JAI SREE GURUDEV

SRI ADICHUNCHANAGIRI FIRST GRADE COLLEGE

Sub : PHYSICS

Intenship Topic : UV-Vis Spectroscopy

Submitted To:

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Introduction

Spectroscopy is the branch of science dealing the study of interaction of electromagnetic radiation with matter. It is the measurement of electromagnetic radiation (EMR) absorbed or emitted when molecules or ions or atoms of a sample move from one energy state to another energy state. Spectroscopy is the most powerful tool available for the study of atomic & molecular structure and is used in the analysis of a wide range of samples.

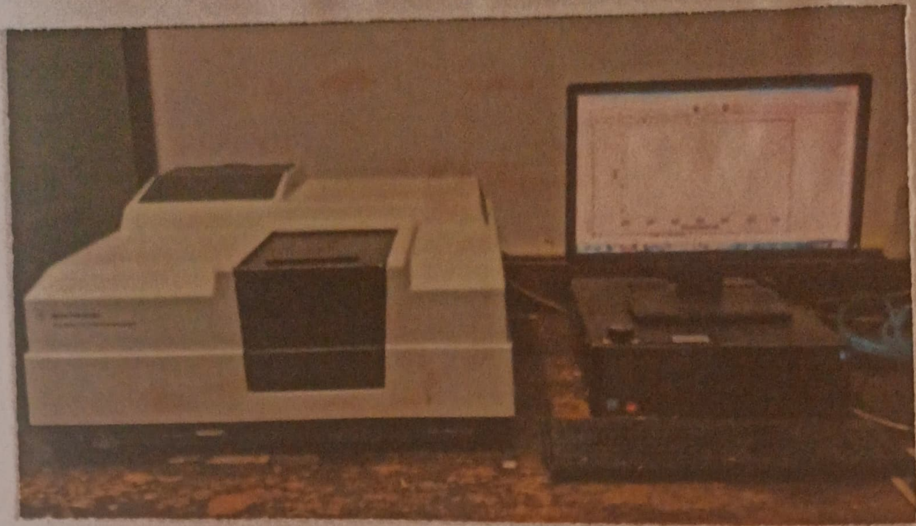
The principle is based on the measurement of spectrum of a sample containing atoms / molecules. Spectrum is a graph of intensity of absorbed or emitted radiation by sample verses frequency (ν) or wavelength (λ). Spectrometer is an instrument design to measure the spectrum of a compound.

UV-Visible spectroscopy is also known as electronic spectroscopy. In which the amount of light absorbed at each wavelength of UV and Visible region of electromagnetic spectrum is measured. This absorption of electromagnetic radiation by the molecules leads to molecular excitation. This is called Electronic spectroscopy since it involves the promotion of electron from the ground state to higher energy state. It is very useful to measure the number of conjugated double bond and also aromatic conjugated within the various molecule. It is also distinguishes between conjugated & non-conjugated system; α, β-unsaturated carbonyl compound from β,γ-analogues, homoannular and heteroannular conjugated diens etc. Excitation occurs in the range 200-800 mu.

UV-Vis Spectroscopy

UV spectroscopy is an analytical technique that meas-
es the amount of discrete wavelengths of UV or visible
ght that are absorbed by or transmitted through a
mple in comparison to a reference or blank sample.
his property is influenced by the sample composition,
tentially providing information on what is in the
ample and at what concentration. Since this spectrospoy
echnique relies on the use of light, let's first consider
he properties of light.

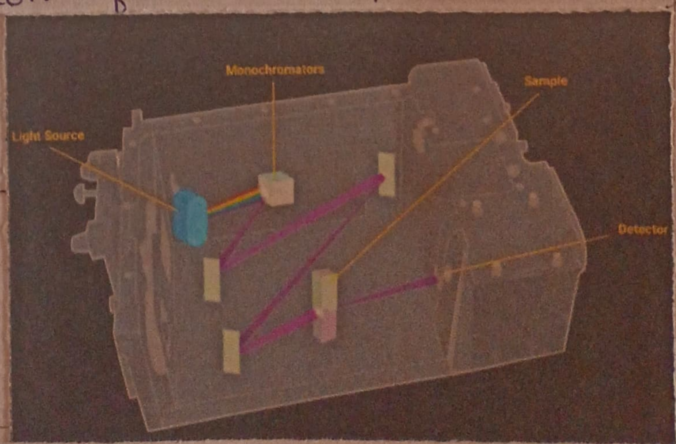
Light has certain amount of energy which is
nversely proportional to its wavelength. Thus, shorter
avelengths of light carry more energy and longer
avelengths carry less energy. A specific amount of
ergy is needed to promote electrons in a substance
o a higher energy state which we can detect as
bsorption. Electrons in different bonding environments in
substance require a different specific amount of energy
promote the electrons to a higher energy state. This
s why the absorption of light occurs for different
avelengths in different substances. Humans are able to
e a spectrum of visible light, from approximately
80nm, which we see as violet, to 780nm, which we see
s red. UV light has wavelengths shorter than that of
isible light has to approximately 100nm. Therefore,
ight can be described by its wavelength, which can
e useful in UV-Vis spectroscopy to analyze or identify
ifferent substances by locating the specific wavelengths
orresponding to maximum absorbance



UV (Ultraviolet-visible) spectroscopy refers to the
sorption spectroscopy in the ultraviolet-visible spectral
gion. Many molecules absorb ultraviolet or visible radiati-
as they move between energy levels. The wavelengths
radiation that is absorbed directly affects the percei-
d colour of the chemicals involved and is related
molecular structure. Different molecules absorb
adiation of different wavelengths; therefore an absorption
ectrum can be used to qualitatively identify compounds.

UV-Vis spectroscopy is mainly used in the quanti-
tive analysis of compounds. When a substance absorbs
isible light, it appears coloured. The human eye does
ot see the colour that is absorbed by the sample,
owever, what is seen is the complement of the
bsorbed colour. For example, a solution of copper (II)
ulphate appears blue because the absorption energy
omes from the orange region of visible spectrum.

chematic diagram of a
uvette-based UV-Vis
pectroscopy system. ←

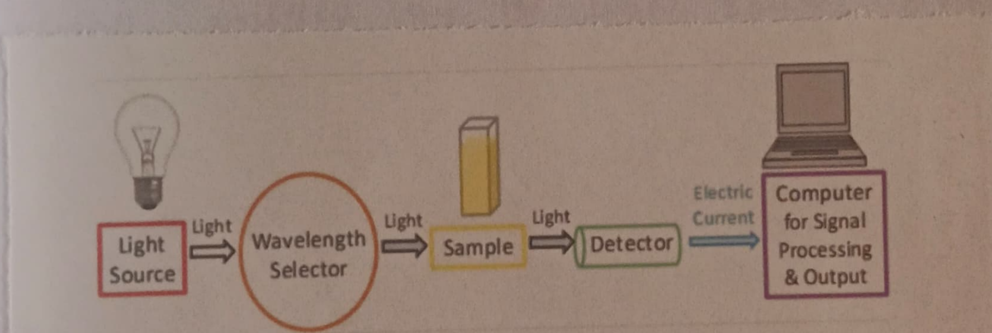


Principle of UV spectroscopy:

UV visible spectroscopy measures the response of sample to ultra violet and visible range of ectromagnetic radiation.
olecule have either n, π or σ-sigma electrons. These ectrons absorb UV radiation and undergoes transition om ground state to excited state.

orking of UV spectrophotometer:

There are many variations on the UV-Vis spectro-hotometer, to gain a better understanding of how an V-Vis spectrophotometer works, let us consider the main omponents.



ght source

As a light-based technique, a steady source able o emit light across a wide range of wavelengths is sential. A single xenon lamp is commonly used as a igh intensity light source for both UV and visible anges. Xenon lamps are, however, associated with igher costs and are less stable in comparison to ungsten and halogen lamps.

For instruments employing two lamps, a tungsten r halogen lamp is commonly used for visible light, hilst a deuterium lamp is the common source of V light. As two different light sources are needed to can both the UV and visible wavelengths, the light ource in the instrument must switch during neasurement. In practice, this switchover typically occurs uring the scan between 300 and 350 nm where the ight emission is similar from both light sources and he transition can be made more smoothly.

Vavelength selection

In the next step, certain wavelengths of light suit- d to the sample type and analyte for detection must e selected for sample examination from the broad vavelengths emitted by the light source. Available nethods for this include:

: Monochromators - A monochromator separates light into narrow band of wavelengths. It is most often based n diffraction gratings that can be rotated to choose ncoming and reflected angles to select the desired vavelength of light. The diffraction grating's groove requency is often measured as the number of grooves er mm. A higher groove frequency provides a better ptical resolution but a narrower usable wavelength ange. A lower groove frequency provides a larger usable wavelength range but a worse optical resolution. 300 to 2000 groves per mm is usable for UV spectroscopy urposes but a minimum of 1200 grooves per mm is typical. The quality of the spectroscopic measurements is ensitive to physical imperfections in the diffraction rating and in the optical setup. As a consequence, ruled diffraction gratings tend to have more defects than blazed holographic diffraction gratings. Blazed holographic diffraction gratings tend to provide significantly better quality measurements.

- Absorption filters: Absorption filters are commonly made of colored glass or plastic designed to absorb particular wavelengths of light.

- Interference filters: Also called dichroic filters, these commonly used filters are made of many layers of dielectric material where interference occurs between the thin layers of materials. These filters can be used to eliminate undesirable wavelengths by destructive interference, thus acting as a wavelength selector.

- Cutoff filters: Cutoff filters allow light either below (shortpass) or above (longpass) a certain wavelength to pass through. These are commonly implemented using interference filters.

- Bandpass filters: Bandpass filters allow a range of wavelengths to pass through that can be implemented by combining shortpass and longpass filters together.

Monochromators are most commonly used for this process due to their versatility. However, filters are often used together with monochromators to narrow the wavelengths of light selected further for more precise measurements and to improve the signal-to-noise ratio.

Sample Analysis

Whichever wavelength selector is used in the spectrophotometer, the light then passes through a sample. It is important to be aware of the materials and conditions used in UV-Vis spectroscopy experiments. Glass can act as a filter, often absorbing the majority of UVC (100-280nm)[2] and UVB (280-315nm)[2] but allowing some UVA (315-400nm)[2] to pass through. Therefore, quartz sample holders are required for UV examination because quartz is transparent to the majority of UV light. Air may also be thought of as a filter because wavelengths of light shorter than about 200nm are absorbed by molecular oxygen in the air.

Preparation of UV sample

Aim: To determine the mass of Fe^{3+} in a sachet of a dietary supplement using UV-Visible spectroscopy.

Materials:

- 2.000×10^{-4} M Fe^{3+} standard solution
- 4 M HNO_3 solution
- 10% KSCN solution
- 10% H_2O_2 solution
- Iron dietary supplement (5mg/25mL)
- Distilled water
- 5 x 25mL volumetric flasks
- 2 x small beakers
- 6 x Pasteur pipettes
- Autopipette
- Spectrophotometer
- 6 x cuvettes
- Safety goggles
- Spectrometer.

Method :

ɹt 1: Preparation of the callibration curve.

The beaker labelled 'iron standard solution' was
ɹnsed with a small amount of the standard solution
nd then 40mL of the solution was placed into beaker.
Five 25mL volumetric flasks were labelled with no 1 to 5.
An autopipette was used to transfer the amounts of
on standard solution listed in the table to flasks.
The 4M HNO_3 and 10% KSCN solution were added to
ch of the flasks.
The solutions were mixed by stoppering and shaking,
en the color variations in the flasks were checked by
ye.
5 cuvette were filled with each of the solutions using a
ean Pasteur pipette each time and were arranged in
der of concentration.

ution	Fe^{3+} solution	4M HNO_3 solution	10% KSCN solution	Distilled water	Fe^{3+} concentration.
1.	0mL	2mL	2mL	To line	0M
2.	1mL	2mL	2mL	To line	0.000008M
3.	2mL	2mL	2mL	To line	0.000016M
4.	3mL	2mL	2mL	To line	0.000024M
5.	4mL	2mL	2mL	To line	0.000032M.

ɹt 2: Frequency of light absorbed

A spectrophometer was used to record the spectrum
the solution in flask 4 over a range of approx
400- 700nm

Part 3: Determining the iron content of supplement

i) Preparation of sample solution

1) The content of the sachet were emptied into a small beaker.

2. An autopipette was used to add 1.0 mL of this liquid into a 250 mL volumetric flask.

3. 1 mL of 10% H_2O_2 solution was added to the flask.

4. 10 mL of 4 M HNO_3 and 10 mL of KNCS was added to soln.

5. The flask was made up to market with distilled water using a clean plastic pipette.

ii) Analysis of the stock solution

1. The sample was transferred to a cuvette and the absorbance was measured at 473.0 nm.

Part 4: Amount of light absorbed.

1. The spectrometer was set to 473.0 nm

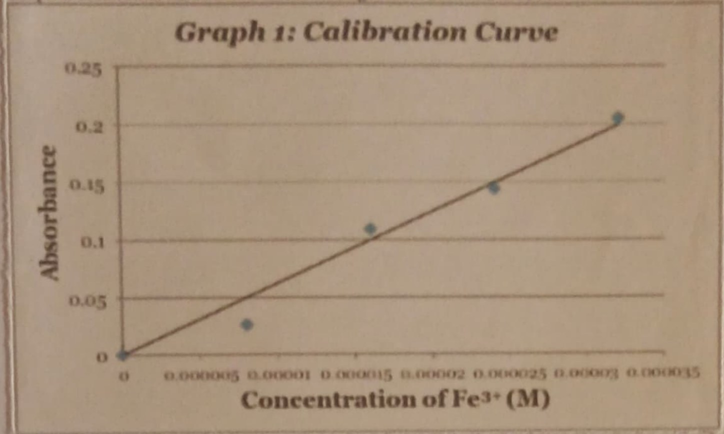
2. The absorbance of each solution was measured

3. Using this information, a calibration curve was plotted.

Results:

Solution	Fe^{3+} concentration (M)	Absorbance
1.	0	0
2.	0.000008	0.026
3.	0.000016	0.109
4.	0.000024	0.144
5.	0.000032	0.205
Sample	unknown	0.098

Graph 1: Calibration curve of the absorbance against concentration of Fe^{3+}



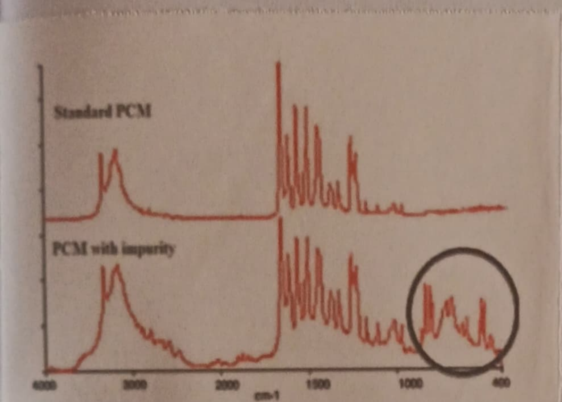
Detection

After the light has passed through the sample, a detector is used to convert the light into a readable electronic signal. Generally, detectors are based on photoelectric coatings or semiconductors. A photoelectric coating ejects negatively charged electrons when exposed to light. When electrons are ejected, an electric current proportional to the light intensity is generated. A photomultiplier tube (PMT) is one of the more common detectors used in UV-Vis spectroscopy. A PMT is based on the photoelectric effect to initially eject electrons upon exposure of light, followed by sequential multiplication of the ejected electrons to generate a larger electric current. PMT detectors are especially useful for detecting very low levels of light.

Applications of UV Spectroscopy:

1. Detection of Impurities

UV absorption spectroscopy is one of the best methods for determination of impurities in organic molecules. Additional peaks can be observed due to impurities in the sample and it can be compared with that of standard raw materials. By also measuring the absorbance at specific wavelength, the impurities can be detected.



This shows the UV spectra of a Paracetamol (PCM)

2. Structure elucidation of organic compounds

UV spectroscopy is useful in the structure elucidation of organic molecules, the presence or absence of unsaturation, the presence of hetero atoms. From the location of peaks & combination of peaks, it can be concluded that whether the compound is saturated or unsaturated.

3. Quantitative Analysis

UV absorption spectroscopy can be used for the quantitative determination of compounds that absorb UV radiation. This determination is based on Beer's law which is as follows.

$$A = \log I_0 / I_t = \log 1/T = -\log T = abc = \varepsilon bc$$

1. Qualitative Analysis

UV absorption spectroscopy can characterize those types of compounds which absorbs UV radiation. Identification is done by comparing the absorption spectrum with the spectra of known compounds

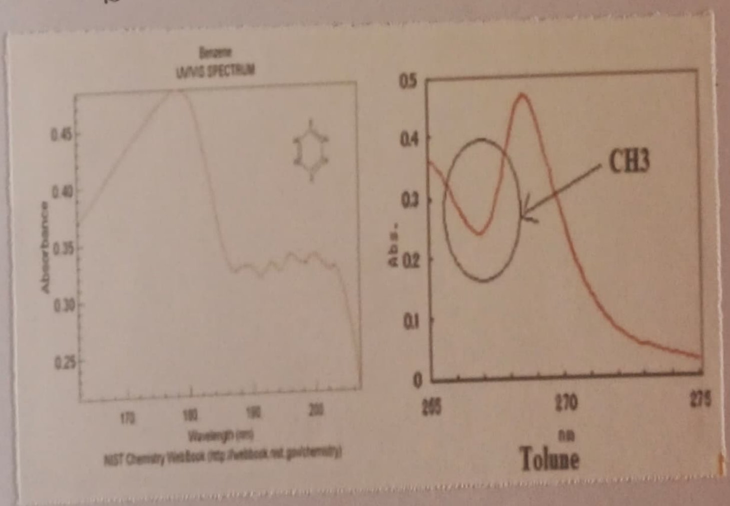
. Chemical Kinetics

Kinetics of reaction can also be studied using UV spectroscopy. The UV radiation is passed through the reaction cell and the absorbance changes can be observed.

. Detection of Functional Groups.

This technique is used to detect the presence or absence of functional group in the compound. Absence of a band at particular wavelength regarded as an evidence for absence of particular group.

Benzene Tolune



7. As HPLC Detector

A UV/Vis spectrophotometer may be used as a detector for HPLC.

Conclusion

We can conclude that UV spectroscopy is best method which routinely used in analytical chemistry for the quantitative determination of different analytes, such as transition metal ions, highly conjugated organic compound and biological. The proposed method development of UV-Visible spectroscopy was found to be least rapid, precise, accurate and sensitive in comparison to other.

The advent of new types of UV-Vis instrumentation and sample presentation options has aided in the development of new possibilities to monitor many processes in biological samples. It is a cost-effective, simple, versatile, non-destructive, and analytical technique, which is suitable for a large spectrum of organic compounds and some inorganic species.

Government of Karnataka

Model Curriculum

Program Name	BSc in Physics		Semester	VI
Course Title	Electronic Instrumentation & Sensors **(Theory)**			
Course Code:	PHY C16 - T		No. of Credits	04
Contact hours	**60 Hours**		Duration of SEA/Exam	**2 hours**
Formative Assessment Marks	**40**		Summative Assessment Marks	**60**

Course Pre-requisite(s):

Course Outcomes (COs): After the successful completion of the course, the student will be able to:

- Identify different types of tests and measuring instruments used in practice and understand their basic working principles.
- Get hands on training in wiring a circuit, soldering, making a measurement using an electronic circuit used in instrumentation.
- Have an understanding of the basic electronic components viz., resistors, capacitors, inductors, discrete and integrated circuits, colour codes, values and pin diagram, their practical use.
- Understanding of the measurement of voltage, current, resistance value, identification of the terminals of a transistor and ICs.
- Identify and understand the different types of transducers and sensors used in robust and hand-held instruments.
- Understand and give a mathematical treatment of the working of rectifiers, filter, data converters and different types of transducers.
- Connect the concepts learnt in the course to their practical use in daily life.
- Develop basic hands-on skills in the usage of oscilloscopes, multimeters, rectifiers, amplifiers oscillators and high voltage probes, generators and digital meters.
- Servicing of simple faults of domestic appliances: Iron box, immersion heater, fan, hot plate, battery charger, emergency lamp and the like.

Contents	60 Hrs
Power supply AC power and its characteristics, Single phase and three phase, Need for DC power supply and its characteristics, line voltage and frequency, Rectifier bridge, Filters: Capacitor and inductor filers, L-section and π-section filters, ripple factor, electronic voltage regulators, stabilization factor, voltage regulation using ICs. (5 hours) **Basic electrical measuring instruments** Cathode ray oscilloscope- Block diagram, basic principle, electron beam, CRT features, signal display. Basic elements of digital storage oscilloscopes. Basic DC voltmeter for measuring potential difference, Extending Voltmeter range, AC voltmeter	

using rectifiers
Basic DC ammeter, requirement of a shunt, Extending of ammeter ranges.

Topics for self-study: (5 hours)
Average value and RMS value of current, Ripple factor, Average AC input power and DC output power, efficiency of a DC power supply. Multirange voltmeter and ammeter.

Activities (3 hours)

1. Design and wire your own DC regulated power supply. Power output: 5 V, 10 V, ± 5 V. Components required: A step down transformer, semiconductor diodes (BY126/127), Inductor, Capacitor, Zener diode or 3-pin voltage regulator or IC. Measure the ripple factor and efficiency at each stage. Tabulate the result.
2. Extend the range of measurement of voltage of a voltmeter (analog or digital) using external component and circuitry. Design your own circuit and report.
3. Measure the characteristics of the signal waveform using a CRO and function generator. Tabulate the frequency and time period. Learn the function of Trigger input in an CRO.
4. Learn to use a Storage Oscilloscope for measuring the characteristics of a repetitive input signal. Convince yourself how signal averaging using Storage CRO improves S/N ratio.

Unit-II: Wave form generators and Filters

Basic principle of standard AF signal generator: Fixed frequency and variable frequency, AF sine and square wave generator, basic Wein-bridge network and oscillator configuration, Triangular and saw tooth wave generators, circuitry and waveforms.

(5 hours)

Passive and active filters. Fundamental theorem of filters, Proof of the theorem by considering a symmetrical T-network. Types of filters, Circuitry and Cut-off frequency and frequency response of Passive (RC) and Active (op-amp based) filters: Low pass, high pass and band pass.

(5 hours)

Activities (3 hours)

1. Measure the amplitude and frequency of the different waveforms and tabulate the results.

Required instruments: A 10 MHz oscilloscope, Function generators (sine wave and square wave).

2. Explore where signal filtering network is used in real life. Visit a nearby telephone exchange and discuss with the Engineers and technicians. Prepare a report.
3. Explore op-amp which works from a single supply biasing voltage (+15V). Construct an inverting/non-inverting amplifier powered by a single supply voltage instead of dual or bipolar supply voltage.
4. Op-amp is a linear (analog) IC. Can it be used to function as logic gates? Explore, construct and implement AND, OR NAND and NOR gate functions using op-amps.

Verify the truth table. Hint: LM3900 op-amp may be used. The status of the output may be checked by LED.

Unit-III: Data Conversion and display

Digital to Analog (D/A) and Analog to Digital (A/D) converters – A/D converter with pre-amplification and filtering. D/A converter - Variable resistor network, Ladder type (R-2R) D/A converter, Op-amp based D/A converter.

(4 hours)

Digital display systems and Indicators- Classification of displays, Light Emitting Diodes (LED) and Liquid Crystal Display (LCD) – Structure and working.

(3 hours)

Data Transmission systems – Advantages and disadvantages of digital transmission over analog transmission, Pulse amplitude modulation (PAM), Pulse time modulation (PTM) and Pulse width modulation (PWM)- General principles. Principle of Phase Sensitive Detection (PSD).

(3 hours)

Topic for self-study: Lock-in amplifier and its application, phase locked loop.

Activities (3 hours)

1. Explore where modulation and demodulation technique is employed in real life. Visit a Radio broadcasting station. (Aakashavani or Private). Prepare a report on different AM and FM stations.
2. Explore and find out the difference between a standard op-amp and an instrumentation op-amp. Compare the two and prepare a report.

Unit-IV: Transducers and sensors

Definition and types of transducers. Basic characteristics of an electrical transducer, factors governing the selection of a transducer, Resistive transducer-potentiometer, Strain gauge and types (general description), Resistance thermometer-platinum resistance thermometer. Thermistor. Inductive Transducer-general principles, Linear Variable Differential Transducer (LDVT)- principle and construction, Capacitive Transducer, Piezo-electric transducer, Photoelectric transducer, Photovoltaic cell, photo diode and phototransistor – principle and working.

(10 hours)

Activities (3 hours)

1. Construct your own thermocouple for the measurement of temperature with copper and constantan wires. Use the thermocouple and a Digital multimeter (DMM). Record the emf (voltage induced) by maintaining one of the junctions at a constant temperature (say at 0° C, melting ice) and another junction at variable temperature bath. Tabulate the voltages induced and temperatures read out using standard chart (Chart can be downloaded from the internet).
2. Observe a solar water heater. Some solar water heaters are fitted with an anode rod (alloy of aluminium). Study why it is required. Describe the principle behind solar water heater.

Pedagogy: Lecture/ PPT/ Videos/ Animations/ Role Plays/ Think-Pair-Share/ Predict-Observe-Explain/ Demonstration/ Concept mapping/ Case Studies examples/ Tutorial/ Activity/ Flipped Classroom/ Jigsaw/ Fie[ld] based Learning/ Project Based Learning/ Mini Projects/ Hobby Projects/ Forum Theatre/ Dance/ Proble[m] Based Learning/ Game Based Learning/ Group Discussion/ Collaborative Learning/ Experiential Learning / Se[lf] Directed Learning etc.

Formative Assessment for Theory	
Assessment Occasion/ type	**Marks**
Total	**40 Marks**
Formative Assessment as per UNIVERSITY guidelines are compulsory	