

**SHREE H. N. SHUKLA INSTITUTE OF
PHARMACEUTICAL EDUCATION AND
RESEARCH**



B.PHRAM

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**SUBJECT NAME: INSTRUMENTAL METHOD OF
ANALYSIS**

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CHAPTER 1: UV VISIBLE SPECTROSCOPY

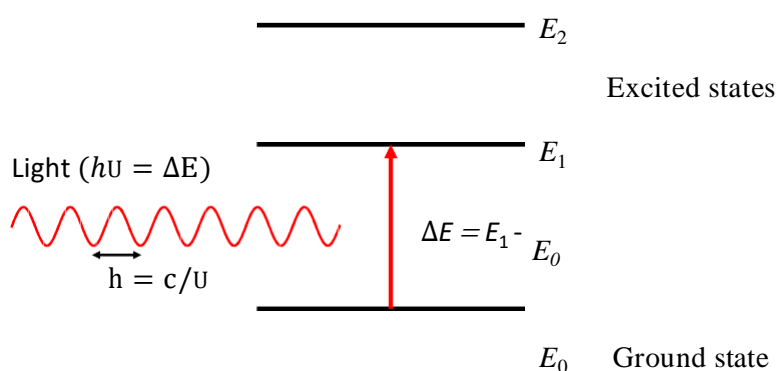
UV/VIS Spectroscopy

1 Introduction

Absorption of Radiation

Electronic orbitals of atoms and molecules have characteristic energies, giving rise to a set of discrete energy levels. An electron is able to change from an occupied orbital

to another orbital, gaining or losing energy only in amounts exactly corresponding to the difference between two levels: The transition from the ground state (lowest possible



energy) at energy E_0 to a higher level at energy E_n is possible if the molecule absorbs electromagnetic radiation of the corresponding wavelength $\lambda = hc/(E_n - E_0)$, where c is the speed of light and h is Planck's constant. Excited states usually exist only for a very short period of time (femtoseconds to microseconds), because the higher energy state is unstable and the extra energy is lost through relaxation processes such as emission of light (see the Experiment Fluorescence Quenching). The typical energy difference between the ground and the first excited levels of many molecules corresponds to electromagnetic waves of the ultra-violet (UV) and visible regions of the electromagnetic spectrum.

The Electromagnetic Spectrum

The UV-visible range is only a small part of the total electromagnetic spectrum, and is generally defined from wavelengths of 190 nm at the high energy UV end to about 750 nm at the low energy red end of the spectrum. Light in other regions of the spectrum gives rise to different types of transitions and is the subject of different types of spectroscopy. For example, IR radiation is usually not energetic enough to cause electronic transitions but can excite vibrations of molecules. The wavelength λ is the distance between adja-

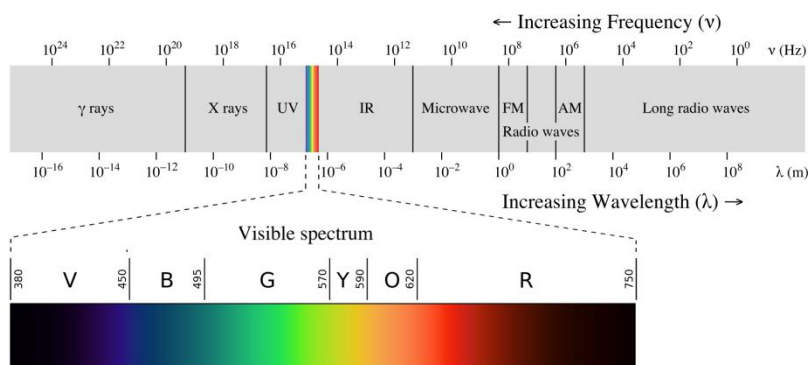


Figure 1: The electromagnetic spectrum.

Source: http://upload.wikimedia.org/wikipedia/commons/3/30/EM_spectrumrevised.png

cent peaks (or troughs) in the time-frozen electromagnetic wave, and is given in meters, centimetres or nanometres (10^{-9} meters). Visible wavelengths cover a range from approximately 400 to 750 nm. The frequency ν is the number of wave cycles that travel past a fixed point per unit of time, and is usually given in cycles per second, or Hertz (Hz). Frequency and wavelength are related via

$$\lambda = c/\nu = \frac{2\pi c}{\omega} \quad (1)$$

where c is the speed of light. The angular frequency $\omega = 2\pi\nu$ (radians per second) is often used instead of ν . When polychromatic or 'white' light passes through or is reflected by a coloured substance, a characteristic portion of the spectrum is absorbed. The remaining light will then exhibit the complementary colour to the wavelength(s) absorbed. Thus, absorption of blue light between 420-430 nm renders a substance yellow, and absorption of green, 500-520 nm light makes it red. Green, to which our eyes are most sensitive, is unique in that it can be created by absorption close to 400 nm as well as absorption near 800 nm.

Absorbance and Electronic Transitions

The Lambert-Beer Law

After crossing a thin slice Δx of absorbing sample, the light intensity $I(\Delta x)$ is smaller by an amount proportional to the initial intensity I_0 and the thickness of the slice. We can thus write:

$$I(\Delta x) = I_0 - \alpha I_0 \Delta x \quad (2)$$

This can be recast into the form:

$$\frac{I(\Delta x) - I_0}{\Delta x} = -\alpha I_0 = \frac{dI}{dx} \quad (3)$$

which is a differential equation with the solution

$$I(x) = I_0 \exp(-\alpha x) \quad (4)$$

By convention, this Lambert-Beer law is usually written in decadic form:

$$I(x) = I_0 10^{-ax} \quad (5)$$

with the proportionality constant $a = \alpha/\ln(10)$. The higher the concentration c of molecules in the sample, the larger is a . Writing $a = sc$ obtain for the light intensity after a sample of thickness d :

$$I(d) = I_0 10^{-scd} \quad (6)$$

The wavelength dependent quantity $s(\lambda)$ is called molar extinction coefficient. It is a measure of the probability that an electronic transition takes place when a molecule interacts with light of wavelength λ . By convention, the sample thickness d is given in cm, the concentration in mol/L. Consequently, the unit of s is

$$[s] = \frac{\text{L}}{\text{mol cm}} = \text{M}^{-1}\text{cm}^{-1} \quad (7)$$

UV-visible spectrometers usually display the absorbance $A(\lambda)$, which is the negative log- ratio of transmitted (sample in beam) over incident (no sample in beam) intensities:

$$A(\lambda) = - \log_{10} \frac{I(d)}{I_0} = s(\lambda)cd \tag{8}$$

When more than one species with concentration c_i is present in the sample, the absorbance is the sum of the different contributions:

$$A(\lambda) = (s_1(\lambda)c_1 + s_2(\lambda)c_2 + . . .) d \tag{9}$$

An absorption spectrum $A(\lambda)$ shows us the wavelength at which a molecule can absorb light and thus provides information about electronic state energies. Consequently, absorption spectroscopy in the UV-visible spectral region is sometimes called "electronic spectroscopy". From the magnitude of $A(\lambda)$ we can obtain information about sample composition or the probability of an electronic transition. Depending on the nature of the ground and excited state orbitals this probability can be very different.

The Transition Dipole Moment

The probability of an electronic transition is proportional to the square of the electronic transition dipole moment, which is defined as

$$\mu_{0n} = e \int \psi_0(\vec{r}) \vec{r} \psi_n(\vec{r}) \tag{10}$$

where ψ_n is the wavefunction of electronic state n and ψ_0 is the ground state wavefunction. You can think of equation 10 as a measure for the overlap between orbitals in the ground state and in the excited state. ¹

Absorption bands are never infinitely narrow but extend over a certain wavelength range. In solution, interactions with the solvent can modify the energy gap of individual molecules leading to a distribution of transition energies. Vibrational excitations also contribute to the broadening of an electronic transition. The overall transition probability should be independent of these broadening effects, and is extracted by integrating over the absorption band. This integral provides is experimental measure for the transition dipole moment:

$$|\mu_{0n}|^2 = \kappa \int_{\lambda}^{\lambda_{max}} \epsilon(\lambda) d\lambda \approx \kappa \epsilon(\lambda_{max}) \frac{\Delta\lambda}{\lambda_{max}} \tag{11}$$

with λ_{max} : central wavelength
 $\Delta\lambda$: full width at half-maximum (FWHM, see Fig.2)
 $\epsilon(\lambda_{max})$: extinction coefficient at λ_{max}

When s is in $M^{-1} cm^{-1}$, and the transition dipole moment μ_{0n} is in Debye (1 Debye = $= 3.335641 \cdot 10^{-30} C m$), the constant $\kappa = 0.0092$.

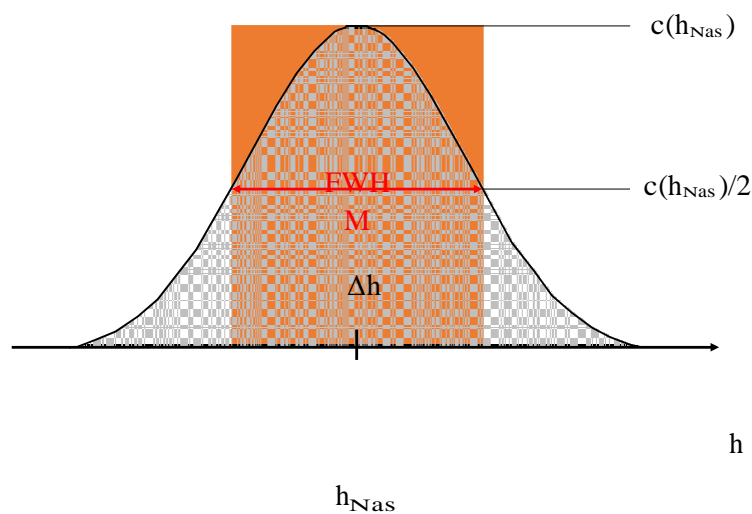


Figure 2: Absorption band and full width at half maximum. The orange area is an approximation for the integral over the full spectrum.

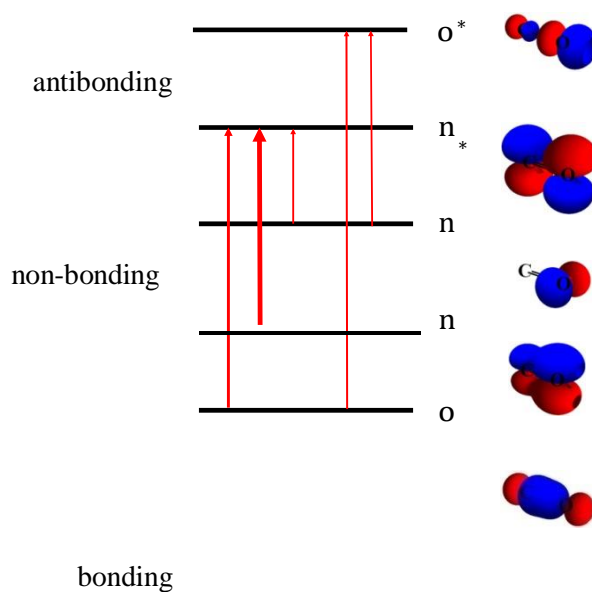


Figure 3: Illustration of different types of electronic transitions.

Different types of Electronic Transitions

A rigorous discussion of transition probabilities is beyond the scope of this lab course, but one obvious factor is the overlap of the orbitals involved in the electronic excitation. This is nicely illustrated by the two transitions of an isolated carbonyl group. The $n \rightarrow \pi^*$ transition is lower in energy ($\lambda_{max} \approx 290 \text{ nm}$) than the $\pi \rightarrow \pi^*$ transition ($\lambda_{max} \approx 180$

nm), but the transition probability of the former is a thousand times smaller than that of the latter. The spatial distribution of these orbitals suggests why this is so. As illustrated in Figure 3, the n -orbitals do not overlap at all well with the π^* orbital, so the probability of this excitation is small. The $\pi \rightarrow \pi^*$ transition, on the other hand, involves orbitals that have significant overlap, and the transition probability is very large.

Transitions between electronic states can be divided into the following categories:

¹The quantity is called transition dipole moment because of the analogy with a static dipole moment (see that Experiment): in quantum mechanics, the charge distribution of the electrons in the ground

- $\pi \rightarrow \pi^*$ transitions: For molecules that possess π bonds like alkenes, alkynes, aromatics, acryl compounds or nitriles, light can promote electrons from a π bonding molecular orbital to a π anti-bonding molecular orbital. This is called a $\pi \rightarrow \pi^*$ transition and is usually strong (high extinction coefficient ϵ). Groups of atoms involved in π bonding are thus often called **chromophores**. The transition energy (or absorption wavelength) can be an indication for different types of π bonds (carbon-carbon, carbon oxygen or carbon-nitrogen in a nitrile group).
- $n \rightarrow \pi^*$ transitions: Lone pair electrons that exist on oxygen and nitrogen atoms may be promoted from their non-bonding molecular orbital to a π anti-bonding molecular orbital. This is called an $n \rightarrow \pi^*$ transition and requires less energy (longer wavelength) compared to a $\pi \rightarrow \pi^*$ transitions within the same chromophore. However, the transition probability is usually much lower.
- $n \rightarrow \sigma^*$ transitions: Saturated compounds with substituents containing lone-pairs such as water, ammonia, hydrogen disulfide only have $n \rightarrow \sigma$ and $\sigma \rightarrow \sigma^*$ transitions in the UV-visible range.
- $d-d$ transitions: Many transition metal ion solutions are coloured as a result of their partially filled d -levels, which allows promotion of an electron to an excited state (change of d -level occupation) by the absorption of relatively low energy visible light. The bands are often broad and strongly influenced by the chemical environment. They are also usually very weak.
- Charge transfer transitions: Much stronger absorption is found when complexing the metal ion with some suitable organic chelating agent to produce a charge-transfer complex. Electrons may be transferred from the metal to the ligand or vice versa. The high transition probability is exploited to quantitatively detect ions in solution. There are numerous chelating agents available which may or may not complex selectively where there is more than one type of metal ion present. For example 1,10-phenanthroline is a common chelate for the analysis of Fe(II).

2 Experiment

Components of optical spectrometers

Light Sources

Radiation sources need to be continuous over the range of wavelengths of interest. The earliest sources were simply tungsten filament lamps (light-bulbs!) but these have since been replaced by tungsten-halogen lamps. Such light sources cover the wavelength range from 300-900 nm. To reach further into the UV an additional source is needed. This is usually a deuterium arc lamp, which has a continuous spectrum below 400 nm.

Monochromator

A monochromator is used to select the wavelength at which an absorption measurement is made. In fact, it is not possible to select a 'single' wavelength, but rather a narrow

range of wavelengths, which defines the spectral resolution of the spectrometer. There are two main choices for dispersing light into its different components: a prism, or a diffraction grating. Most modern instruments employ gratings, because it is easier to achieve high spectral resolution. However, gratings have the disadvantage of giving rise to more than one order of diffraction. This means that if the monochromator is set to 600 nm for example, then it will also pass 300 nm (second order) radiation. This problem is easily overcome by the use of additional filters to remove the unwanted radiation. A typical monochromator design is shown in Figure 4. It consists of the diffraction grating (dispersing element), slits, and curved mirrors, which image the entrance slit onto the exit slit and produce a parallel beam at the grating. During a scan, the grating is slowly rotated, and light of different wavelengths will emerge from the exit slit and pass through the sample to the detector. Thus the spectrum is obtained sequentially as the grating is rotated to select the wavelength and the detector observes the transmitted radiation intensity. The spectral resolution can be varied by changing the size of the slits. Narrower slits allow for higher resolution at the expense of light intensity, which can result in larger noise.

Detectors

The following detectors are commonly used in UV/Vis spectroscopy:

1. **Photomultipliers:** A photomultiplier consists of a photocathode and a series of dynodes in an evacuated glass enclosure. Light that strikes the photocathode causes the ejection of electrons due to the photoelectric effect. The electrons are accelerated towards a series of additional electrodes called dynodes. These electrodes are each maintained at a more positive potential. Additional electrons are generated at each dynode. This cascading effect creates 10^5 to 10^7 electrons for each photon hitting the first cathode depending on the number of dynodes and the accelerating voltage. This amplified signal is finally

collected at the anode where it can be measured.

2. Semiconductor Photodiodes: When a photon strikes a semiconductor, it can promote an electron from the valence band (filled orbitals) to the conduction band (unfilled orbitals) creating an electron(-) - hole(+) pair. The concentration of these

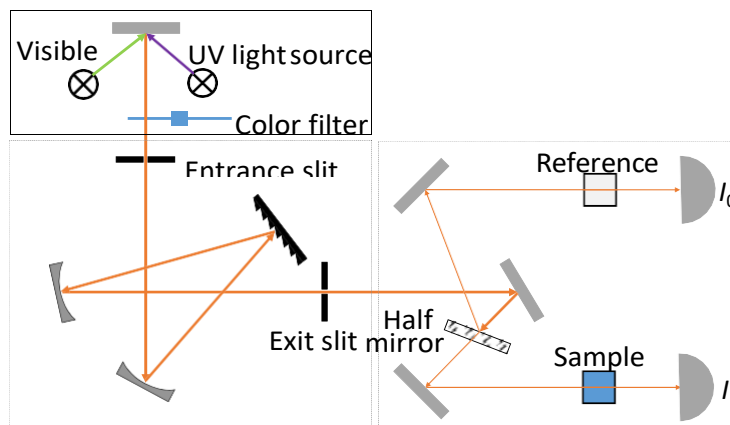


Figure 5: Schematic view of a dual beam spectrophotometer.

electron-hole pairs is dependent on the amount of light striking the semiconductor, making the semiconductor suitable as an optical detector. Photovoltaic detectors contain a p-n junction that causes the electron-hole pairs to separate to produce a voltage that can be measured. Photodiode detectors are not as sensitive as PMTs but they are small, cheap and robust.

3. Charge-coupled devices (CCD): A CCD is an integrated-circuit chip that contains an array of capacitors that store charge when light creates electron-hole pairs. The charge accumulates and is read in a fixed time interval. CCDs are used in similar applications as arrays of photodiodes but the CCD is much more sensitive for measurement of low light levels. They can replace the exit slit of a monochromator which disperses light only after it has passed a sample. In this way, full spectra can be accumulated very quickly without moving any optics.

Dual Beam Spectrophotometers

A diagram of the components of a typical dual beam spectrophotometer is shown in Figure 5. A beam of light from either the visible or UV light source is separated into its component in a monochromator. An additional filter suppresses light at shorter wavelengths to avoid interference from second order diffraction. The monochromatic (narrow bandwidth) beam is then split into two beams of equal intensity by a half-mirror or beam splitter. One beam, the sample beam, passes through the cuvette containing a solution of the compound being studied. The other beam, the

reference, passes through an identical cuvette containing only the solvent. The intensities of these light beams are then measured by photo detectors and compared. The intensity of the reference beam, which should have suffered little or no light absorption but the same reflection losses as the sample beam, is defined as I_0 . The intensity of the sample beam is defined as I . During a wavelength scan, intensity changes and fluctuations are equally sensed by the two detectors and normalized out by the division of I by I_0 . However, even if both cuvettes contain the same solution, these two intensities may not be exactly the same, for example because of different detector

efficiencies or spatial beam drifts. This leads to a small background spectrum, which can even be negative in some frequency ranges. Like with a single beam spectrometer (no reference beam) it is thus important to first record the background spectrum with only solvent in the sample cell. This spectrum must then be subtracted from the one recorded with the sample solution. If you do this, the reference compartment may even be left empty.

Data acquisition

The earliest instruments simply directly connected the amplified detector signal to a chart recorder. Today, all experimental settings are controlled by a computer and the detector signals are digitized, processed and stored. Nevertheless it is important that you note parameters which you set via the instrument software (slit width, scan range, scan speed, single beam/dual beam) into your laboratory journal, along with the name of the file containing the data (and its path). Otherwise it can become very difficult to find or reproduce a measurement after other users have changed these settings!

Quantitative work

A frequent analytic application of UV-vis spectroscopy is the precise determination of concentration. You should already be familiar with this method from your first year lab course. There, you have determined the Manganese concentration in steel by measuring the absorbance (at a single wavelength) of a solution of permanganate ions that you produced from your steel sample. In addition, you recorded the absorbance of a series of permanganate solutions of known concentration. This allowed you to make a calibration graph of absorbance vs. concentration and fit a straight line with slope sd (molar extinction coefficient times the path length). The unknown concentration of sample could then be simply read from the graph or calculated using the value of s determined from the slope. Note that the calibration points and the slope of the straight line usually have errors, which must be taken into account when calculating the final uncertainty of the concentration.

Indicators

pH-Indicators are molecules whose colour depends on their protonation state, i.e. they have different colours in different pH-regions. Although the structural changes accompanying protonation and deprotonation may be quite complex, we may simply denote the deprotonated

indicator by Ind and the protonated form by $HInd$ and write down the simple equilibrium equation



This equation has an equilibrium constant $K_a = \frac{[Ind^-][H_3O^+]}{[HInd]}$, so that we get:

$$pK_a = pH - \log_{10} \frac{[Ind^-]}{[HInd]}$$

It is therefore possible to determine the pK_a of the indicator by measuring the concentration ratio of protonated and unprotonated indicator at different pH values. This concentration ratio can be determined from the UV-vis spectra: At low pH the indicator is almost completely in the protonated form and the absorption is due to $HInd$ only. Likewise, at high pH the indicator is completely deprotonated and the absorption is due

to Ind^- . We can therefore determine the extinction coefficient of the pure protonated and deprotonated forms of the indicator. At a pH close to the pK_a , the solution contains appreciable concentrations of both $HInd$ and Ind^- , and their relative contributions to the absorption spectrum can be calculated with the help of the individual extinction coefficients.

3 Experimental Tasks

Understanding the Instrument

- One of the spectrometers will be opened for you. Make sure you understand the beam path and the role of the different optical elements and make a sketch in your notebook (a photograph alone is not enough, it needs to be labelled!).
- Learn to change measuring parameters using the software and make sure you know what they mean.
- Record a spectrum with only solvent in the cuvette and then repeat the same measurement. Try this for at least two different parameter settings (e.g. scan speed, slit width). Import your data into a data analysis program (Origin or Mathematica) and plot the spectra. How big is the difference between two consecutive scans and thus the instrumental error in your data?

Quantitative measurements

Measure the absorption spectrum of a pH indicator (Bromothymol blue or other) at different pH, and determine its equilibrium constant K_a .

- Prepare 50mL of a $5 \cdot 10^{-4}$ M Bromothymol blue solution in distilled water. The molar mass of Bromothymol blue is $624.38 \text{ g mol}^{-1}$. Prepare this solution carefully, as you will use it to make all your other solutions. Note: The assistant may decide to give you a different indicator and will provide the necessary information!
- Prepare four aqueous solutions with different pH, < 5 , two near 7 (close to the pK_a of the indicator) and > 9 , by titrating distilled water with HCl and/or NaOH solutions. Estimate the concentration $[HInd]$ and $[Ind^-]$ at the different pH values, based on an approximate value for the pK_a of the indicator (≈ 7.3 for Bromothymol blue). Convince yourself that only one form of the indicator contributes to the absorption spectrum in your high and low pH solutions (use equation 13 and $[HInd] + [Ind^-] = \text{const}$).
- For your low and high pH solutions make a dilution series with 5 different indicator concentrations (between $c = 10^{-4}$ and $c = 2 \cdot 10^{-6}$). When you take a small volume of the indicator solution and dilute it in a bigger volume (typically more than 5 times) of the aqueous solutions, you can assume that the pH does not change significantly.
- Record absorption spectra starting with the most dilute solution.
- Record absorption spectra of the indicator at the four different pH (low, high and near the pK_a), always with the same indicator concentration. For the two solutions with pH near the pK_a of the indicator re-measure the pH immediately after the absorption measurements.

4 Data Analysis

- Plot the optical density at the maximum of the absorption bands as a function of concentration at basic and acidic pH. Calculate the molar extinction coefficient at the absorption maximum $\epsilon(\lambda_{\text{max}})$ for the two forms of the indicator, using linear regression. Provide error limits for $\epsilon(\lambda_{\text{max}})$.
- Determine the transition dipole moment of the protonated and deprotonated forms of the indicator. Provide error limits.
- Plot the spectra recorded at different pH in a single graph, find the wavelength of the isosbestic point and calculate the equilibrium constant and the pK_a . Use your errors for the extinction coefficients to calculate errors for concentrations and pK_a . Also consider the uncertainty in pH.

1. Which statement is false about Chromophore ?

A. It is co-valent unsaturated compound.

B. It is contain π electron.

C. They required high energy for electron transition.

D. They required low energy for electron transition.

2. Which statement is true about Auxochrome ?

A. It is only saturated compound.

B. It is only unsaturated compound.

C. It is Decreased intensity of radiation.

D. They are saturated and unsaturated compound.

3. Which group act as a Chromophore?

A. -N=N-

B. -OH

C. -OR

D.- NH₂

4. DNA denaturation is example of which type of shift ?

A. Bathochromic

B. Blue shift

C. Hyperchromic shift

D. Red shift

5. λ_{max} increase with absorption of radiation in which shift ?

A. Red shift

B. Blue shift

C. A and B

D. None of this

6. Due to deformation of compound due to attached some other functional group it is example of

A. Red shift

B. Blue shift

C. Hyperchromic shift

D. Hypochromic shift

7. Auxochrome have which type of electron ?

A. Non bonded

B. π electron

C. σ electron

D. None of this

Answer key

1. C

2. D

3. A

4. C

5. A

6. C

7. A

3. what is role of slit in uv-visible spectroscopy ?

A. Monochromatic radiation to polychromatic radiation.

B. Polychromatic radiation to monochromatic radiation.

C. A and B

D. None of this

4. Which radiation source has electrode in its construction ?

A. Tungsten lamp

B. Hydrogen discharge lamp

C. Xenon Discharge Lamp

D. Mercury lamp

5. Which device is used to isolate the radiation of the desired wavelength from wavelength of the continuous spectra ?

A. Monochromator

B. Radiation source

C. Recorder

D. None of this

6. Diffraction grating is consists of a

A. Glass

B. Quartz

C. Alkyl halide

D. All of the above

7. The work of Entrance slit is?

- A. To get narrow source
- B. To render light parallel**
- C. To disperse radiation
- D. To fall on sample cell

8. Collimator is used for?

- A. To reform the image of the entrance slit.
- B. To get narrow source**
- C. To fall on sample cell
- D. To disperse radiation

9. Exit slit used for

- A. To reform the image of the entrance slit.**
- B. To get narrow source**
- C. To fall on sample cell
- D. To disperse radiation

10. Which type of vapour is stored in Mercury lamp ?

- A. Mercury vapour
- B. Hydrogen vapour**
- C. Xe
- D. Ozone

Answer key**1. B****2. C****3. B****4. B****5. A****6. D****7. A****8. A****9. C****10. A****6. Which compound is increased uv absorption ?****A. Auxochrome****B. Chromophore****C. Inorganic compound****D. All of the above****7. If solvent is polar and electron transition is $n \rightarrow \pi^*$ which type of shift are seen in graph ?****A. Hypsochromic shift****B. Red shift**

C. Blue shift

D. A and C

8.If solvent is non polar and electron transition is $n \rightarrow \pi^*$ which type of shift are seen in graph ?

A. Hypso chromich shift

B. Red shift

C. Blue shift

D. A and C

9.If solvent is non polar and electron transition is $\pi \Rightarrow \pi^*$ which type of shift are seen in graph ?

A. Hypso chromich shift

B. Red shift

C. Blue shift

D. A and C

10. If solvent is polar electron transition is $\pi \Rightarrow \pi^*$ which type of shift are seen in graph ?

A. Hypso chromich shift

B. Red shift

C. Blue shift

D. A and C

Answer key

1.C

2.C

3.D

4.C

5.C

6.D

7.D

8.A

9.B

10.D

4. Beer's law states that the intensity of light decreases with respect to

- a) Concentration
- b) Distance**
- c) Composition
- d) Volume

Answer: a

5. Lambert's law states that the intensity of light decreases with respect to

- a) Concentration**
- b) Distance
- c) Composition
- d) Volume

Answer: b

6. The representation of Beer Lambert's law is given as $A = abc$. If 'b' represents distance, 'c' represents concentration and 'A' represents absorption, what does 'a' represent?

- a) Intensity
- b) Transmittance
- c) Absorptivity
- d) Admittance

Answer: c

9. What is the unit of absorbance which can be derived from Beer Lambert's law?

- a) $L \text{ mol}^{-1} \text{ cm}^{-1}$
- b) $L \text{ gm}^{-1} \text{ cm}^{-1}$
- c) Cm
- d) No unit

Answer: d

10. What is the unit of molar absorptivity or absorptivity which is used to determine absorbance A in Beer Lambert's formula?

- a) $L \text{ mol}^{-1} \text{ cm}^{-1}$
- b) $L \text{ gm}^{-1} \text{ cm}^{-1}$
- c) Cm
- d) No unit

Answer: a

