Module-6 Unit-4
UV-Vis Spectroscopy

Spectroscopy
Spectroscopy involves investigating the interaction of electromagnetic field with matter. Historically, it originated as the study of wavelength based dispersion of visible light by a prism. The idea was expanded to include all the interactions involving radiative energy variation with wavelength or frequency. Spectroscopic data is represented as an emission spectrum of wavelength or frequency dependent response. Spectroscopy can be broadly classified into two categories – (a) techniques based on energy transfer between photon and sample, and (b) reflections, refraction, diffraction, dispersion, or scattering from the sample altering the amplitude, phase angle, polarization, or direction of propagation of the electromagnetic radiation.
Over the years, spectroscopy has evolved as a potential tool for experiments and analyses conducted in research laboratories and industries. This technique is essentially considered by analysts as an apparent solution. The objective should also be to use these spectroscopic techniques in control and industrial laboratories and to develop fully recognised spectroscopic techniques.
This chapter reviews the interactions of ultraviolet, visible, and infrared radiations with matter. Irrespective of differences in the instrumentation, all spectroscopic techniques have many common attributes. The similarities as well as differences between various spectroscopic techniques have been outlined.

1. **Principles and instrumentation for UV-Vis-IR**

Ultraviolet (UV) spectroscopy is an important physical tool which exploits light in ultraviolet, visible, and near infrared range of electromagnetic spectrum. Beer-Lambert law establishes a linear relationship between absorbance, concentration of absorbers (or absorbing species) in the solution and the path length. Therefore, UV-Vis spectroscopy can be employed for determining the concentration of the absorbing species, for a fixed path length [1]. This is a very simple, versatile, fast, accurate and cost-effective technique. Instrument employed for ultraviolet−visible (or UV-Vis) spectroscopy is called UV−Vis−NIR Spectrophotometer. This can be used to analyze liquids, gases and solids by using radiative energy corresponding to far and near ultraviolet (UV), visible (Vis) and near infrared (NIR) regions of electromagnetic spectrum. Consequently, predetermined wavelengths in these regions have been defined as: UV: 300 - 400 nm; Vis: 400 - 765 nm; and NIR: 765 - 3200 nm.

1.1 Principle: A light beam is passed through an object and wavelength of the light reaching the detector is measured. The measured wavelength provides important information about chemical structure and number of molecules (present in intensity of the measured signal). Thus, both quantitative and qualitative information can be gathered. Information may be obtained as transmittance, absorbance or reflectance of radiation in 160 to 3500 nm wavelength range [2,3]. The absorption of incident energy promotes electrons to excited states or the anti-bonding orbitals. For this transfer to occur, photon energy must match the energy needed by electron to be promoted to next higher energy state. This process forms the basic operating principle of absorption spectroscopy. Potentially, there may be three types of ground state orbitals involved:

1. σ (bonding) molecular orbital

2. π (bonding) molecular orbital

3. n (non-bonding) atomic orbital

Besides, the anti-bonding orbitals are:

i. σ* (sigma star) orbital

ii. π* (pi star) orbital

A transition involving excitation of an electron from s bonding orbital to σ anti-bonding orbital is called σ to σ* transition. Likewise, π to π* represents the excitation of an electron of a lone pair (non-bonding electron pair) to an antibonding π orbital. Electronic transitions occurring due to absorption of UV and visible light are:

σ to σ*;

n to σ*;

n to π*;

π to π*.

The transitions s to σ* and n to σ* involve higher energies and thus usually occur in far UV region or weakly in 180 to 240nm region. Thus, saturated groups do not show strong absorption in UV region. Molecules with unsaturated centres undergo n to π* and π to π* transitions; these transitions involve lesser energies and thus occur at longer wavelengths than transitions to σ* anti-bonding orbitals.

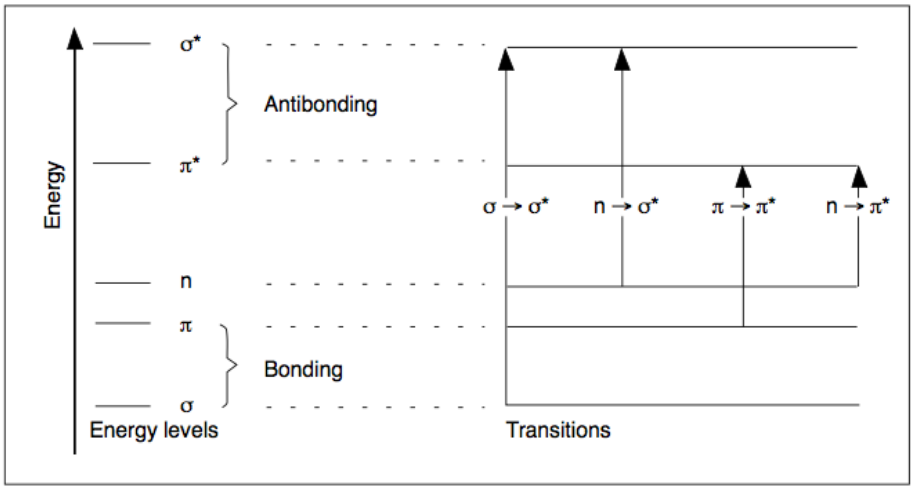


Figure 1 Electron transitions in UV-Vis spectroscopy.

When light having specific wavelength and energy is focused onto the sample, it absorbs some energy of the incident wave. A photodetector measures energy of transmitted light from sample, and registers absorbance of the sample. The absorption or transmission spectrum of the light absorbed or transmitted by the sample against the wavelength is formed. Bouguer−Beer law or the Lambert−Beer rule (**Figure 2**) is basic principle of quantitative analysis, and it establishes that absorbance of a solution scales directly with analyte concentration. For a given wavelength, the absorbance (unit less) A is described as the molar absorptivity of the absorbing species (M^{-1} cm^{-1}), b is path length of sample holder (normally 1 cm), and c is the concentration of the solution (M).

$$A = a \cdot b \cdot c \qquad (1)$$

UV−Vis−NIR spectrometer can monitor absorbance or transmittance in UV – visible wavelength range. The relation between incident light of intensity 'I_o' and transmitted light of intensity 'I' is described as follows.

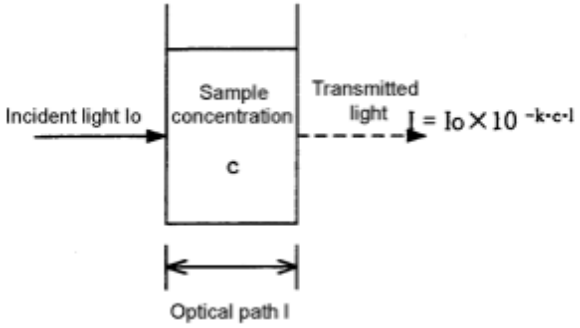


Figure 2 Bouguer−Beer Rule.

Transmittance (T) is given by I/I_o and $(I/I_o)*100$ gives transmission rate (T%). Absorbance (abs) is the inverse of transmittance and given by log $(1/T) = log(Io/I)$.

$$T = I/I_o = 10^{-kcl} \quad (2)$$

$$abs = \log (1/T) = \log(I_o/I) = -kcl \quad (3)$$

here, k represents constant of proportionality. Transmittance does not depend on sample concentration, whereas absorbance shows proportionality with concentration of sample (Beer's law) and optical path (Bouguer's law). Additionally, when optical path is 1 cm and concentration of targeted material is 1mol/l, k is described as molar absorption coefficient and denoted as 'ε'. Under specific conditions, molar absorption coefficient is typical of the material. Bouguer−Beer rule assumes the absence of any stray, generated, scattered, or reflected light.

The UV-Vis spectrum can by recorded via the following types of absorbance instruments:

a. Single bram spectrometer

b. Double beam spectrometer

c. Simultaneous spectrometer

Light source (mostly tungsten lamps), small holder and detector are common to all the three type of spectrometers. However, a filter may be used, in addition, to select one wavelength at a time. This filter is often termed as the monochromator. Single beam spectrometer (shown in **Figure 3**) includes a monochromator between the light source and specimen. The specimen is analysed individually for all wavelengths. Double beam spectrometer (**Figure 4**) uses a single light source, monochromator, a splitter and a series of mirrors, to direct the beam towards the reference and the sample under investigation. Whereas, a simultaneous spectrometer (**Figure 5**) uses an array of diodes for simultaneous detection of absorbance at all wavelengths. This is the fastest and most efficient of the three.

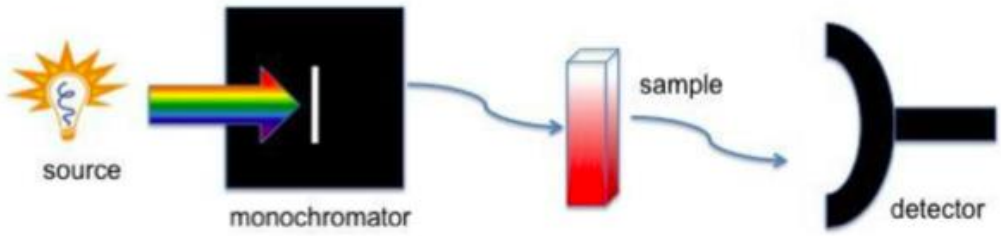


Figure 3 Schematics of a single beam UV-Visible spectrometer.

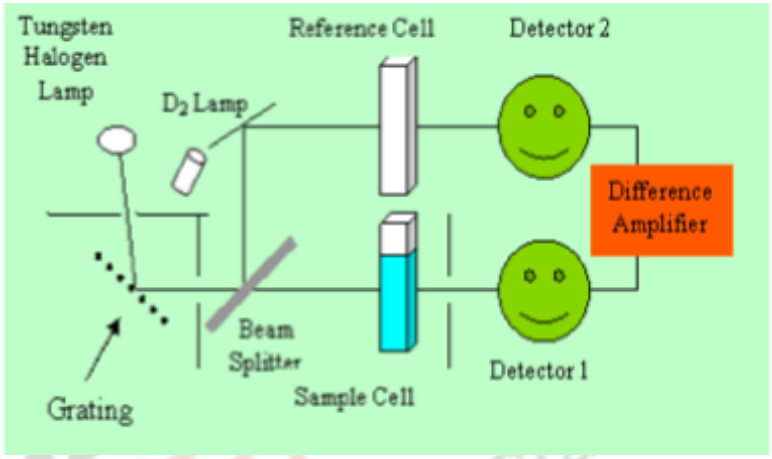


Figure 4 Double beam UV-Visible spectrometer.

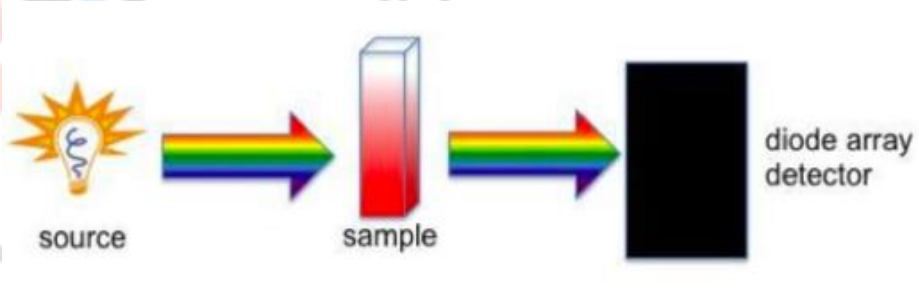


Figure 5 Simultaneous UV-Visible spectrometer.

1.2 Instrumentation: The basic components of a spectrometer include: light source (UV and visible), monochromator (wavelength selector), sample stage, and detector. A tungsten filament, continuous over UV region is generally used as light source. Detector is usually a photodiode or CCD. Photodiodes go with monochromators to filter light of a particular wavelength, to be fed to the detector. While monitoring the absorbance in UV spectrum, the visible lamp must be turned off, and vice-versa. **Figure 6** includes schematic UV−Vis−NIR Spectrometer.

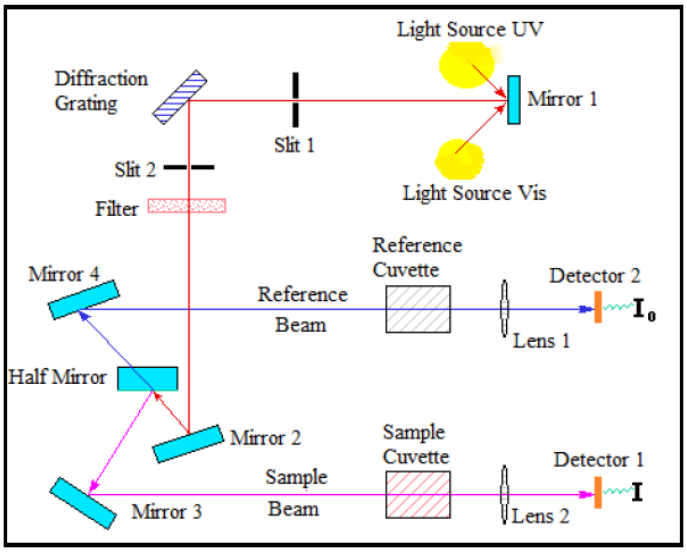


Figure 6 UV−Vis−NIR Spectrometer.

Instrumental components

1. **UV Source**

The power of radiating source should not vary in its operating wavelength range. Continuous UV spectrum is produced by electrically exciting deuterium or hydrogen at low pressures. The mechanism for generation of UV light includes creating an excited molecular species, that breaks into two atomic species and a UV photon. The emission wavelengths of both deuterium and hydrogen lamps are in 160 to 375 nm range. The material of the cuvettes needs to selected such that it does not absorb the light incident, because this will result in errors in obtained absorption spectrum. Thus, quartz is usually used.

2. **Visible Light Source**

Tungsten filament lamp is used as visible light source. This lamp can produce light in 350 to 2500 nm wavelength range. In a tungsten filament lamp, energy emitted is proportional to the fourth power of the operating voltage. Thus, in order to get stable emission, a highly stable voltage must be applied to the lamp. The stability of voltage is ensured by using electronic voltage regulators or constant-voltage transformers. Tungsten/halogen lamps include small quantities of iodine embedded within a quartz 'envelope', which also contains the tungsten filament. The iodine reacts with gaseous tungsten, formed by sublimation, and produces a volatile compound WI_2. As WI_2 molecules hit the filament, they decompose, and redeposit tungsten back on the filament. The tungsten/halogen lamps usually have lifetime twice to the conventional tungsten filament lamp. Tungsten/halogen lamps are used in modern spectrophotometers owing to their high efficiency, and their output extends to UV region as well.

3. **Cuvettes**

Monochromator source is used; before reaching sample, light is divided in two parts of similar intensity with a half-mirror splitter. One part (or sample beam), travels via the cuvette having the solution of material to be examined in transparent solvent. Second beam, or reference beam, travels via similar cuvette having only solvent. Reference and sample solution containers have to be transparent towards passing beam.

4. **Detectors**

Detector detects intensity of light transmitted by cuvettes and sends this data to a meter to record and

display the values. Electronic detectors calculate and compare the intensities of light beams. Several UV−Vis spectrophotometers have two detectors – a phototube and a photomultiplier tube, and reference and sample beams are monitored simultaneously. The photomultiplier tube is the extensively used detector in UV-Vis instruments. It includes a photoemissive cathode (electrons are emitted from the cathode when photons strike it), several dynodes (a dynode emits multiple electrons when one electron strikes it) and an anode. The incident photon, after entering the tube, strikes the cathode. The cathode then emits multiple electrons, which are then accelerated towards the first dynode (whose potential is 90V more positive than cathode). The electrons strike the first dynode, leading to the emission of several electrons for each incident electron. These electrons are then accelerated towards the second dynode, to produce more electrons which are accelerated towards dynode three and so on. All the electrons are eventually collected at the anode. By this time, each original photon has produced $10^6 – 10^7$ electrons. The resulting current is amplified and measured. Photomultipliers are highly sensitive towards UV and visible radiations and have fast response times. However, photomultipliers are used only at low power radiation as high power light may damage them.

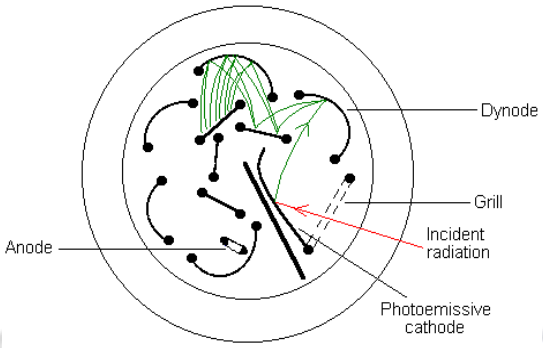


Figure 7 Cross-section of a photomultiplier tube.

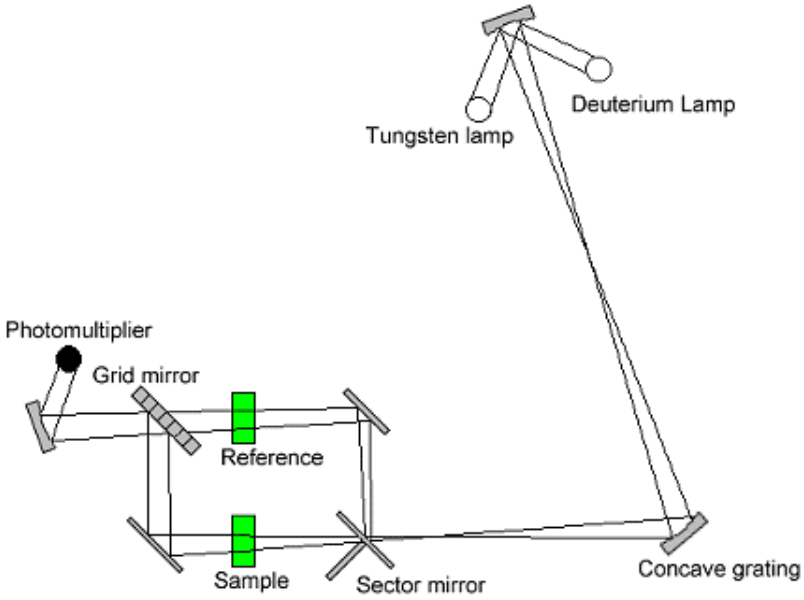
The linear photodiode array is an example of a multichannel photon detector. These detectors can simultaneously measure all elements of a beam of dispersed radiation. A linear photodiode array consists of several small silicon photodiodes created on a single silicon chip. The number of photodiodes can vary between 64 to 4096 sensor elements on a chip, however, 1024 photodiodes is most common. For each diode, there is also a storage capacitor and a switch. The individual diode-capacitor circuits can be sequentially scanned.

Charge-Coupled Devices (CCDs) are like diode array detectors, but instead of diodes, they consist of an array of photocapacitors [4].

Reference beam intensity, should suffer little or no absorption, and termed I_0 whereas that of sample beam is called I. The spectrophotometer automatically examines all wavelength components in a short time. This technique is good to evaluate the concentration as well as molecular structure or structural changes. It may also be used to examine the vibrational and conformational energy levels alterations before and after an interaction with a substrate, or a molecule.

Review your learning

Now, you have an understanding of the separate components of a spectrophotometer, and how they are configured in the equipment. Following figure shows schematics Hitachi 100-60 manual double-beam spectrophotometer. Can you explain the working of each component?



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3. International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use, Validation of analytical procedures, 2000.
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DO YOU KNOW?

1. In order to acquire a UV-Vis spectrum, the solid has to be completely dissolved in a suitable solvent. Note that not every solvent is suitable for UV-Vis spectroscopy. Generally, spectrograde solvents are used that have undergone special purification steps to remove trace impurities of aromatic and other compounds. For instance, 95% ethanol is generally not suitable as solvent for UV-Vis measurements because it often contains aromatic compounds as denaturing agents.
2. Cuvettes also have limited optical windows. While cuvettes made from fused quartz allow measurements as far down as 200 nm, Pyrex cuvettes already show a significant absorption around 260 nm. Cuvettes made from acrylic plastic or ordinary glass absorb already at higher

wavelengths. In addition, many of the plastic cuvettes are not compatible with most organic solvents (the windows are not translucent anymore).

TIME LINE:

In the 1930s, vitamin research indicated that several vitamins, particularly vitamin A, absorb ultraviolet (UV) light. Spurred by the American government's interest in measuring vitamin content in soldiers' rations using ultraviolet and visible (UV-Vis) light, this research culminated in the commercial launch of UV-Vis spectrophotometers in the early 1940s. Of these, the Beckman DU spectrophotometer—first sold in 1941—distinguished itself from competing products by delivering more accurate results and reducing analysis time from hours, or even weeks, to minutes. Although modern UV-Vis spectrometers differ greatly from the first DUs, all operate on the same basic principle. Light intensity is measured from UV-Vis source lamps before and after the light passes through a sample. The amount of light absorbed corresponds to the molecular concentration in the sample.

Whether as standalone instruments or high performance liquid chromatography (HPLC) detectors, UV-Vis spectrophotometers are indispensible for measuring analyte concentrations—in scientific research, academic teaching, and QA/QC laboratories studying pharmaceuticals, proteins, DNA, solar panels, semiconductors, and coatings. New instrument designs and accessories have expanded the range of UV-Vis samples— from liquids traditionally measured in milliliter volumes in 1-cm cuvettes to today's micro volume.

Nobel laureate Bruce Merrifield referred to the UV-Vis spectrophotometer as "probably the most important instrument ever developed toward the advancement of bioscience."

1930s

In **1935**, Arnold O. Beckman founds National Technologies Laboratories—later named Beckman Instruments.

1940s

The first commercially available UV-Vis spectrophotometers are introduced. In **1940**, Beckman and colleagues at National Technologies Laboratories make their first laboratory spectrophotometer. Howard H. Cary, who leads the project, would later form the Cary Instrument Company.

In **1941**, Beckman introduces the DU UV-Vis spectrophotometer, which has higher resolution and lower stray light in the ultraviolet region than any other commercial instrument.

In **1946**, Cary Instruments is founded by Howard Cary, George W. Downs, and William C. Miller under the name Applied Physics Corporation. Previously, Howard Cary was vice president in charge of development for Beckman Instruments.

In **1947**, Applied Physics Corporation delivers the first commercially available recording UV-Vis spectrophotometer, the Cary 11, to the Mellon Institute in Pittsburgh, PA.

1950s

1950s – 1970s Mass production reduces the cost of UV-Vis spectrophotometers. New photodiode arrays collect all wavelengths simultaneously, reducing the time required to scan a spectrum from minutes to seconds. In 1950, National Technologies Laboratories changes its name to Beckman Instruments, Inc.

In **1953**, Bausch & Lomb introduces the SPECTRONIC 20 UV-Vis spectrophotometer, the first massproduced, low-cost UV-Vis spectrophotometer.

In **1954**, Applied Physics Corporation launches the Cary 14 spectrophotometer, the first commercially available double-beam spectrophotometer. The doublebeam design greatly simplifies and speeds up sample analysis by simultaneously measuring sample and solvent transmittance over the wide spectral range of ultraviolet, visible, and near infrared wavelengths.

1960s

In **1963**, JASCO introduces the ORD\UV-5 with double-beam UV-Vis capabilities.

In **1966**, Applied Physics Corporation is purchased by Varian Medical Systems, becoming the Cary Instruments division of Varian.

In **1969**, Cecil Instruments introduces the CE 212, the world's first commercially available variable wavelength detector for HPLC, allowing users to select—without changing filters or lamps—detection wavelengths on a single detector.

1970s

In **1979**, Hewlett-Packard launches the first commercially available diode-array spectrophotometer, the

8450A. Unlike traditional scanning spectrophotometers with a single photomultiplier tube that scans one wavelength at a time, the 8450A utilizes an array of photodiodes to scan simultaneously the full spectrum of wavelengths in seconds.

1980s

The proliferation of personal computers in the **1980s** improves data acquisition and instrument control.

In **1980**, Bausch & Lomb introduces the Spectronic 2000 UV-Vis spectrophotometer, the first microprocessor-controlled double-beam UV-Vis spectrophotometer. Now, instead of measuring sample and solvent transmittance separately, which the single-beam spectrophotometers required, the double-beam design greatly simplifies and speeds up sample analysis by simultaneously measuring sample and solvent transmittance.

In **1987**, Pye Unicam Corporation. introduces the PU-8700 UV-Vis spectrophotometer, the first mouse-driven, graphical interface UV-Visible spectrophotometer.

In **1989**, Dr. Arnold O. Beckman, now 88 years old, receives the National Medal of Science for his leadership in analytical instrumentation development.

1990s

1990s, External software now provides PC control, onscreen spectra display, and spectra reprocessing and storage. Fiber optics reduce instrument size, and fiber optic sampling accessories allow sample measurement outside the UV-Vis spectrophotometer's sample compartment, eliminating the need to fill sample cells and cuvettes.

In **1995**, Hewlett-Packard launches the 8453A, the first small-footprint and full-featured diode-array spectrophotometer.

In **1997**, Beckman Instruments, Inc. acquires Coulter Corporation, the leading manufacturer of systems for blood and cell analysis. In 1998, the company is renamed Beckman Coulter, Inc.

In **1999**, Hewlett-Packard announces a strategic realignment to create an independent measurement company, Agilent Technologies.

2000s

2000s, Significant progress is made in the ability to measure micro volume liquid samples (< 1 μL) in biotechnology and pharmaceutical applications. UV-Vis spectroscopy is applied to alternative energy R&D such as solar energy. Instrument manufacturers start to miniaturize instruments and develop dedicated instruments for specific applications, such as biological applications.

In **2000**, Thermo Scientific introduces the GENESYS 10 instruments with out-of-plane optics that minimize stray light and reduce noise caused by instrument optics.

In **2002**, Varian Inc. releases the 6000i UV-Vis-NIR spectrophotometer. The Cary 6000i uses an InGaAs detector that improves signal-tonoise ratio over conventional lead sulfide detectors. Its operating range of 175 nm to 1800 nm is applicable to materials science research.

In **2003**, Thermo Scientific introduces the Evolution 300 spectrophotometer, the first double-beam xenon lamp-based spectrophotometer. The double-beam design simplifies and speeds up sample analysis. Xenon flash lamps provide a high-energy light source with a shorter warm up time and longer lamp life than traditional tungsten and deuterium lamps.

In **2004**, Shimadzu introduces the SolidSpec-3700/3700DUV series of UV-Vis-NIR spectrophotometers, the first UV-Vis-NIR spectrophotometer with three detectors—a photomultiplier for the UVVis region, and an InGaAs detector and a cooled PbS detector for the NIR region.

In **2005**, the NanoDrop ND-1000 UV-Vis spectrophotometer (from NanoDrop Technologies) for micro-quantitation of only 1 μl of sample enters the market. The sample is directly pipetted onto a fiber optic measurement surface where it is held in place by surface tension, eliminating the need for cuvettes or capillaries.

In **2006**, JASCO manufactures a new range of UV-Vis-NIR spectrophotometers with compatible accessories for life sciences, materials analysis, and semiconductor R&D.

In **2008**, Shimadzu launches the UV- 1800 compact UV-Vis spectrophotometer. It occupies a 15% smaller footprint than the model it replaces, the UV-1700.

Also in **2008**, Perkin Elmer releases the LAMBDA Bio UV-Vis spectrophotometer pre-configured with standard methods for biological applications including protein assays, cell density measurements, as well as DNA, RNA, and oligonucleotides concentration and purity.

2010s

In **2010**, Agilent Technologies acquires Varian Inc. and continues to offer the Cary spectrophotometer

series under the Cary name.
Also in **2010**, Thermo Scientific introduces the Evolution 200 Series spectrophotometer. Its application-focused beam geometry tailors the instrument's optical system to specific applications for microcells, solid sampling, and fiber optics.
Also in **2010**, JASCO offers the SAH-769 One Drop accessory to measure micro volume samples of proteins and nucleic acids with UVVis spectrophotometers.
In **2011**, Agilent Technologies releases the Cary 60 UV-Vis spectrophotometer with low cost of ownership—the xenon lap typically lasts 10 years—and remote sampling options that minimize sample handling.

SELF-ASSESSMENT:

Objective Questions:

1) Absorption occurs at...

a) All wavelengths in the spectrum.
b) A characteristic wavelength dependent on the molecule
c) The UV region
d) The visible region

2) Transmittance is...

8) The amount of radiation absorbed by the sample
8) The amount of radiation initially divided by the amount of radiation passing through a sample.
8) The amount of radiation passing through the sample divided by the initial amount
8) The wavelength used that promotes an electron

3) The Beer-Lambert Law...

a) Relates absorbance, concentration, path length and molar absorption coefficient
b) Tells us the volume of the sample
c) Relates frequency and wavelength
d) Allows us to calculate how conjugated the system is

4) Conjugated systems tend to absorb in the visible region because...

a) electrons are coloured
b) overlapping pi orbitals increase the energy gap between orbitals
c) overlapping pi orbitals reduce the energy gap between orbitals
d) 100% transmittance occurs

5) DE is the...

a) difference in energy between the HOMO and the LUMO
b) The energy of the HOMO
c) The energy of the LUMO
d) The energy of the HOMO plus the energy of the LUMO.

6) UV-Visible spectrometer uses a prism to...

a) Focus all wavelengths on the sample simultaneously
b) Separate radiation into its constituent wavelengths
c) Reduce the amount of radiation passing through the sample
d) Stop any radiation going through the sample

7) UV-Vis. Spectroscopy of organic compounds is usually concerned with which electronic transition(s)?

a) s to s*
b) s to s*
c) n to p* and p to p*

8) Ultraviolet-visible spectroscopy or ultraviolet-visible spectrophotometry **(**UV-Vis or UV/Vis**)** involves the spectroscopy of _____in the UV-visible region.

• Atom • Photon • Standard Model • Electron

9) The basic parts of a spectrophotometer are a light source, a holder for the sample, a _____ or monochromator to separate the different wavelengths of light, and a detector.

• Diffraction grating • Holography • Dispersion (optics) • Optics

1) _____, especially those with a high degree of conjugation, also absorb light in the UV or visible regions of the electromagnetic spectrum.

• Biochemistry • Carbon • Organic compound • Organic chemistry

Subjective Questions:

1) What is a fundamental difference between IR detectors and UV/vis detectors?

2) Write the applications of UV-Visible spectroscopy.

3) How does delocalisation of electron helped in getting a molecule to absorb UV-Visible radiation and displaying the UV-Visible absorption spectra?

4) Explain in short non-bonding electron excitation and UV spectra.

5) Write a brief note on: intensity of vibrational, rotational and electronic spectra.