

GPAT Online Class for B.Pharm Students



Ananthapuramu Local Branch

Pharmaceutical Analysis – Part 1 (1st July 2020)

By

Dr P Ramlingam, *M.Pharm., PhD*
Director – Research

Raghavendra Institute of Pharmaceutical Education and Research – Autonomous
Affil: **JNT University Anantapur**, Anantapur, Andhra Pradesh, India

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Pharmaceutical Analysis – Part 1 Pharmacopoeia Considerations, Spectroscopy Fundamentals, UV-Spectroscopy

By

Dr P Ramlingam, *M.Pharm., PhD*

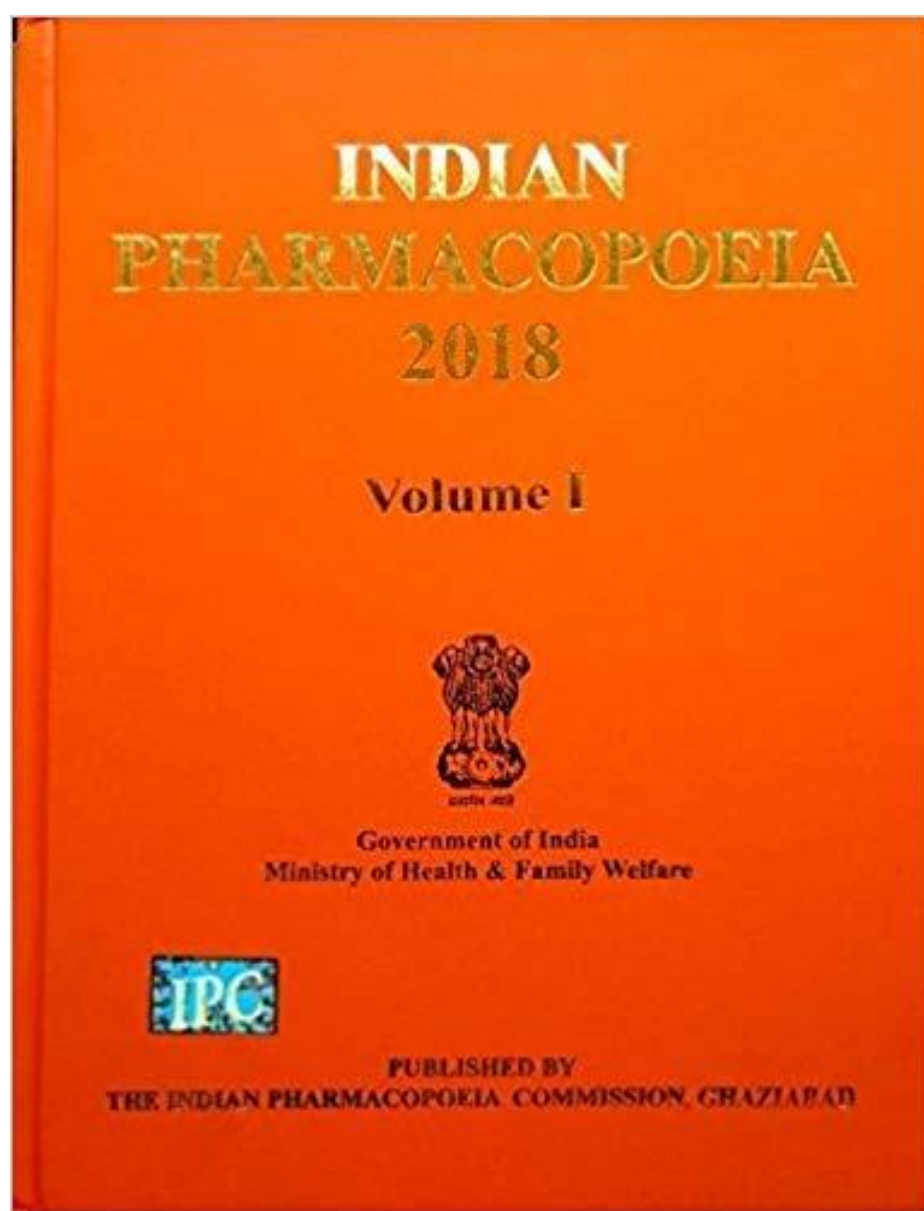
Director – Research

Raghavendra Institute of Pharmaceutical Education and Research – Autonomous

Affil: JNT University Anantapur, Anantapur, Andhra Pradesh, India



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**Recent Edition
(8th Edition)**

Released by
**Ministry of Family Welfare,
Govt. Of India**

Published by
**Indian Pharmacopoeia
Commission (IPC), Ghaziabad**

Currently : 4 volumes



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What is new in IP 2018 ?

- Most of the existing Assays and Related Substances test methods are upgraded by HPLC methods.
- Pyrogen test - replaced by Bacterial Endotoxin test (BET) in parenteral preparations and other monographs.
- Specifications on Volumetric Glassware, Conductivity, Dissolution test, Disintegration test, Dimensions of Hard Gelatin Capsule Shells etc. have been revised.
- General chapter on Maintenance, Identification, Preservation and Disposal of Microorganism have been revised.



Types of Monograph in IP

General monograph :

Address the manufacturing & testings as per cGMP

Individual Monograph :

Address the specifications and limit for quality control of

- APIs
- Pharmaceutical products
- Pharmaceutical additives and excipients

Indian Pharmacopoeia is regulated under

- Drugs & Cosmetic Act 1945
- GMP

IP-2018 has been brought 220 new monographs Includes,

- Chemical Monographs (170),
- Herbal Monographs (15),
- Blood and Blood related products (10),
- Vaccines and immuno sera for Human use (02),
- Radiopharmaceutical monographs (03),
- Biotechnology Derived Therapeutic Products (06),
- Veterinary monographs (14),

Along with 366 revised monographs and 7 omissions



Types of Glassware as per IP

1. Glass A (Quantitative) – Assay, estimations etc.,
2. Glass B (Qualitative /Routine use)

The above category is based on tolerance limits (Specified in IP).
(maximum allowed error @ $25 \pm 2^\circ \text{C}$)

For example: 10 ml volumetric flask

Actual volume is 10.01 ml;

Hence the volume error = $10.01 - 10 = 0.01 \text{ ml}$ (Glass A)

As per IP; For 10 ml volumetric flask:

Tolerance limit for Glass A is not more than 0.02 ml

Tolerance limit for Glass B is not more than 0.04 ml



Analytical Fundamental terms in Indian Pharmacopoeia

Alcohol : 95 % Ethanol

Ethanol: 100 % Ethanol (Absolute alcohol)

Weigh : with error less than 0.1 %

Accurately weigh: with error less than 0.01 %

Weigh about: $\pm 50\%$ of the prescribed amount.

Water used in Buffer preparation: CO₂ free water

Buffer can be stored for : 3 months (if no turbid)



Freshly prepared: Solution prepared in less than 24 hours

OVI : Organic Volatile impurities (Residual solvents)

Solubility (“g” per “ml” of solvents as per USP)

Descriptive term	Part of the solvent required per part of solute
Very soluble	Less than 1
Freely soluble	From 1 to 10
Soluble	From 10 to 30
Sparingly soluble	From 30 to 100
Slightly soluble	From 100 to 1000
Very slightly soluble	From 1000 to 10,000
Practically insoluble	10,000 and over



Types of Organic Solvents

Oxygenated solvents

- **Alcohols** (e.g. ethanol)
- **Esters** (e.g. ethyl acetate)
- **Ketones** (e.g. acetone)
- **Glycols** (e.g. ethylene glycol)
- **Ethers** (e.g. diethyl ether)

Hydrocarbon solvents

- **Non and low aromatic petroleum solvents** (e.g. hexane)
- **Polyaromatic hydrocarbons**

Halogenated solvents

- **Chlorinated hydrocarbons** (e.g. dichloromethane)
- **Hydrofluoroalkanes**
- **Hydrofluoroethers**

Strictly limited and restricted use

Types of solvents as per Indian Pharmacopoeia /ICHQ3

Residual Solvents are classified according to their Risk Assessments to human health to 3 main classes:

Class 1 solvents
Solvents to be avoided

Halogenated solvents
Benzene

Class 2 solvents
Solvents to be limited

Methanol
Chloroform
DMF
Acetonitrile
Pyridine
Hexane
Ethylene glycol
Dioxane

Class 3 solvents
Solvents with low toxic potential

Acetone
Acetic acid
Ethanol
Ethyl acetate /esters
Ethyl ether
Formic acid
DMSO
Butanols

1. **COLD:** 2°C to 8°C.
2. **COOL:** 8°C and 25°C.
3. **ROOM TEMPERATURE:** The temperature prevailing in a working area.
4. **WARM:** between 30°C and 40°C.
5. **EXCESSIVE HEAT:** above 40°C.
6. **LIGHT RESISTANT CONTAINERS:** protect the content from the effect of actinic light.
7. **WELL CLOSED CONTAINER:** prevent from contamination by extraneous liquid and from loss of the article under normal condition of handling, shipment, storage and distribution.
8. **TIGHTLY CLOSED CONTAINERS:** protects the contents from contamination by extraneous liquid and solids or Vapour from loss or deterioration of the article from effervescence, deliquescent or evaporation.



**If the storage is not specified on label the product, then
It should be stored in, ????**

- a) protect from moisture and store not more than 40 °C and do not freeze,
- b) protect from moisture, light store not more than 40° C.
- c) protect from moisture and store not more than 30 °C.
- d) protect from moisture, light and store not more than 40° C and do not freeze.



Monograph & Analytical Techniques

Parameter	Technique	Approach
Molecular Structure	UV, IR, NMR, Mass, X-Ray Crystallography	Qualitative
Molecular weight	Mass Spectroscopy	Qualitative
Molecular formula	Mass Spectroscopy & Elemental analysis	Qualitative
Identification	Chemical test, UV, IR, TLC, HPLC	Qualitative
Test for purity	Chemical Tests /HPLC	Qualitative



Monograph & Analytical Techniques

Parameter	Technique	Approach
Limit test (anion & cation)	Comparison Tests (Nessler cylinders)	Semi-Quantitative
Moisture content	Karl-Fisher technique, Coulometry	Quantitative
Metal impurities	Atomic absorption & Emission spectroscopy	Quantitative
Impurity levels /Related substance	HPLC, UPLC, LC-MS	Quantitative
Assay	HPLC, UPLC, HPTLC, Polarimetry, Electrochemical methods, UV (A1%1cm) method, Bio-assays	Quantitative



Analytical Methods

- **Classical Methods:** Wet chemical methods such as precipitation, extraction, distillation, boiling or melting points, gravimetric and titrimetric measurements.
- **Instrumental Methods:** Analytical measurements (conductivity, electrode potential, light absorption or emission, mass-to-charge ratio, fluorescence etc.) are made using instrumentation.



Analytical method approach

Qualitative analysis (what?)

measured property indicates presence of analyte in matrix

Classical

identification by colors,
boiling points, odors

Instrumental

chromatography, electrophoresis,
spectroscopy, electrode potential, etc

Quantitative analysis (how much?)

magnitude of measured property is proportional to concentration of analyte in matrix

Classical

mass or volume
(e.g., gravimetric, volumetric)

Instrumental

measuring property and
determining relationship to concentration



Modern Instrumental techniques

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Technique	Principle	Example
Spectroscopy	Based on light (EMR), electron, charged species interaction with analyte	Absorption: UV, IR, NMR, ESR, AAS, MASS Emission: Fluorimetry / Phosphorimetry, AES.
Chromatography	Separation of mixture using stationary & Mobile phase	Column, TLC, Paper, HPLC, GC etc,
Electrochemical methods	Based on electrical property of analyte like electro-ox-reduction potentials	Potentiometry, conductometry, polarography, Coulometry,



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Modern Instrumental techniques

Technique	Principle	Example
Thermal methods	Based on endo/exo thermal behaviour of anayte under external heat	Differential thermal analysis Differential calorimetric Thermogravimetry etc.
Electrophoresis	Separation on under field based on charge	Gel, Paper, capillary electrophoresis
X-Ray methods	Interaction with X-ray	X-ray diffraction X-ray crystallography X-ray fluorescence
Radio-immunological methods	Based on radio-labelled antigen-antibody complex formation	ELISA



Broad classification of Spectroscopy

- **Electromagnetic spectroscopy** involves interactions of matter with electromagnetic radiation, such as light.
- Electron spectroscopy involves interactions with electron beams.
- Mass spectrometry involves the interaction of charged species with magnetic and/or electric fields
- Acoustic spectroscopy involves the frequency of sound.



Types of Electromagnetic Spectroscopy

- **Atomic Spectroscopy**

It deals with the interaction of EMR with atoms.

Atomic Emission spectroscopy

Flame photometry
ICP-AES

Atomic Absorption Spectroscopy

ICP-AAS
AAS

- **Molecular Spectroscopy**

It deals with interaction of EMR with molecules.

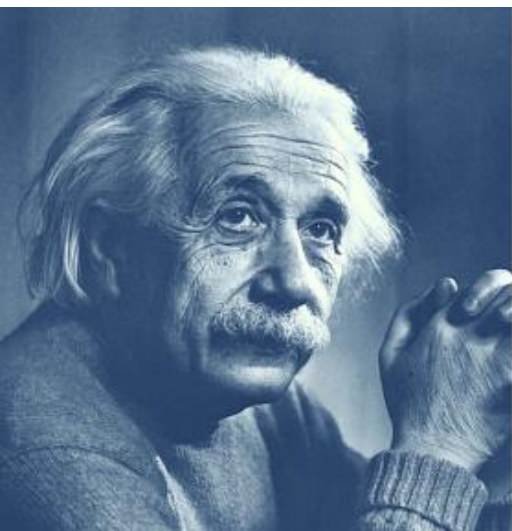
Molecular Absorption Spectroscopy:

UV-Visible, IR, NMR, ESR spectroscopy

Emission Spectroscopy:

Fluorimetry
Phosphorimetry





Electromagnetic Radiation

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I have no special talent.
I am only passionately
curious.

ALBERT EINSTEIN

This also called Planck's
relation

Energy of light (or)
Photon (E) is inversely
proportional to
Wavelength (Lambda)

$$E = h\nu = \frac{hc}{\lambda}$$

E = Energy of a single photon

$h = 6.626 \times 10^{-34} \text{ J} \cdot \text{s}$ (Planck's constant)

ν = frequency (Hz)

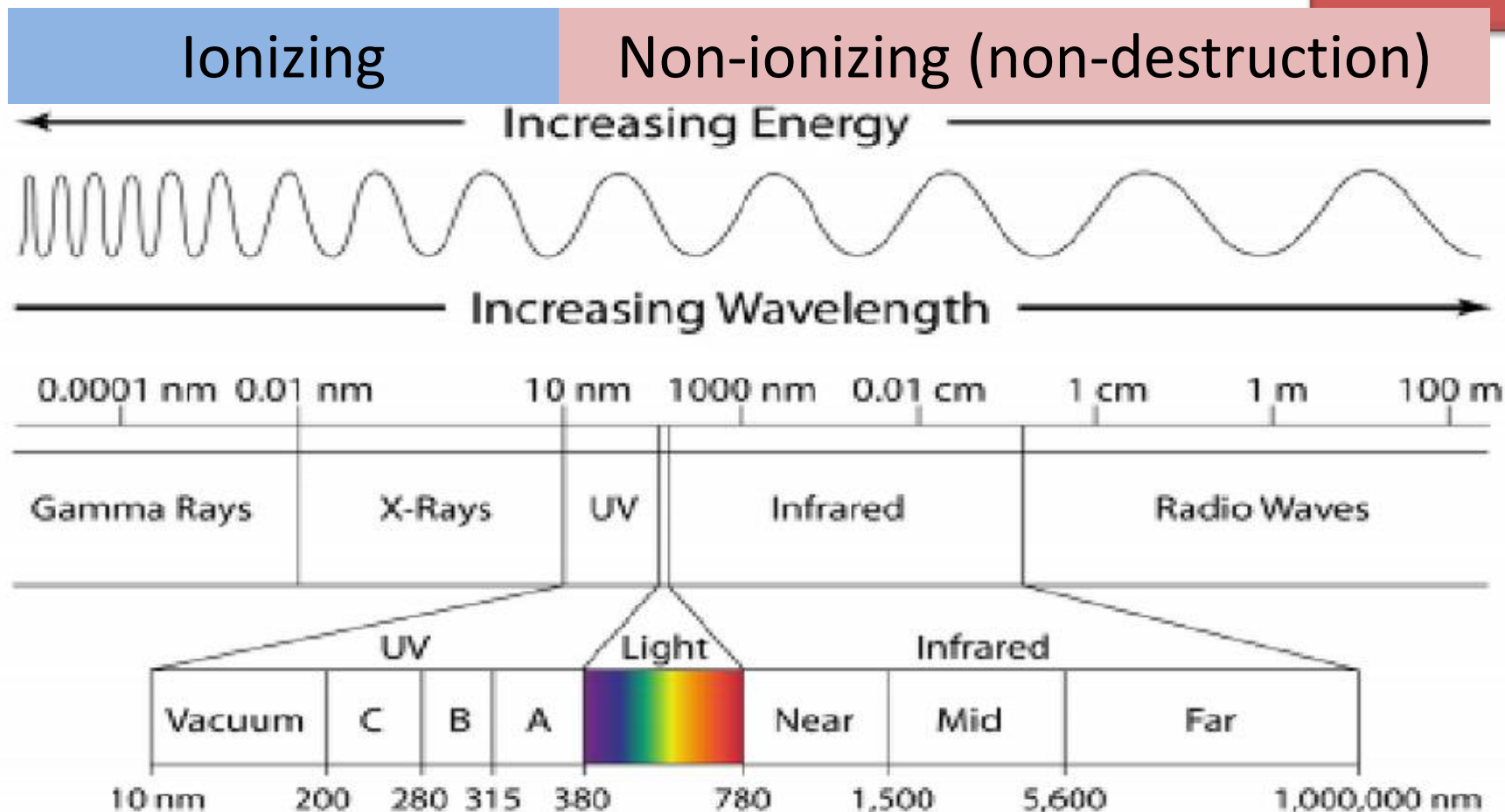
λ = wavelength (m)

$c = 2.998 \times 10^8 \text{ m/s}$ (speed of light)



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Types of Electromagnetic radiation



EMR Vs Types of Spectroscopy

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Spectroscopy	EMR	Interaction	Purpose
UV	190-380 nm (UV-Region)	Absorbed by valence electron	Conjugation detection, position of double bonds and of colourless samples
Visible	380- 790 nm (Visible region)	Absorbed by valence electron	Conjugation detection, position of double bonds of coloured sample
IR	2.5 to 25 micron (Middle IR)	Absorbed by bond	Functional groups and identification
NMR	60-800 MHZ (Radio-waves)	Absorbed by Spinning nuclei	Structure elucidation and nuclei environment in chemical structure
ESR	0.04 – 25 cm (Microwaves)	Absorbed by odd electrons	Reaction mechanism, free radical nature, etc.

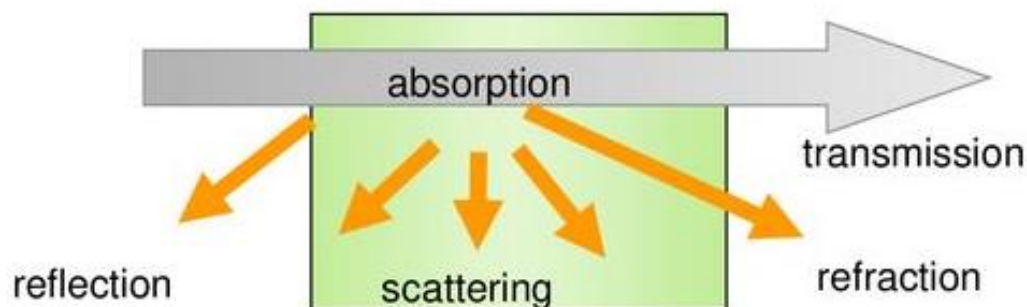


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Spectroscopy is deals with the measure of Light energy (EMR) interaction with matter (Analyte)

In spectroscopy the light energy interact in different ways with matter like

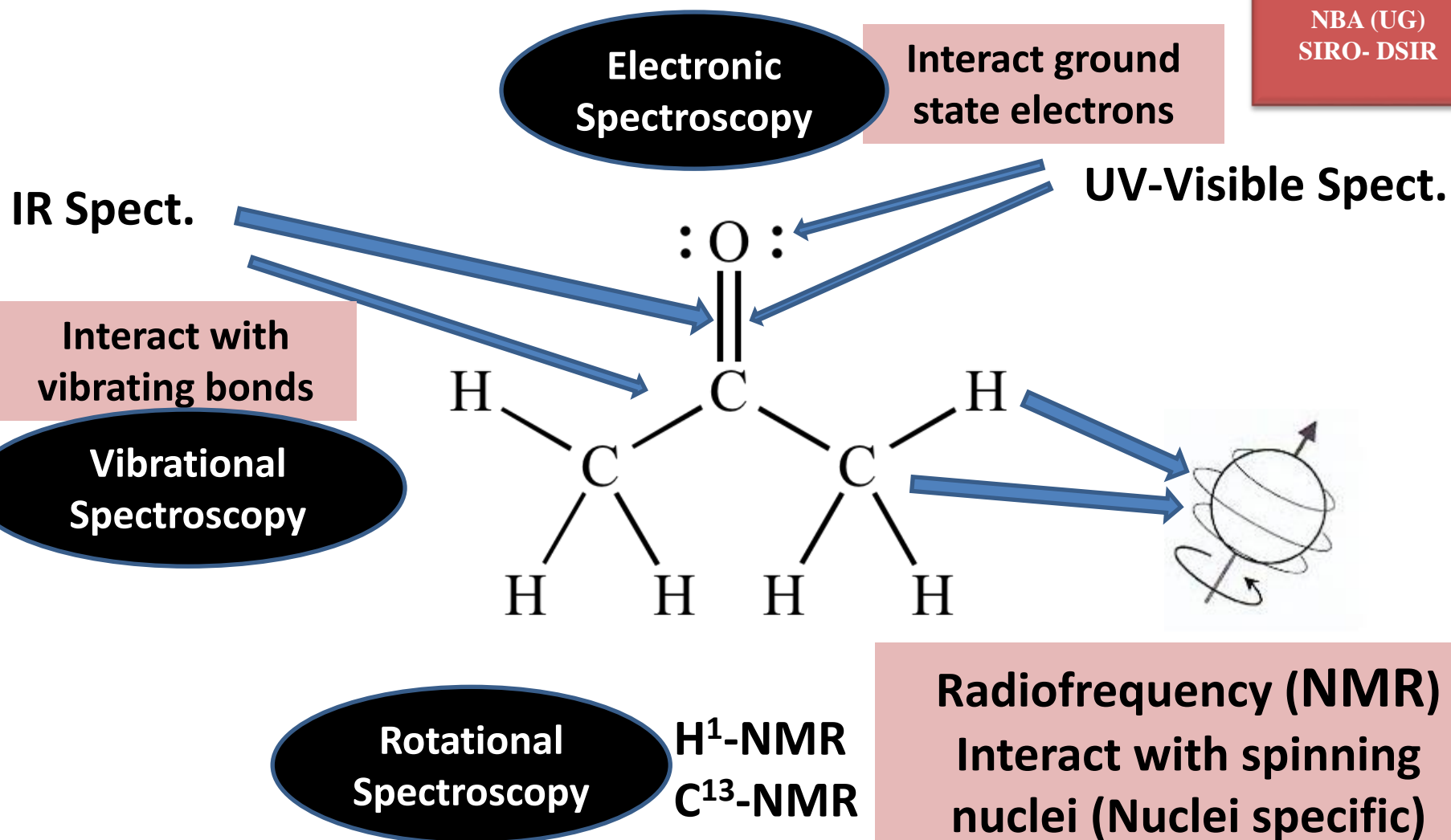
- Absorption
- Emission
- Reflection
- Transmission
- Scattering
- Refraction



- Each interaction can disclose certain properties of the matter and different type information about matter can be obtained

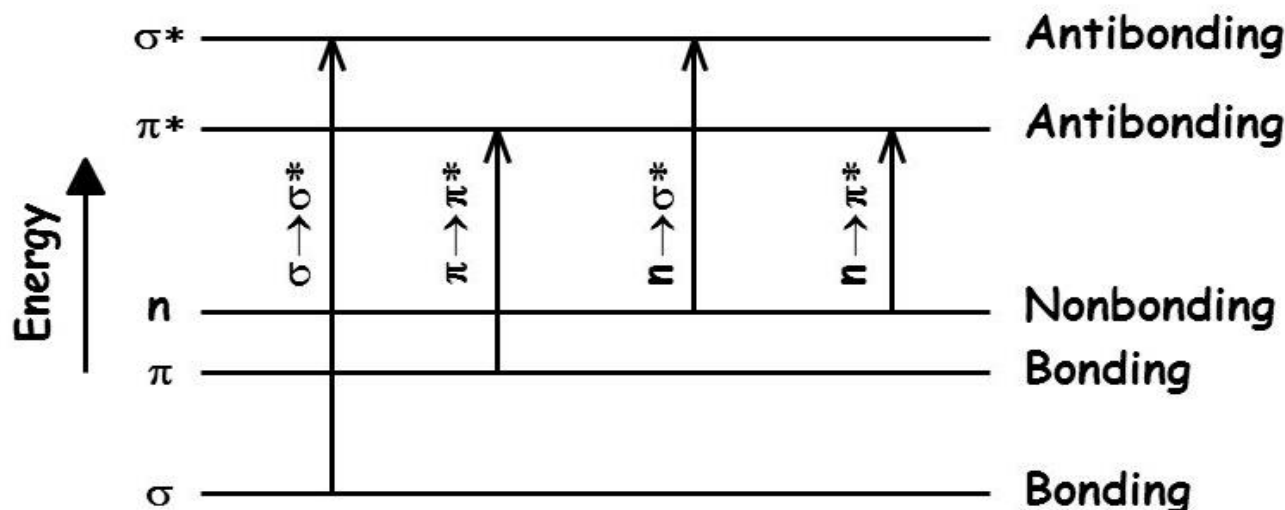
Interaction of EMR with Molecules

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Ultraviolet-Visible Spectroscopy

Bonding electrons appear in σ and π molecular orbitals
nonbonding in n



Excited state
Electron unpaired
& opposite spin

Ground state
Electron paired &
opposite spin

Electronic transitions can occur between various states.
The energy of the transitions increases in the following order:

$$(n \rightarrow \pi^*) < (\pi \rightarrow \pi^*) < (n \rightarrow \sigma^*) < (\sigma \rightarrow \sigma^*)$$

$\sigma \rightarrow \sigma^*$ band

- Excitation from the ground state to the excited state requires EM radiation with a wavelength of 150 nm.
- Not useful for routine spectroscopy

$n \rightarrow \sigma^*$ band

- The number of organic functional groups with $n \rightarrow \sigma^*$ peaks in the UV region is small.
- Excitation requires EM radiation with a wavelength in the range 150 - 250 nm.

$\pi \rightarrow \pi^*$ band
 $n \rightarrow \pi^*$ band

- Most absorption spectroscopy of organic compounds is based on these transitions.
- The absorption peaks for these transitions fall in an experimentally convenient region of the spectrum (200 - 700 nm).
- These transitions need an unsaturated group in the molecule to provide the p electrons.

UV Solvents:
Water,
alcohols,
Ethers,
CHCl₃,
Dioxane

Unsaturated,
aromatic,
heterocyclic ,
Carbonyl,
compounds

The Frank-Condon principle

During an electronic transition the atoms do not move; whereas electrons, including those of the solvent molecules, will reorganize during an electronic transition.

Most transitions result in an excited state which is more polar than the ground state.

Therefore, polar solvents will be able to interact to a greater extent with the more polar excited state via dipole-dipole interactions.

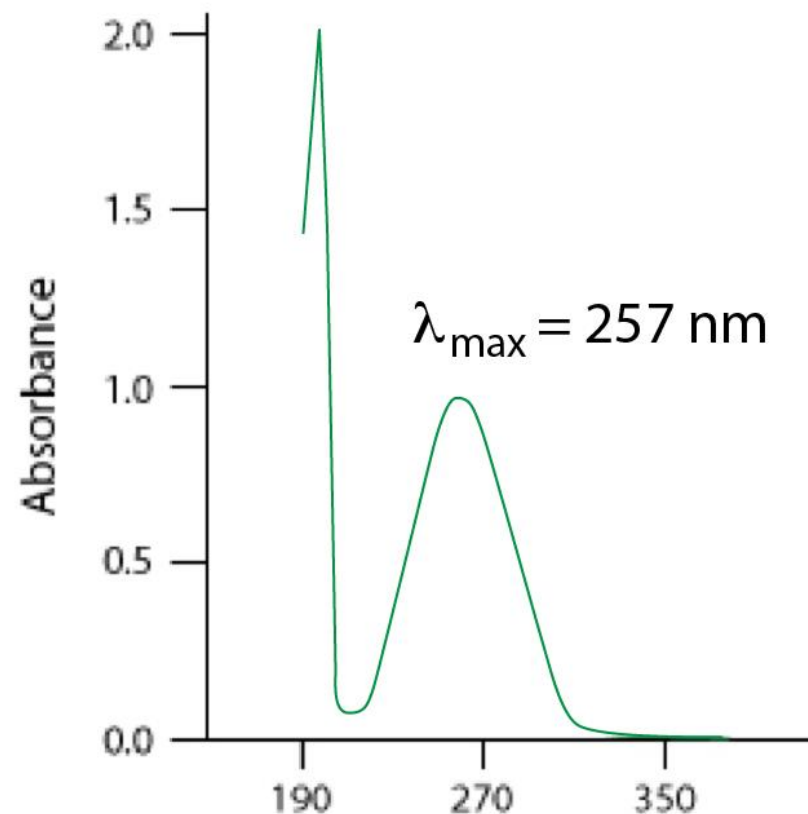
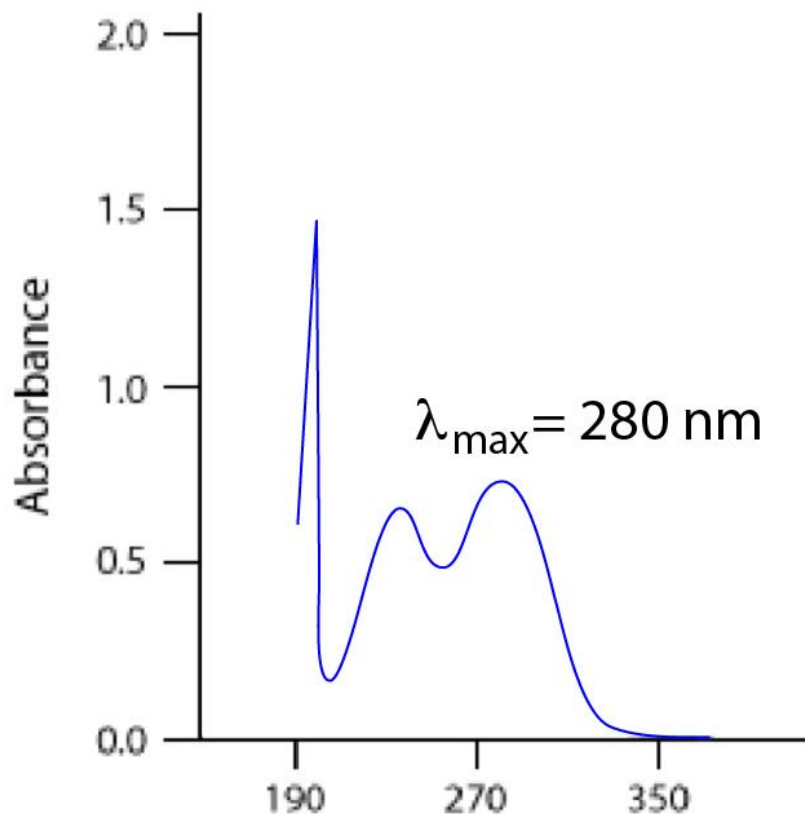
These stabilizing interactions will lower the energy of the excited state but will have a negligible effect on that of the ground state, resulting in a change of absorbance maximum.



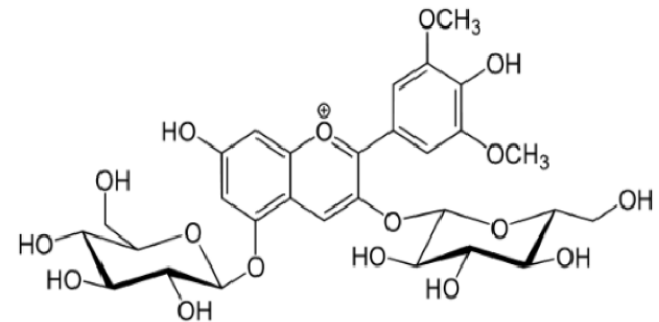
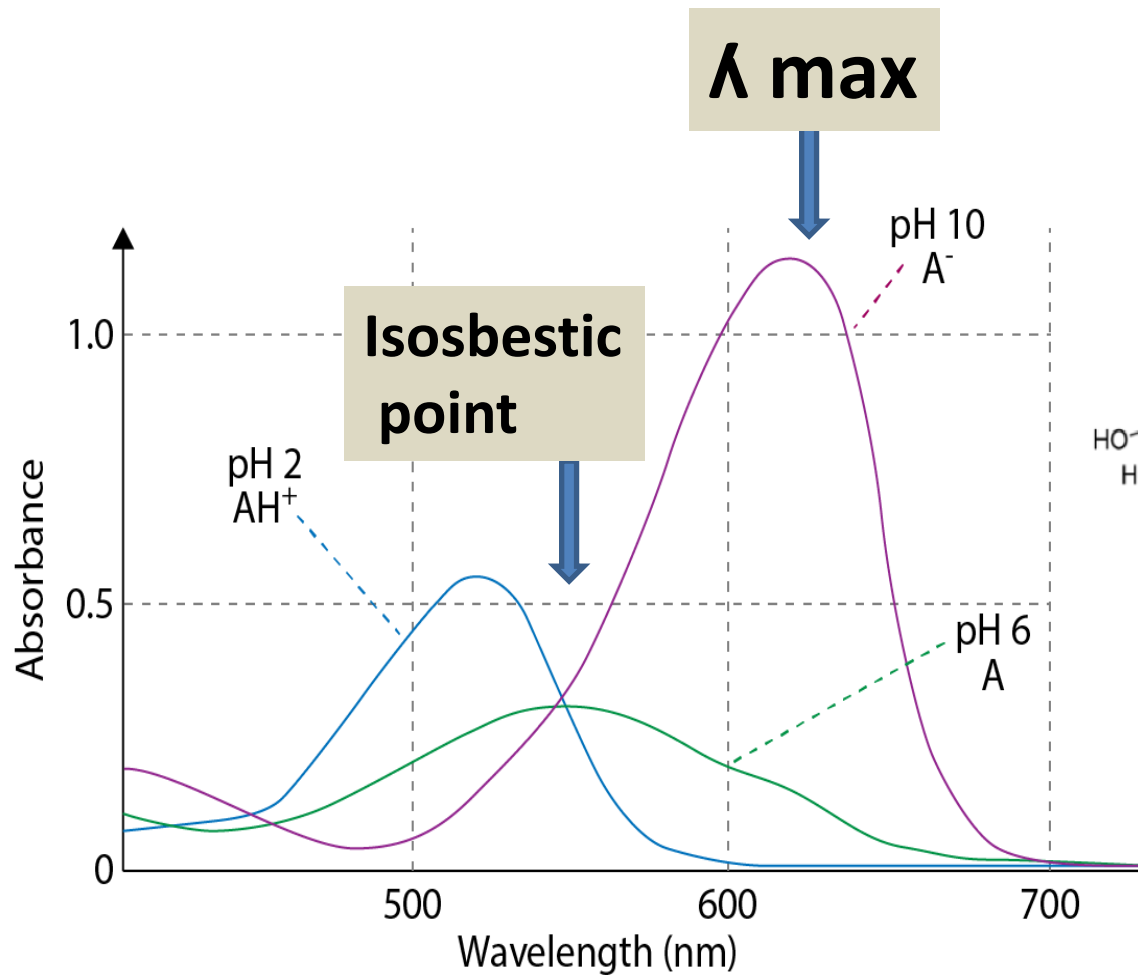
UV Spectrum

UV spectra differ based on chemical structure & double bond positions

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Effect of pH on UV – absorption maximum

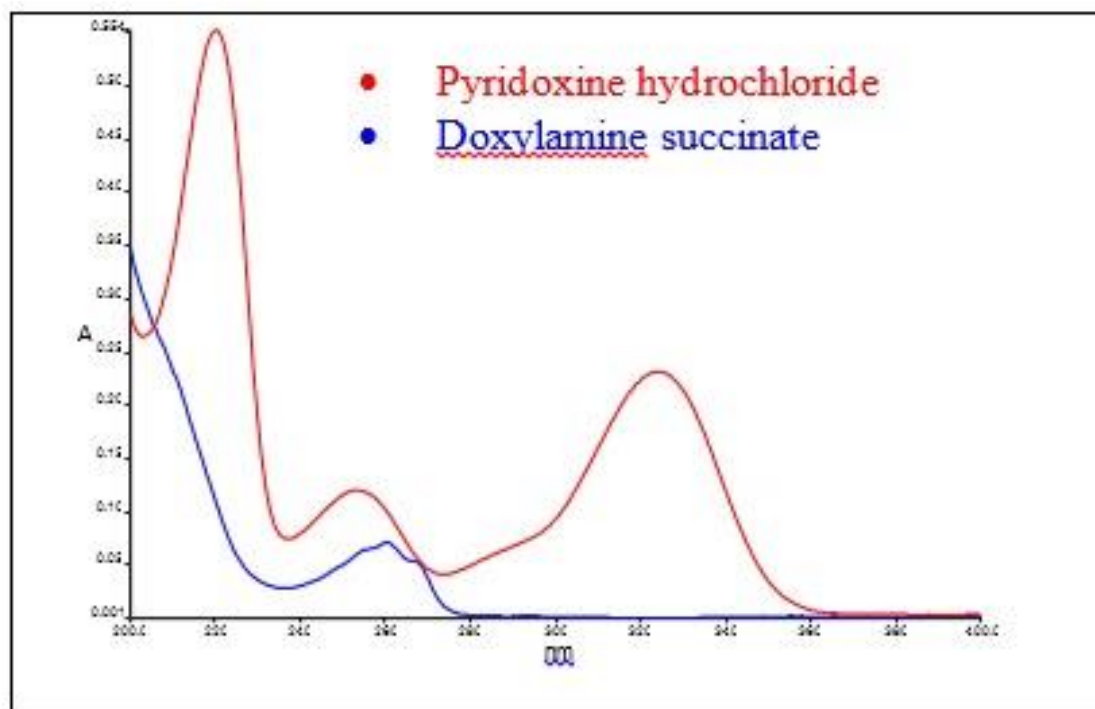
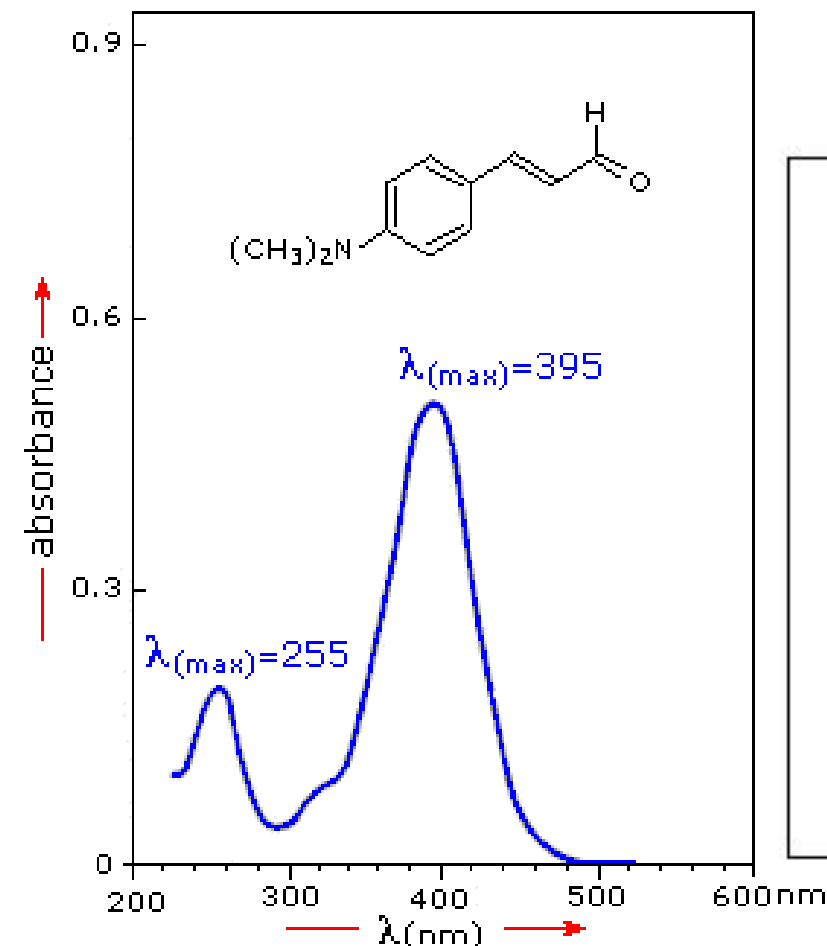


Anthocyanins

Chemical Structure and UV Spectrum

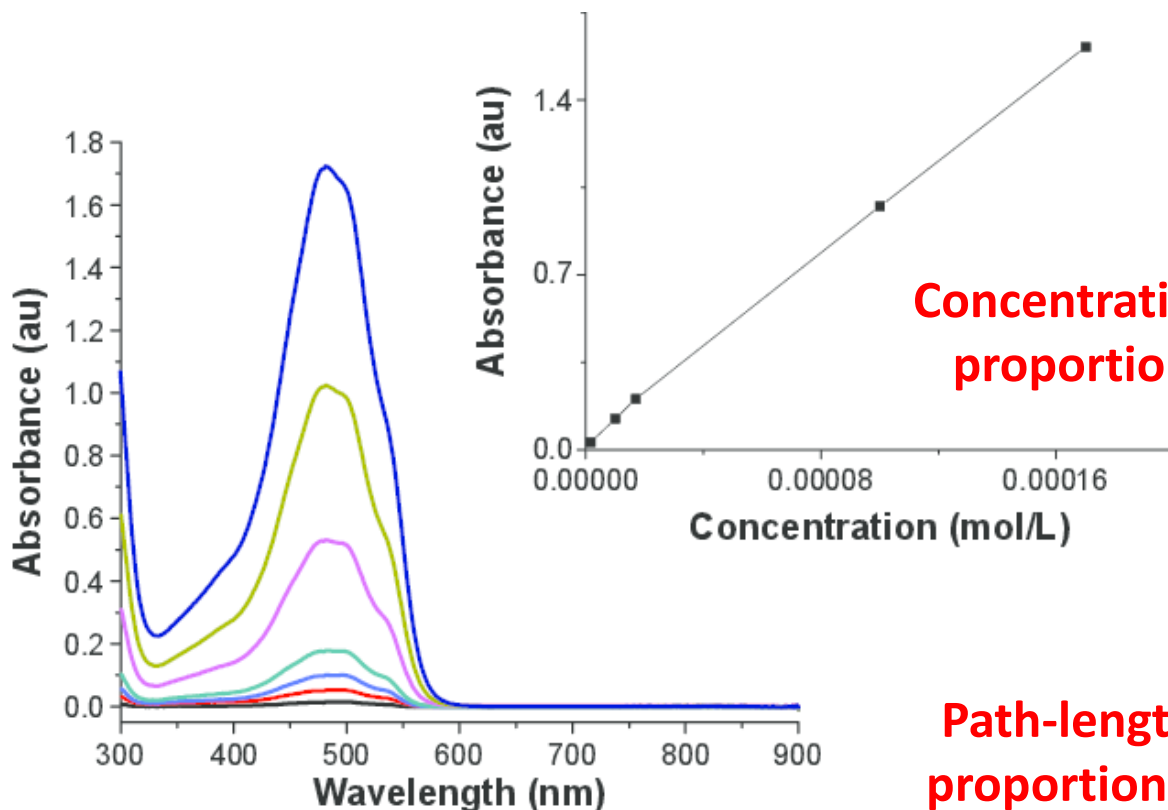
UV-Spectrum & λ_{max} depends on chemical structure

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Concentration and UV spectrum

λ_{max} is independent of concentration



Beer's Law

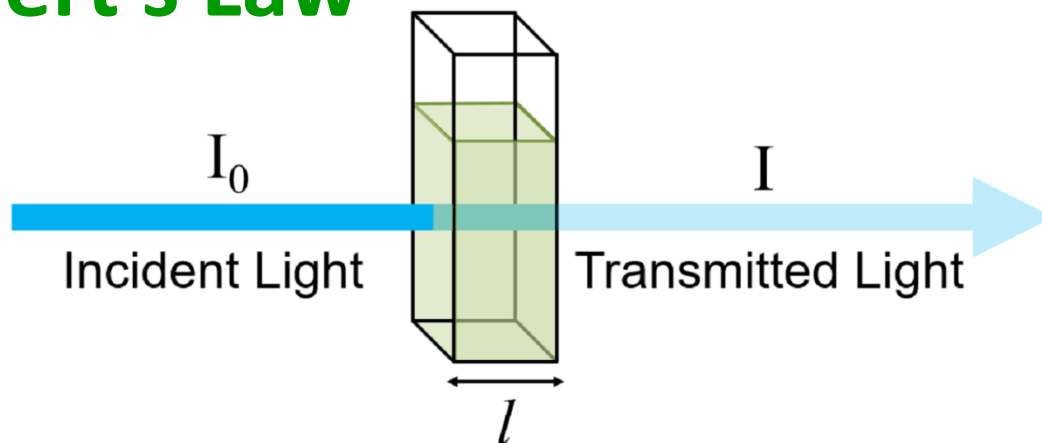
Concentration & absorbance are directly proportional when the path length is constant

Lambert's Law

Path-length & absorbance are directly proportional when the concentration is constant

Beer-Lambert's Law

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Greek letter, epsilon

$$\log_{10} \frac{I_0}{I} = \epsilon l c$$

concentration of solution
(mol dm⁻³)

length of solution the light
passes through (cm)

$$A = \log_{10} \frac{I_0}{I}$$

$$A = -\log_{10} T$$

$$T(\%) = 100 \frac{I}{I_0}$$



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Specific Absorbance (A1% 1cm)

The Beer-Lambert equation

$$A = A^{1\%}_{\text{cm}} \cdot b \cdot c$$

Where

A = Absorbance, $(\log (I_0/I))$

b = Path length and c is concentration (g/100 ml),

A1%cm = The specific absorbance of compound which is constant (it is the absorbance of 1 % w/v solution in 1cm path length).

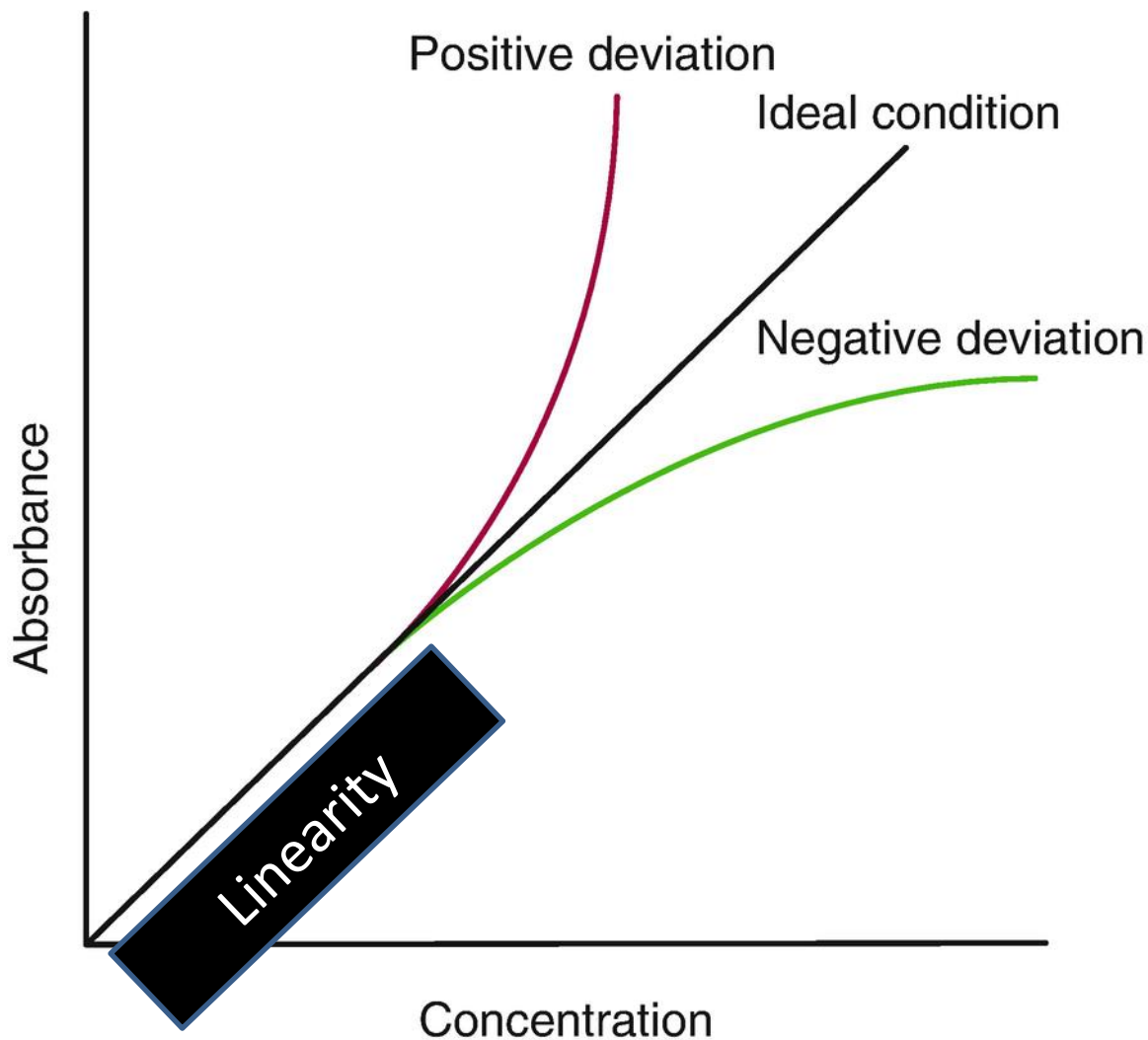
The above formula is recommended by Indian pharmacopoeia (2010, 2014) for the quantification of drug in pharmaceutical dosage form, when standard substance is not available.

Linearity curve or Beer-Lambert curve can be used for quantification if both standard and sample



Beers Law deviation

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Derivation of Beer Lambert Law

This relationship is a linear for the most part. However, under certain circumstances the Beer relationship gives a non-linear relationship.

These deviations from the Beer Lambert law can be classified into three categories:

Real Deviations - These are fundamental deviations due to the limitations of the law itself.

Chemical Deviations- These are deviations observed due to specific chemical species of the sample which is being analyzed.

Instrument Deviations - These are deviations which occur due to how the absorbance measurements are made.

Limitations / Beer-Lambert Law

1) Real Limitations

- Beer-Lambert law is derived on the basis of having a dilute solution. At high concentrations, (>0.01 M) intermolecular interactions can occur between molecules (dipole-dipole interactions), directly altering ϵ .
- Intermolecular interactions can also occur when secondary species (i.e., salts) interact with charged chromophores, thus altering ϵ .
- The value of ϵ is also dependent on refractive index of the medium, thus solvent effects on absorbance can be observed (correct using $\epsilon n/(n^2 + 2)^2$ instead of ϵ).

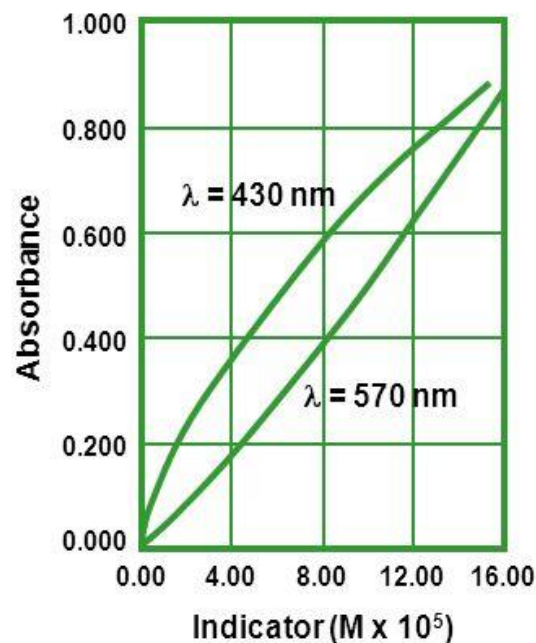


Limitations / Beer-Lambert Law

2) Apparent Chemical Deviations

- Arise as a result of chemical reactions involving the absorbing sample (association, dissociation, oxidation, reduction, addition, elimination, etc...).

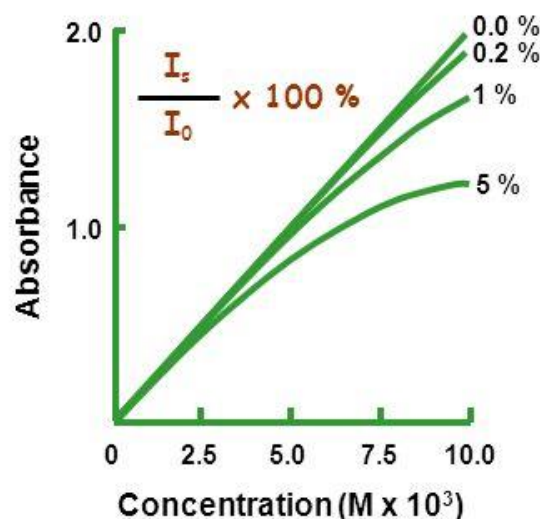
Classic example is pH sensitive chromophores (indicator dyes). Figure shows the absorbance at 430 nm and 570 nm for a dye that undergoes a protic equilibrium that depends on total dye concentration.



Limitations / Beer-Lambert Law

4) Instrumental Deviations due to Stray Light

- Imperfections in monochromator gratings often lead to 0.005 – 0.2% stray light.
- Differs greatly in wavelength from the value of the principal radiation, and may not even pass through sample (i.e., depends on how light-tight the instrument is).
- Observed absorbance in presence of stray light is given by:

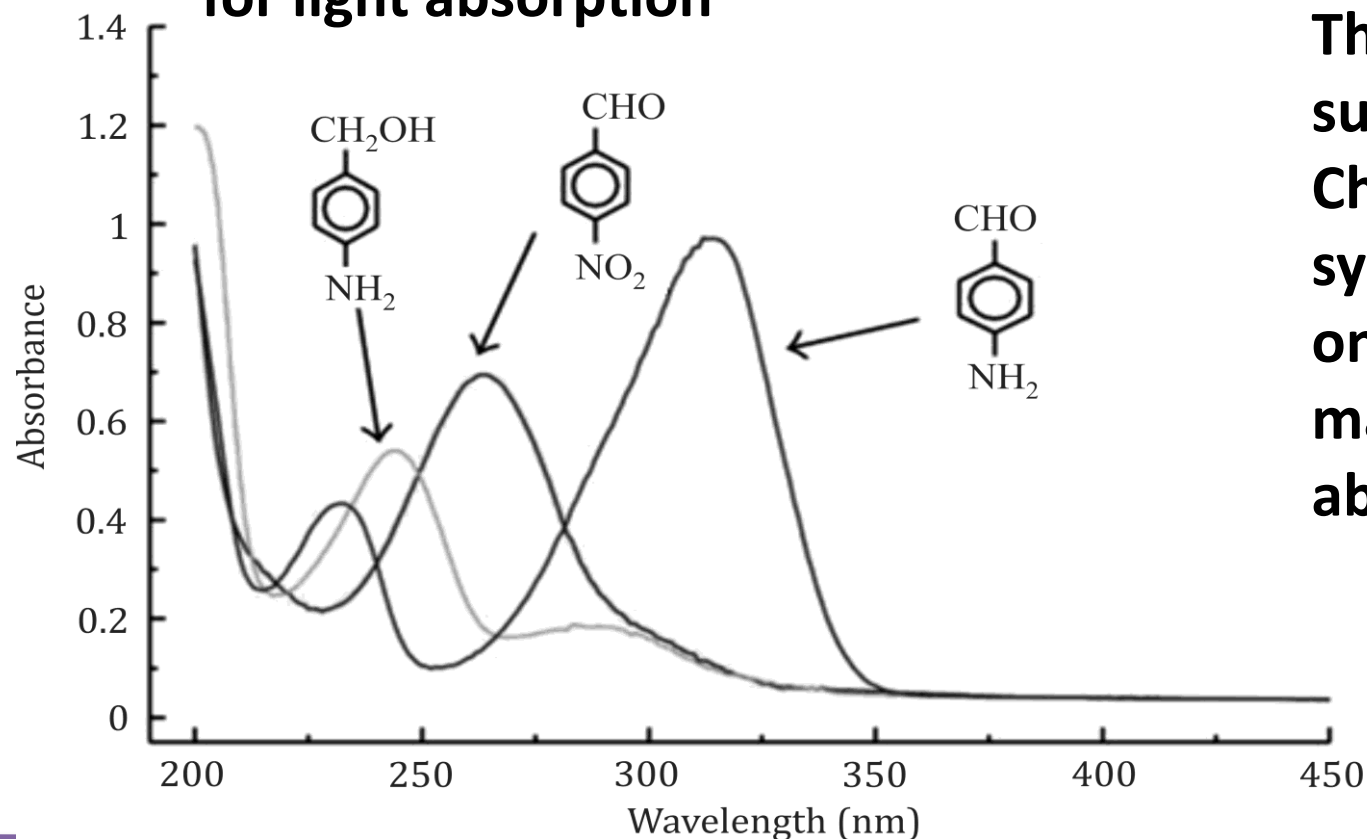


$$A' = \log \frac{I_0 + I_s}{I + I_s}$$

Where I_s is the intensity of non-absorbed stray radiation. This leads to a negative deviation in A vs C , as shown in the Figure

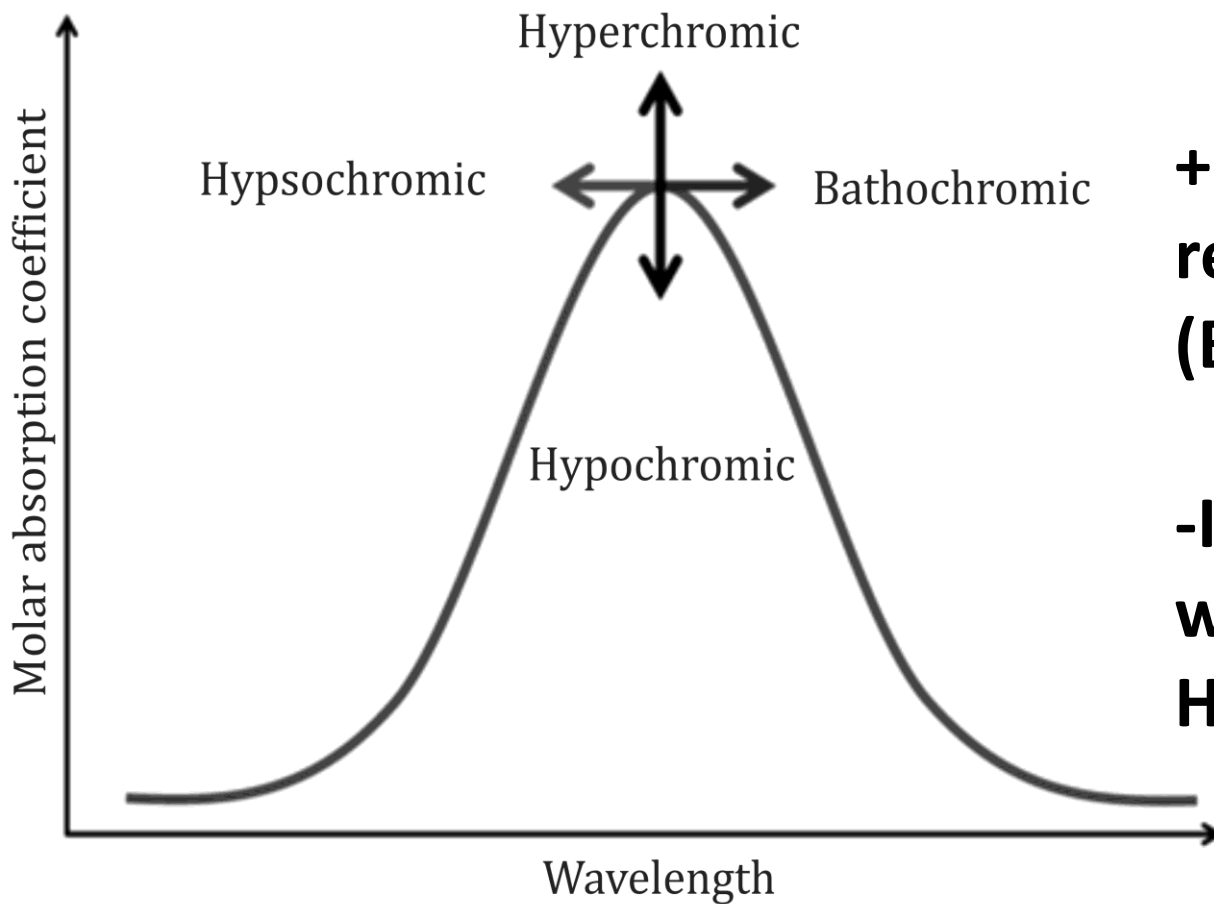
Chromophore and Auxochrome

Chromophore: Double bond or conjugated system in chemical structure responsible for light absorption



Thus the substitution on Chromophore system has effect on both lambda max and absorbance

Auxochrome Effect



**+I group : Electron releasing
(Bathochromic effect)**

**-I group: electron withdrawing
Hypsochromic effect**

Solvatochromism – Solvent effect

Solvatochromism is the ability of a chemical substance to change color due to a change in solvent polarity. **There are two type of effect,**

- **Negative solvatochromism**
- **Positive solvatochromism**

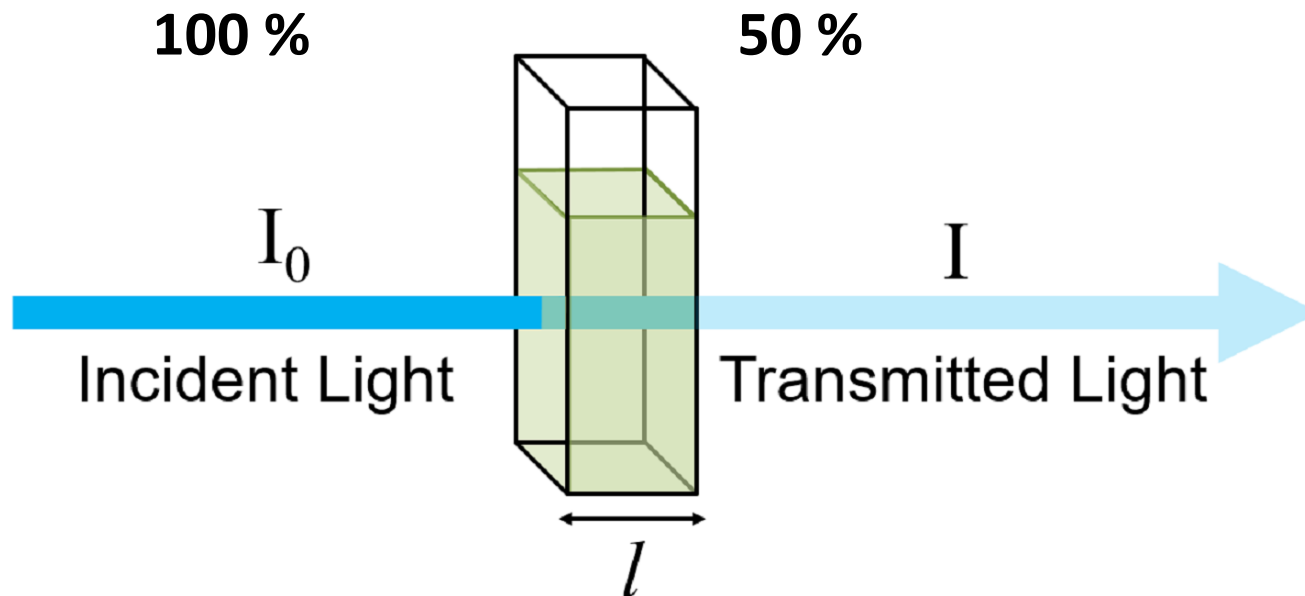
Negative solvatochromism corresponds to hypsochromic shift (or blue shift) with increasing solvent polarity **similarly the bathochromic shift** (or red) is positive solvatochromism.



How to predict the possible electronic transition for the given structure ?

Chemical class	Types electron	Possible transitions (theoretical)
Alkane	Sigma electron only	$\sigma-\sigma^*$
Alkene, Benzene Alkyne	Sigma and Pi electrons	$\pi-\pi^*$ and $\sigma-\sigma^*$
Aldehyde, ketone, heterocyclics, acid, ester, amide, oxime etc. (all compounds containing C=O, C=S, C=N)	Sigma, pi electrons and n' electrons	$\pi-\pi^*$, $n-\pi^*$, $\sigma-\sigma^*$ and $n-\sigma^*$
Metal complexes	n' electrons	Charge transfer
UV-Solvents Eg. Water, Ethanol, Ether, Chloroform	Sigma and n electrons	$\sigma-\sigma^*$ and $n-\sigma^*$

**If the % transmittance reduced to 50 %
What is the absorbance and transmittance ?**



**If the transition is π - π^* ,
what is the effect of more polar solvent
on λ_{max} ?**

Example:

λ_{max} of Acetone in ethanol : λ_1

λ_{max} of Acetone in water : λ_2

Which will be higher for π - π^* transition?

Similarly,

Which will be higher for n - π^* transition?

