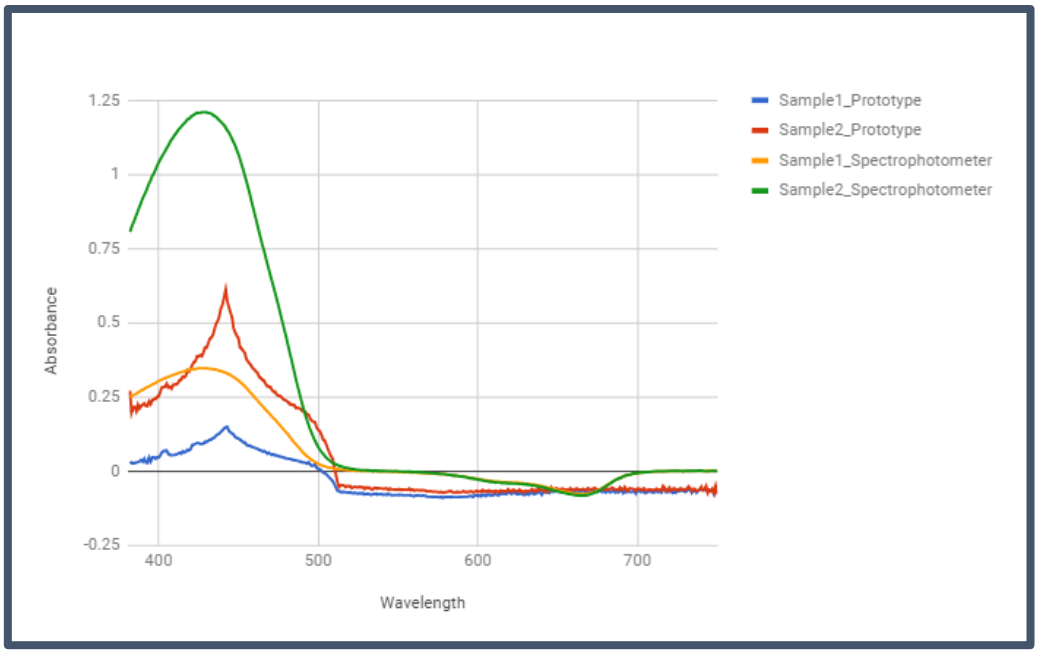


Fig. 2.16 Comparison of Spectrophotometer and Prototype

Observations and Conclusion: It can be seen from the plot that there is a difference of approximately 10nm in the wavelength where the sample shows peak in its absorbance values. The amplitude values also differ for the two spectrophotometers. The amplitude difference arises due to the variance in the sensor and light source being used. Because the ratio of difference in the values of two samples remain same, so it can be calibrated using a proper process.

2.12 PROTEIN ESTIMATION BY LOWRY METHOD:

In biomedical research, the estimation of concentration of proteins in the blood stream is an integral part of disease diagnosis, especially linked with liver. Procedures for measuring concentration of proteins important and have evolved over the time to give more sophisticated and efficient results [9]. Lowry method is one such procedure to determine the concentration of protein in the blood stream. The basic principle of the determination of concentration of proteins by Lowry method is the reaction of peptide nitrogen with the copper compound, which act as source of copper ions, under the alkaline environment and reduction by Folin-Ciocalteay phosphomolybdic and acid to heteropolymolybdenum blue with the oxidation by copper copper-catalysed aromatic acids. The benefit of using this method is that it is sensitive to the low concentrations of proteins.

The equipment used in the process include:

- Cuvettes and test tubes
- Graduated cylinders
- Weight Balance
- Spectrophotometer

The reagents used in the process are:

- 2% of Na_2CO_3 in 0.1N NaOH
- 1% of NaK Tartrate in H_2O
- 0.5% CuSO4.5 H2O in H2O
- Reagent I: 48 ml of A, 1 ml of B, 1 ml C
- Reagent II - 2-part Folin-Phenol [2 N]: 1-part water
- BSA Standard - 1 mg/ ml

The procedure for the experiment is discussed below.

- BSA working standard was taken in 4 different test tubes.
- The BSA was made up-to 1ml by adding distilled water.
- 3 ml of Reagent 1 is added to each test tube
- The samples were left for incubation period of 15 minutes at room temperature and dark conditions.
- 0.3 ml of Reagent II was added to each test tube and samples were processed with vortex machine.

- Samples were again left for incubation for a period of 30 minutes at room temperature and dark conditions.
- The absorbance values at 680nm are noted down using spectrophotometer and the designed prototype.
- The amount of protein present in the blood sample is estimated.

Sample No	Vol of BSA(µl)	Vol of Distilled Water (µl)	Vol of Reagent 1 (ml)	Incubation for 15 minutes	Vol of Reagent 2 (µl)	Incubation for 30 minutes
1	BSA 10 µl	990	3		300	
2	BSA 20 µl	980	3		300	
3	Serum 10 µl	990	3		300	
4	Serum 20 µl	980	3		300	

Table 2.2: Different BSA samples

Sample No	Absorption Values at Original Spectrophotometer	Absorption Values at Designed Prototype	Absorption Values at Designed Prototype (After calibration)
1	0.092	0.052	0.076
2	0.143	0.112	0.136
3	1.253	0.848	0.872
4	1.699	1.264	1.288

Table 2.3: Absorbance values of different samples

Calculations:

For values from actual spectrophotometer:

Protein Concentration in blood = $\frac{1.253}{0.092} = 13.6\ mg/ml$

For values from designed prototype:

Protein Concentration in blood = $\frac{0.872}{0.076} = 11.4\ mg/ml$

Conclusion: The designed spectrophotometer appeared to be less sensitive at wavelength under consideration. The prototype is sensitive enough to detect the changes in the sample in the same way as actual spectrophotometer. But, it needs to be calibrated properly before it can be employed to estimate the concentration of proteins for a laboratory grade test. This calibration can be done by repeating the experiments multiple times and analysing the difference in subsequent results.

CONCLUSION AND FUTURE SCOPE

3.1 CONCLUSION:

With the wide range of applications, it is really useful to have a low-cost replacement for the spectrophotometer. With the reduction in cost and size, it shall become handier to use for a variety of other experiments. For example, it can be used to check adulteration in samples of milk, blood or other solutions. Based on the experiments performed on the prototype made to replace a spectrophotometer, it can be concluded that it is possible to produce a low-cost spectrophotometer that can perform various experiments with accuracy comparable to the actual spectrophotometer. This can be used as a product in high-school and college laboratories to understand the basics of spectroscopy.

Also, it is necessary to block noise from the external environment to provide repeatability and robustness in the experiments. It is important to know and acknowledge the point where the spectrophotometer loses its linearity property, that is, where the transmittance is not linear to the concentration of solution anymore. It's also necessary to address the changes in the apparatus when the components are replaced. A form of calibration thus becomes necessary in such cases.

Based on the experiments performed during the course of internship, it can be stated that besides high-schools and colleges, this prototype can be put to various other uses such as determining the fat content in milk at an industrial level. The wide market for this product can be a source of huge revenue. When calibrated properly, this can be used to perform various classification tests and tests like estimation of protein concentration in human blood.

3.2 FUTURE SCOPE:

With reducing the cost of electronics and increasing computational power of computers, it becomes feasible to make a spectrophotometer that can be used to perform tests on the more regular basis [11]. A lot of work is being done towards making it possible to perform spectrophotometry using smartphones. In this case, the screen of smartphone can be used as a source of light and camera as a sensor to analyse the composition of the solution. A capillary is used in this case to hold the solution instead of the cuvette or test tube [12].

In the current prototype, the work to be done in the future includes running various tests to determine the accuracy and sensitivity of the spectrophotometer [13-14]. The prime target is to be able to find adulteration in milk solution and running simple tests on blood samples. Initially, the focus will be on the binary classification of samples.

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APPENDIX A

Code for analysing sample solution over the wavelength range of 380nm to 750nm:

```
#import gspread
#from oauth2client.service_account import ServiceAccountCredentials
#import json
import pprint
import datetime,time
import math
import RPi.GPIO as GPIO
import smbus
import xlwt

#Gamma Value
gamma = 0.80

#GPIO numbering
GPIO.setmode(GPIO.BOARD)
#Close warnings
GPIO.setwarnings(False)

#Define pins
red=11
green=15
blue=13

#Define pins as Output
GPIO.setup(red, GPIO.OUT)
GPIO.setup(green, GPIO.OUT)
GPIO.setup(blue, GPIO.OUT)

#Frequency for PWM
Freq=100

#Defining the pins that are going to be used with PWM
```

```
RED = GPIO.PWM(red, Freq)

GREEN = GPIO.PWM(green, Freq)

BLUE = GPIO.PWM(blue, Freq)



'''scope = ['https://spreadsheets.google.com/feeds']

creds = ServiceAccountCredentials.from_json_keyfile_name('spectrometer-
c33d876af1af.json', scope)

client = gspread.authorize(creds)

sheet = client.open_by_key("1Y-
RFTgsYlaKE3pAaUddN_9lI8ZPJTSL10WkAtnIOBi8").sheet1

'''


#Function to convert the wavelength into RGB values

def wav2RGB(wavelength):

    w = float(wavelength)


    # colour

    if w >= 380 and w <= 440:

        attenuation = 0.3 + 0.7 * (w - 380) / (440 - 380)

        R = ((-(w- 440) / (440 - 380)) * attenuation) ** gamma

        G = 0.0

        B = (1.0 * attenuation) ** gamma

    elif w >= 440 and w <= 490:

        R = 0.0

        G = ((w - 440) / (490 - 440)) ** gamma

        B = 1.0

    elif w >= 490 and w <= 510:

        R = 0.0

        G = 1.0

        B = (-(w - 510) / (510 - 490)) ** gamma

    elif w >= 510 and w <= 580:

        R = ((w - 510) / (580 - 510)) ** gamma

        G = 1.0

        B = 0.0
```

```
    elif w >= 580 and w <= 645:
        R = 1.0
        G = (-(w - 645) / (645 - 580)) ** gamma
        B = 0.0
    elif w >= 645 and w <= 750:
        attenuation = 0.3 + 0.7 * (750 - w) / (750 - 645)
        R = (1.0 * attenuation) ** gamma
        G = 0.0
        B = 0.0
    else:
        R = 0.0
        G = 0.0
        B = 0.0
    R *= 255
    G *= 255
    B *= 255
    R = int(R)
    G = int(G)
    B = int(B)

    return [R, B, G]

#Function to find absorption and transmitance


def get_values(intensity):
    baseline = int(10)
    #transmittance
    trans = intensity/baseline
    absorb = -math.log10(trans)
    print("Transmittance : " %trans)
    print("Absorbance : " %absorb)
    return [trans, absorb]
```

```
#Main function
def main():
    Running = True
    book= xlwt.Workbook()
    sheet = book.add_sheet('Sheet 2')
    sheet.write(0, 0, 'Wavelength')
    sheet.write(0, 1, 'Red')
    sheet.write(0, 2, 'Green')
    sheet.write(0, 3, 'Blue')
    sheet.write(0, 4, 'Visible1')
    sheet.write(0, 5, 'IR1')
    sheet.write(0, 6, 'Full Spectrum1')
    i=2
    print("Enter the Wavelength (in nm):")
    w=int(input())
    while(w != 751):
        [R,G,B]=wav2RGB(w)
        print("Wavelength :"+str(w)+" nm --> "+"[R:"+str(R)+"
G:"+str(G)+" B:"+str(B)+"]")
        RED.start((R*100)/255)
        GREEN.start((G*100)/255)
        BLUE.start((B*100)/255)
        bus = smbus.SMBus(1)
            # TSL2561 address, 0x39(57)
            # Select control register, 0x00(00) with command register,
0x80(128)
            #        0x03(03)    Power ON mode
            bus.write_byte_data(0x39, 0x00 | 0x80, 0x03)
            # TSL2561 address, 0x39(57)
            # Select timing register, 0x01(01) with command register,
0x80(128)
            #        0x02(02)    Nominal integration time = 402ms
            bus.write_byte_data(0x39, 0x01 | 0x80, 0x02)
        time.sleep(0.5)
            # Read data back from 0x0C(12) with command register,
0x80(128), 2 bytes
```

```
            # ch0 LSB, ch0 MSB
            data = bus.read_i2c_block_data(0x39, 0x0C | 0x80, 2)
            # Read data back from 0x0E(14) with command register,
0x80(128), 2 bytes
            # ch1 LSB, ch1 MSB
            data1 = bus.read_i2c_block_data(0x39, 0x0E | 0x80, 2)
            # Convert the data
            ch0 = data[1] * 256 + data[0]
            ch1 = data1[1] * 256 + data1[0]
            # Output data to screen
            print "Full Spectrum(IR + Visible) :%d lux" %ch0
            print "Infrared Value :%d lux" %ch1
            print "Visible Value :%d lux" %(ch0 - ch1)
        sheet.write(i,0,w)
        #sheet.update_cell(i,2,trans)
        #sheet.update_cell(i,3,absorb)
        #Writing R, G, B values
        sheet.write(i,1,R)
        sheet.write(i,2,G)
        sheet.write(i,3,B)
        #Writing Sensor Values
        sheet.write(i,4,ch0 - ch1)
        sheet.write(i,5,ch1)
        sheet.write(i,6,ch0)
        '''#Finding Average Values
        sheet.write(i,20,xlwt.AVERAGE())
        sheet.write(i,21,xlwt.AVERAGE())
        sheet.write(i,22,xlwt.AVERAGE())
        #time.sleep(0.2)'''
        w=w+1
        i=i+1
    book.save('/home/pi/Desktop/Test.xls')


main()
```

Code to demonstrate change in wavelength using PWM:

```
#defining the RPi's pins as Input / Output
import RPi.GPIO as GPIO

#importing the library for delaying command.
import time

#used for GPIO numbering
GPIO.setmode(GPIO.BCM)

#closing the warnings when you are compiling the code
GPIO.setwarnings(False)

RUNNING = True

#defining the pins
green = 20
red = 21
blue = 22

#defining the pins as output
GPIO.setup(red, GPIO.OUT)
GPIO.setup(green, GPIO.OUT)
GPIO.setup(blue, GPIO.OUT)

#choosing a frequency for pwm
Freq = 100

#defining the pins that are going to be used with PWM
RED = GPIO.PWM(red, Freq)
GREEN = GPIO.PWM(green, Freq)
BLUE = GPIO.PWM(blue, Freq)

try:
    #we are starting with the loop
    while RUNNING:
        #lighting up the pins. 100 means giving 100% to the pin
        RED.start(100)
        GREEN.start(1)
        BLUE.start(1)
        #For anode RGB LED users, if you want to start with RED too the
only thing to be done is defining RED as one and GREEN and BLUE as 100.

            for x in range(1,101):
                # for changing the width of PWM, this command is used
                GREEN.ChangeDutyCycle(x)
                #for anode LED users, just change x with 101-x

                # and for delay time.sleep is used. You can chance the
duration of the colors with changing the time from here
                time.sleep(0.05)

            for x in range(1,101):

                RED.ChangeDutyCycle(101-x)
                time.sleep(0.025)

            for x in range(1,101)
                GREEN.ChangeDutyCycle(101-x)
                BLUE.ChangeDutyCycle(x)
```

```
                time.sleep(0.025)

            for x in range(1,101):
                RED.ChangeDutyCycle(x)
                time.sleep(0.025)

    except KeyboardInterrupt:
        # the purpose of this part is, when you interrupt the code, it
will stop the while loop and turn off the pins, which means your LED
won't light anymore
        RUNNING = False
        GPIO.cleanup()
```

Code to measure intensity of transmitted light at a particular wavelength:

```
#Program asks for user input to determine color to shine.

import time, sys
import RPi.GPIO as GPIO
import smbus
import time

redPin = 11   #Set to appropriate GPIO
greenPin = 15 #Should be set in the
bluePin = 13  #GPIO.BOARD format

def blink(pin):
    GPIO.setmode(GPIO.BOARD)

    GPIO.setup(pin, GPIO.OUT)
    GPIO.output(pin, GPIO.HIGH)

def turnOff(pin):
    GPIO.setmode(GPIO.BOARD)
    GPIO.setup(pin, GPIO.OUT)
    GPIO.output(pin, GPIO.LOW)

def redOn():
    blink(redPin)

def redOff():
    turnOff(redPin)

def greenOn():
    blink(greenPin)

def greenOff():
    turnOff(greenPin)

def blueOn():
    blink(bluePin)

def blueOff():
    turnOff(bluePin)

def yellowOn():
    blink(redPin)
```

```
    blink(greenPin)

def yellowOff():
    turnOff(redPin)
    turnOff(greenPin)

def cyanOn():
    blink(greenPin)
    blink(bluePin)

def cyanOff():
    turnOff(greenPin)
    turnOff(bluePin)

def magentaOn():
    blink(redPin)
    blink(bluePin)

def magentaOff():
    turnOff(redPin)
    turnOff(bluePin)

def whiteOn():
    blink(redPin)
    blink(greenPin)
    blink(bluePin)

def whiteOff():
    turnOff(redPin)
    turnOff(greenPin)
    turnOff(bluePin)

print("""Ensure the following GPIO connections: R-11, G-13, B-15
Colors: Red, Green, Blue, Yellow, Cyan, Magenta, and White
Use the format: color on/color off""")

def main():
    cmd = raw_input("-->")


    if cmd == "red on":
        redOn()
    elif cmd == "red off":
        redOff()
    elif cmd == "green on":
        greenOn()
    elif cmd == "green off":
        greenOff()
    elif cmd == "blue on":
        blueOn()
    elif cmd == "blue off":
        blueOff()
    elif cmd == "yellow on":
        yellowOn()
    elif cmd == "yellow off":
        yellowOff()
    elif cmd == "cyan on":
        cyanOn()
    elif cmd == "cyan off":
        cyanOff()
    elif cmd == "magenta on":
```

```
        magentaOn()
    elif cmd == "magenta off":
        magentaOff()
    elif cmd == "white on":
        whiteOn()
    elif cmd == "white off":
        whiteOff()
    else:
        print("Not a valid command")
    while True:
        whiteOn()
        # Get I2C bus
        bus = smbus.SMBus(1)

        # TSL2561 address, 0x39(57)
        # Select control register, 0x00(00) with command register,
0x80(128)
        #       0x03(03)    Power ON mode
        bus.write_byte_data(0x39, 0x00 | 0x80, 0x03)
        # TSL2561 address, 0x39(57)
        # Select timing register, 0x01(01) with command register,
0x80(128)
        #       0x02(02)    Nominal integration time = 402ms
        bus.write_byte_data(0x39, 0x01 | 0x80, 0x02)

        time.sleep(0.5)

        # Read data back from 0x0C(12) with command register, 0x80(128),
2 bytes
        # ch0 LSB, ch0 MSB
        data = bus.read_i2c_block_data(0x39, 0x0C | 0x80, 2)

        # Read data back from 0x0E(14) with command register, 0x80(128),
2 bytes
        # ch1 LSB, ch1 MSB
        data1 = bus.read_i2c_block_data(0x39, 0x0E | 0x80, 2)

        # Convert the data
        ch0 = data[1] * 256 + data[0]
        ch1 = data1[1] * 256 + data1[0]

        # Output data to screen
        print "Full Spectrum(IR + Visible) :%d lux" %ch0
        print "Infrared Value :%d lux" %ch1
        print "Visible Value :%d lux" %(ch0 - ch1)

    return


main()
```