SUMMER PROJECT REPORT

2019

Submitted to :

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:- BE ece 3rd year

:-UE175119

ACKNOWLEDGEMENT

We would like to thank Dr. Naveen Aggarwal for providing us with an opportunity to undergo this training with Design Innovation Center(DIC). He guided us through our training, provided helpful insights and motivated us to work harder. His constant guidance and willingness to share his vast knowledge made us understand this project and it's manifestation in great depths which helped us in completing the task assigned.

At last, we would like to thank MHRD for sponsoring this project, as without them the opportunity would have never come our way.

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CERTIFICATE OF ORIGINALITY

We hereby declare that we are responsible for the work submitted in this project, this work is our own original work, and that neither the report nor the original work contained therein has been submitted to this or any other institution. All sources used for the project have been fully and properly cited.

Students involved in the project

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Mentor: Dr. Naveen Aggarwal, UIET Panjab University

I verify that that statements provided above are true and the work carried out by them is authentic.

Signature of Mentor:

……………………………… (Signed)

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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.

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.
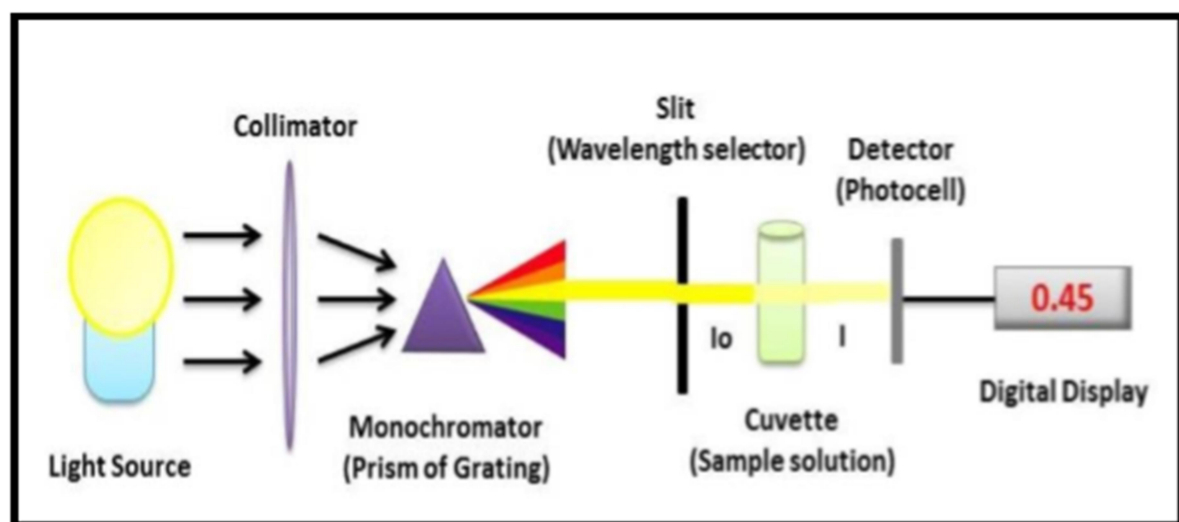

Fig. 1.2 design of spectrophotometer.

INSTRUMENTATION:

The essential components of Spectrophotometer include:

1. Light Source

2. Monochromator

3. Sample Containers

4. Detectors

5. Display device

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

Detail design and 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.

Testing strategy and test result:

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.

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.

CONCLUSION AND FUTURE SCOPE

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.

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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