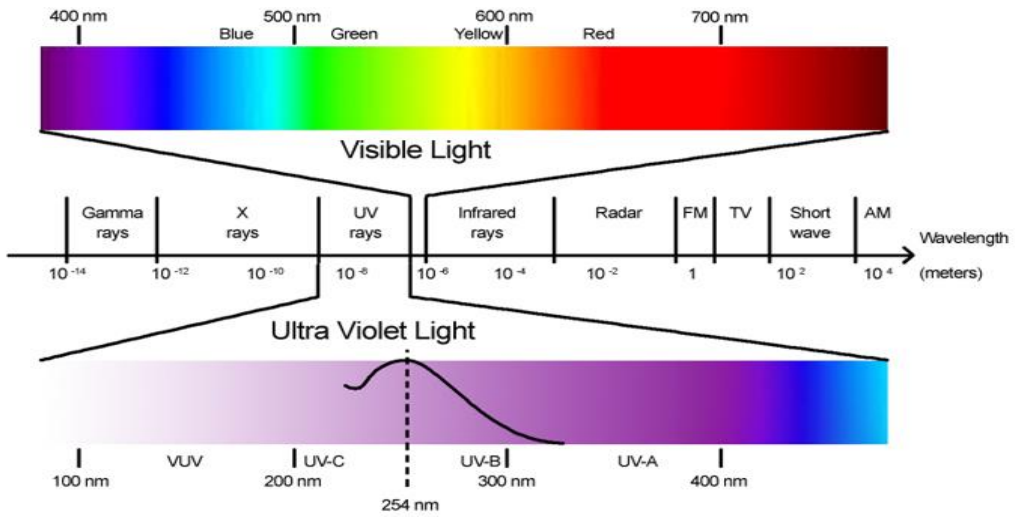
University of Jordan
faculty of pharmacy

Pharmaceutical instrumental analysis laboratory

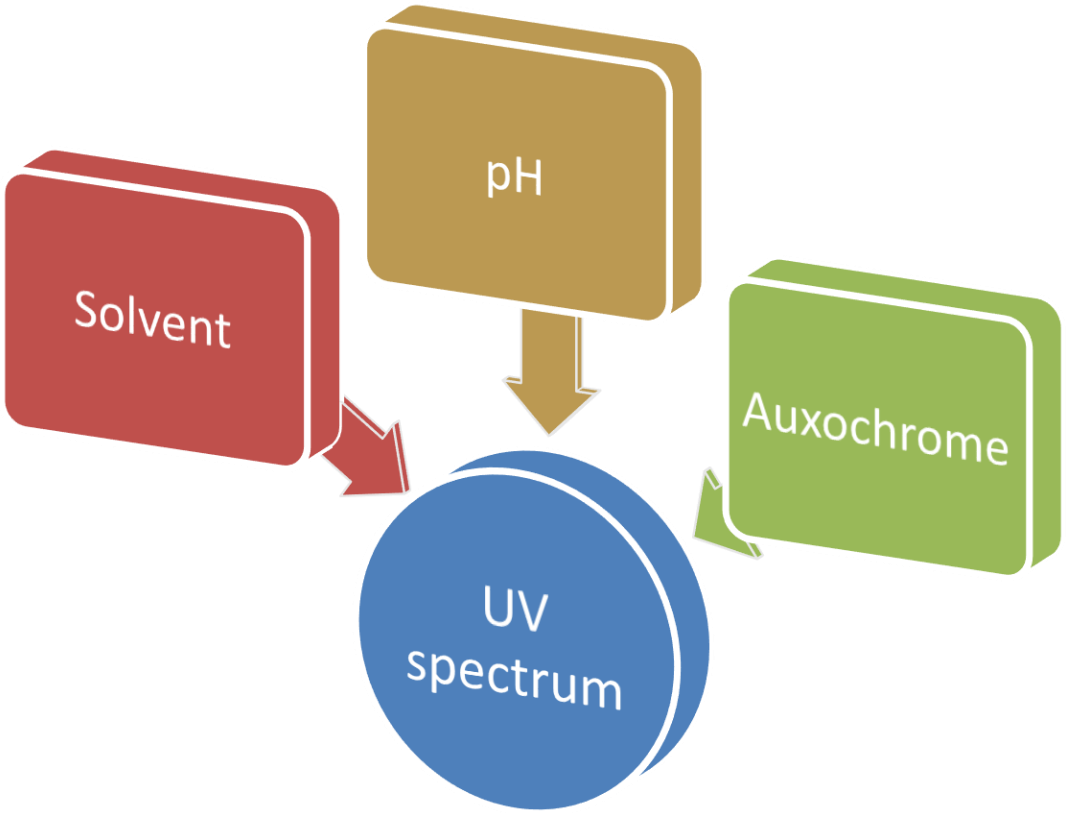
Experiment 1

Effect of Solvent, pH and auxochrome on UV absorbance



Aim of the experiment

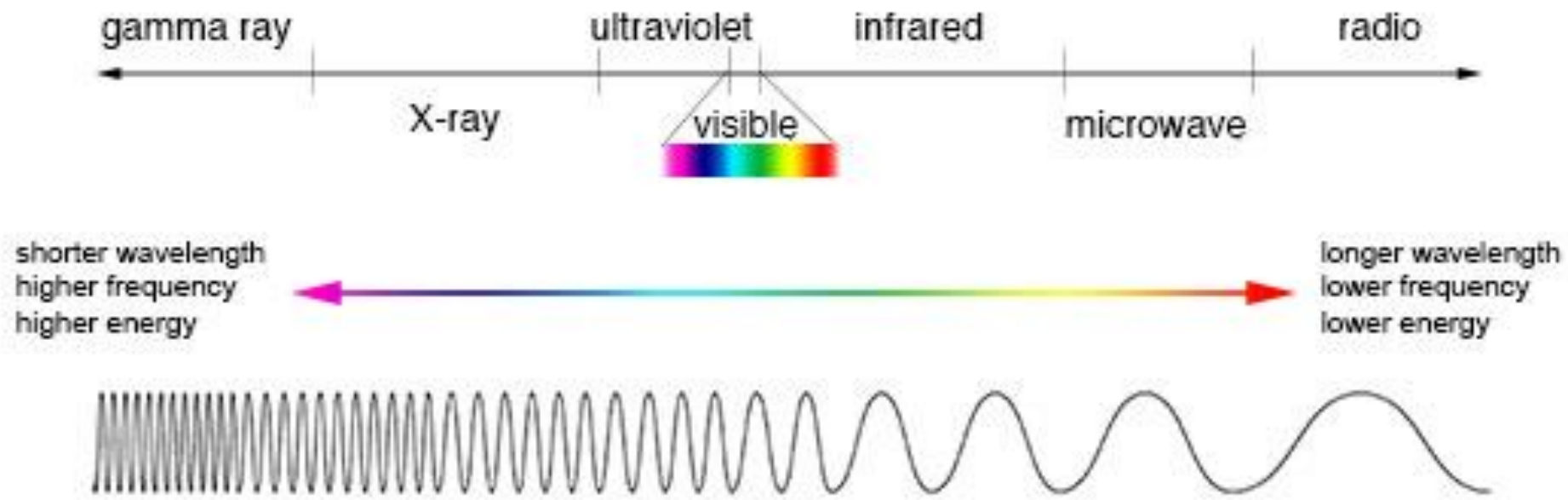
- **To study Effect of Solvent, pH and auxochrome on UV absorbance**



INTRODUCTION AND THEORY

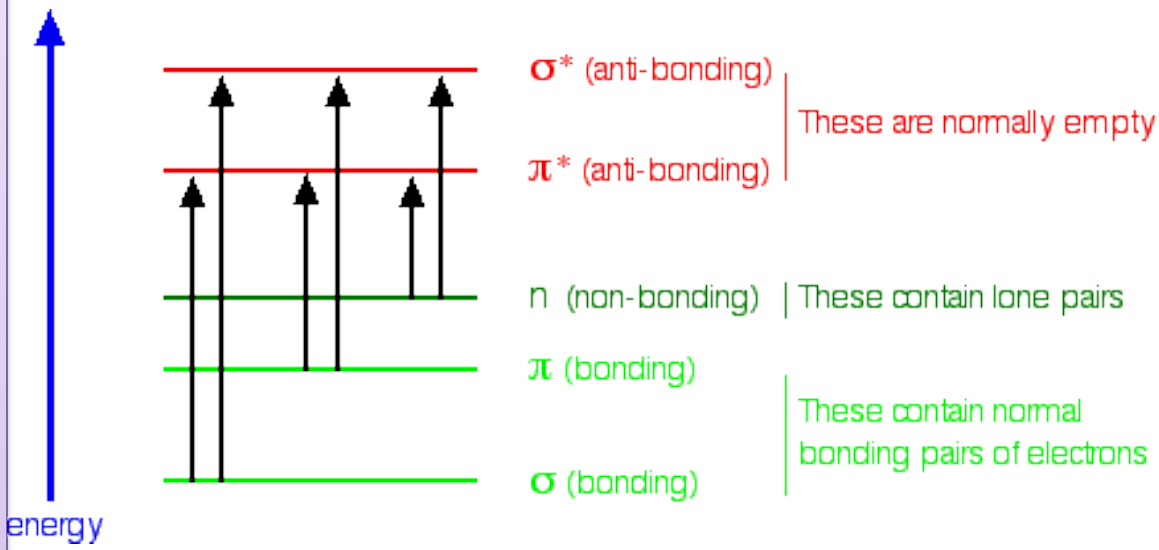
INTRODUCTION AND THEORY

- Ultraviolet-visible spectroscopy or ultraviolet-visible spectrophotometry (UV-Vis or UV/Vis) refers to absorption spectroscopy in the ultraviolet-visible spectral region. This means it uses light in the visible and adjacent (near-UV and near-infrared (NIR)) ranges.



INTRODUCTION AND THEORY

- When light passes through the compound, energy from the light is used to promote an electron from a bonding or non-bonding orbital into one of the empty anti-bonding orbital's.
- An electron is excited from a full orbital into an empty anti-bonding orbital. Each jump takes energy from the light, and a big jump obviously needs more energy than a small one.
- Each wavelength of light has a particular energy associated with it. If that particular amount of energy is just right for making one of these energy jumps, then that wavelength will be absorbed - its energy will have been used in promoting an electron.



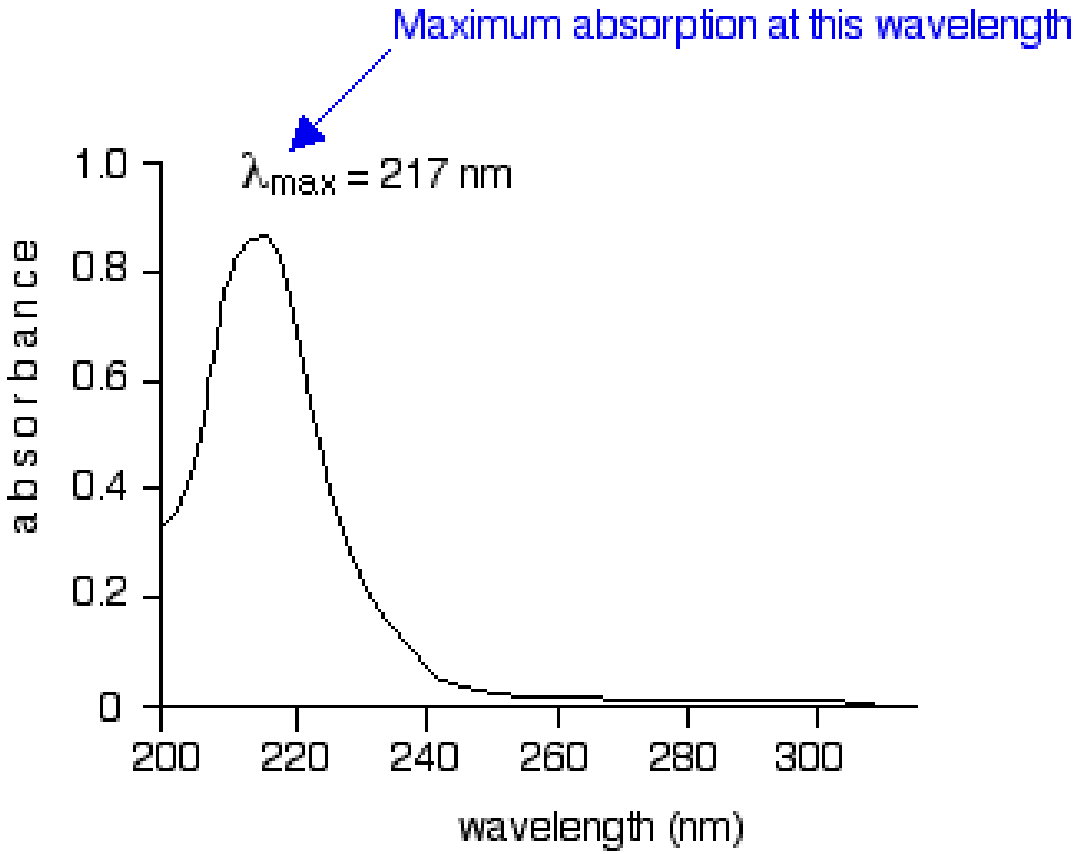
possible electron jumps that light might cause

INTRODUCTION AND THEORY

- The larger the energy jump, the lower the wavelength of the light absorbed.
- In order to absorb light in the region from 200 - 800 nm (UV-vis light range), the molecule must contain either **pi bonds** or **atoms with non-bonding orbitals**. Remember that a non-bonding orbital is a lone pair on, say, oxygen, nitrogen or a halogen.
- Groups in a molecule which absorb light are known as ***chromophores***.

INTRODUCTION AND THEORY

- A plot relating the amount of light absorbed (absorbance) by a molecule at UV range of wavelengths is called the **UV absorption spectrum**.
- Each compound has a characteristic UV-spectrum.



Shifts in UV spectrum

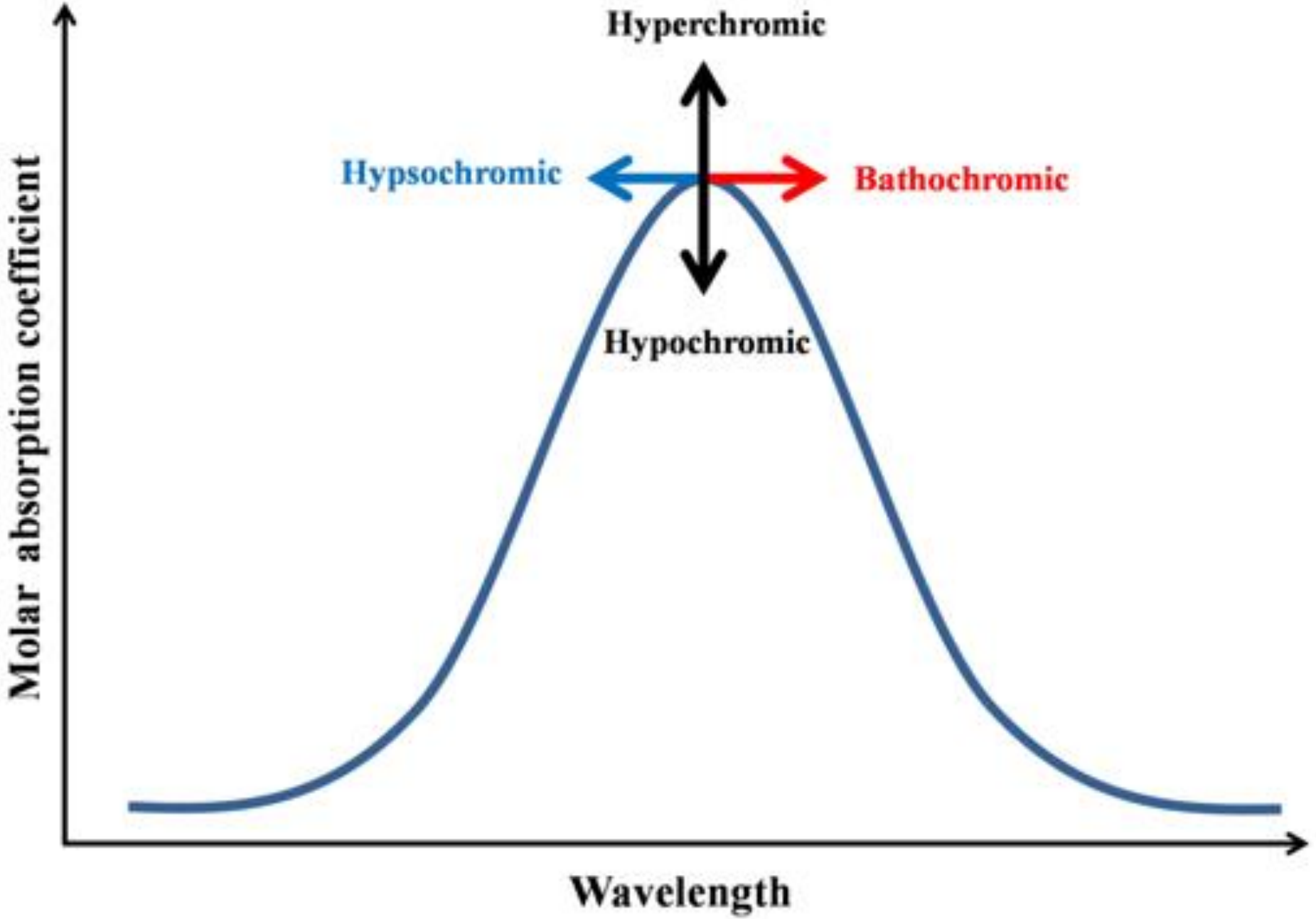
- **Changing certain factors such as (pH, solvent, Auxochrome) can lead to a various shifts in the compound UV spectrum which include:**

➢ **Bathochromic Shift**: shift in absorption to a longer wavelength

➢ **Hypsochromic shift**: shift in absorption to a shorter wavelength

➢ **Hypochromic effect**: a decrease in the intensity of absorption

➢ **Hyperchromic effect**: an increase in the intensity of absorption.


Hyperchromic
Hypsochromic
Bathochromic
Hypochromic
Molar absorption coefficient
Wavelength

Important definitions you must keep

- **Chromophore**: A chemical group on a molecule responsible for electronic absorption
- **Auxochrome**: is a saturated group with non-bonded electrons which when attached to a chromophore alters both the wavelength and intensity of absorption such as -OH, -OR and -NH.

INTRODUCTION AND THEORY

- More detailed explanation can be found in the links below.
- https://www.chemguide.co.uk/analysis/uvvisible/theory.html#top
- http://www.civil.northwestern.edu/EHE/COURSES/eac/exp2/meth2.htm

PRACTICAL PART

Materials

- **Glassware:**
- Nine 100 ml volumetric flasks.
- 0.5, 1, 2ml volumetric pipettes
- **Chemicals:**
- Solvents: Cyclohexane, distilled water, 0.1M NaOH, 0.1M HCl
- Analytes: Acetone, Phenol, Ephedrine HCl

Instrument

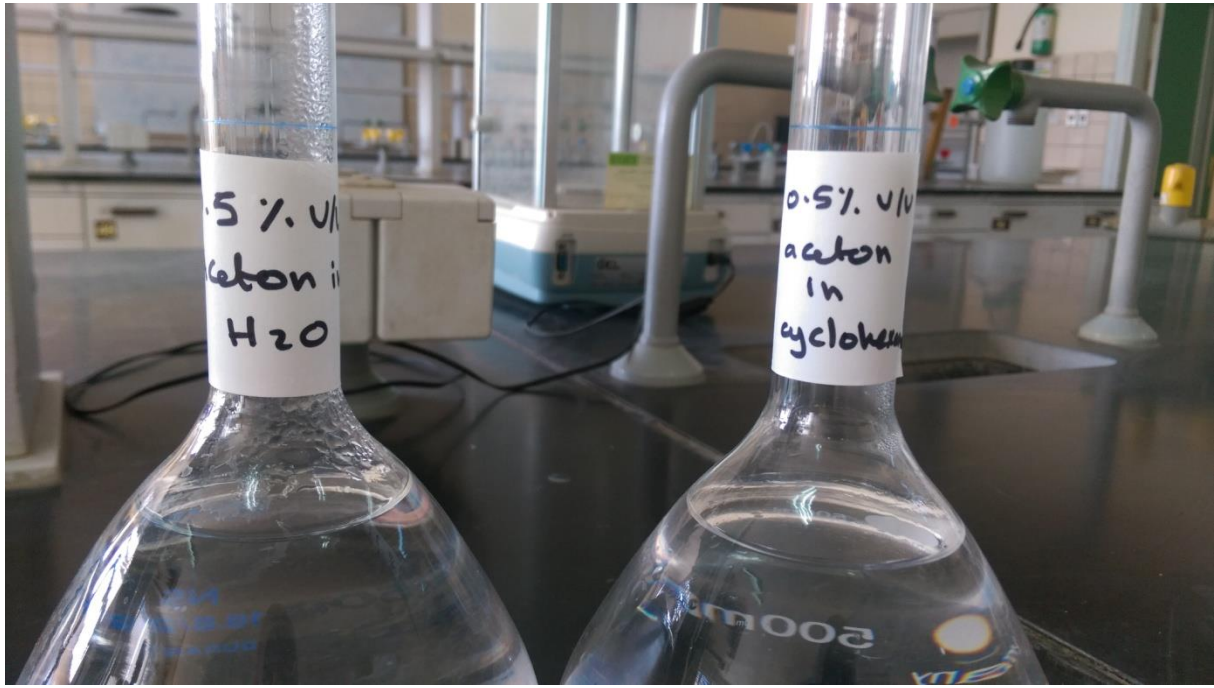
- **Double beam UV-Vis spectrophotometer**

Spectrophotometers devices

- **In our lab we have 2 Spectrophotometers devices**
- **Single Beam Spectrophotometers**
- Here a single beam of light is passed through a single sample container and the resulting light is detected by a detector. Single Beam Spectrophotometers are of the simplest in design hence have lower capital and maintenance prices than other spectrophotometer type.
- **Double Beam Spectrophotometers (will be used in this experiment)**
- Here the light leaving the monochromator is split, using a beam splitter, into a sample beam and a reference beam. After each beam of light is passed through its respective sample/reference (blank) container, each beam is then detected by its own detector.
- The sample and reference are simultaneous/measured/scanned, saving time and providing for optimum accuracy.
- Double beam spectrophotometers ensure that any fluctuations in the light emitted from the lamp are applied equally to both the sample and the reference beams.

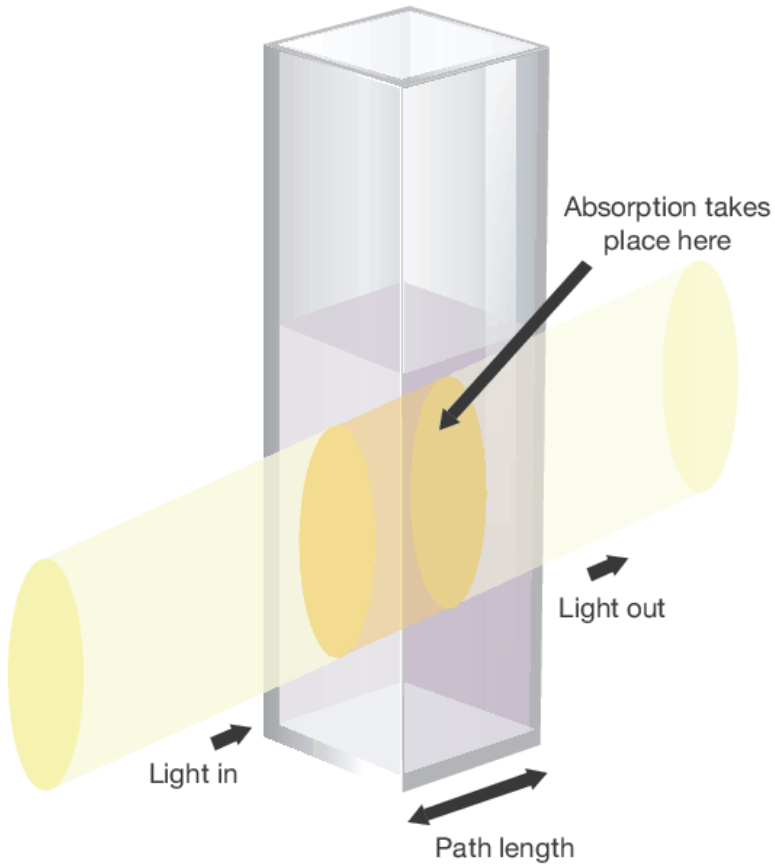
Procedure and results

- **1. Effect of solvent on UV absorbance**
- a. Prepare the following solutions:
- Acetone in H_2O (0.5% v/v) and acetone in cyclohexane (0.5% v/v)
- b. Scan (obtain UV spectrum) for each solution between 200-300nm. (**watch the videos in the next slides**)



Where to put your sample?

- In UV spectroscopy the liquid sample is held in cuvette.
- Cuvette is sealed at one end, and made of a clear, transparent material such as plastic, glass, or fused quartz..



Measuring UV spectrum 1
sample preparation

- **In order to insert your sample in the UV-spectrophotometer for measurement do the followings:**

➢ Clean the cuvette

➢ Place your liquid sample in the clean cuvette .

➢ Clean the transparent sides of your cuvette with a damp paper towel to remove finger prints.

➢ Insert the cuvette in the sample holder in UV-spectrophotometer.

Notes to take into consideration

- The Double beam UV-Vis spectrophotometer have two cuvette holders one for the reference and one for sample .
- Red arrow shows cuvette holder for reference and blue arrow shows cuvette holder for sample.

Notes to take into consideration

- Don’t overfill the cuvette.
- Inside the UV spectrophotometer Pay attention that the transparent sides of the cuvette are facing the direction of light.

Measuring spectrum 2 scanning

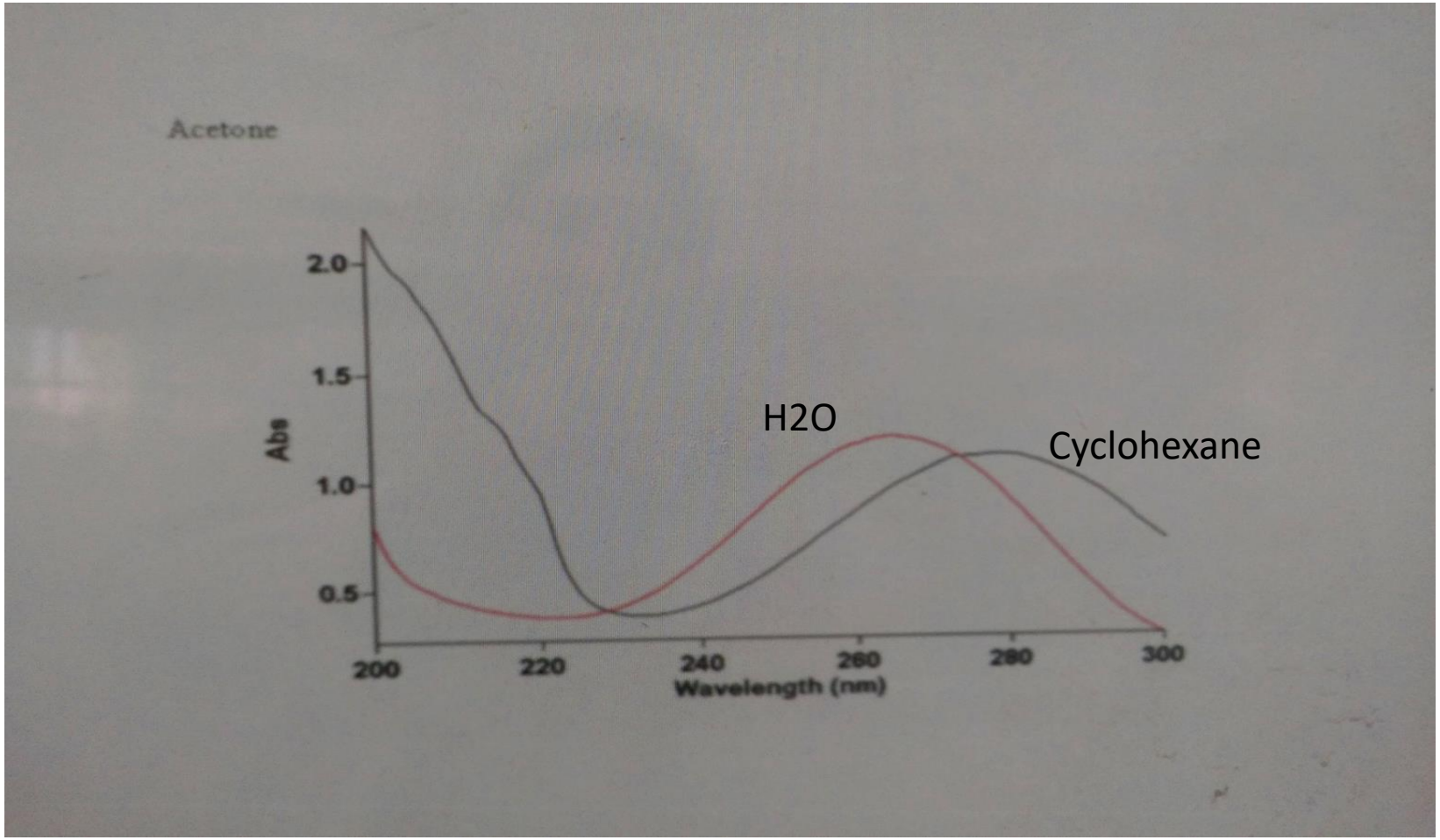
- **In order to scan your sample spectrum**

➢ from start menu choose program**-->** carry WinUV --> scan.

➢ Wait a minute till the device calibrate then from the opened window choose **setup** to set the UV range you want to measure your sample on.

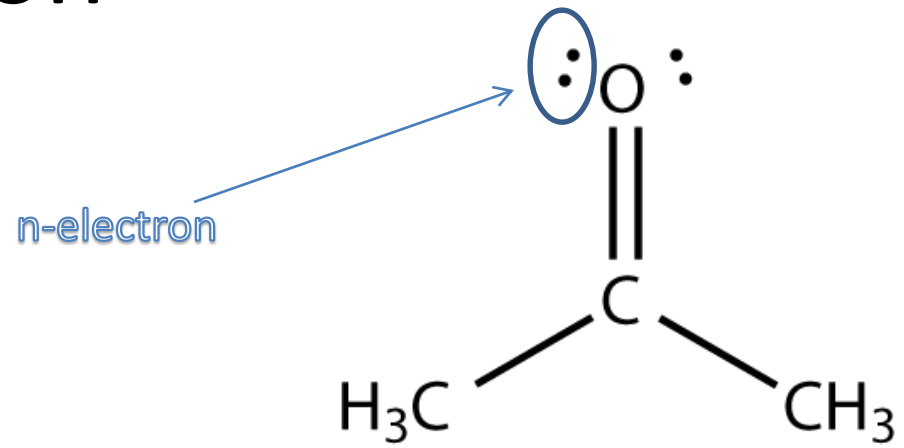
➢ Press start to begin your measurement.

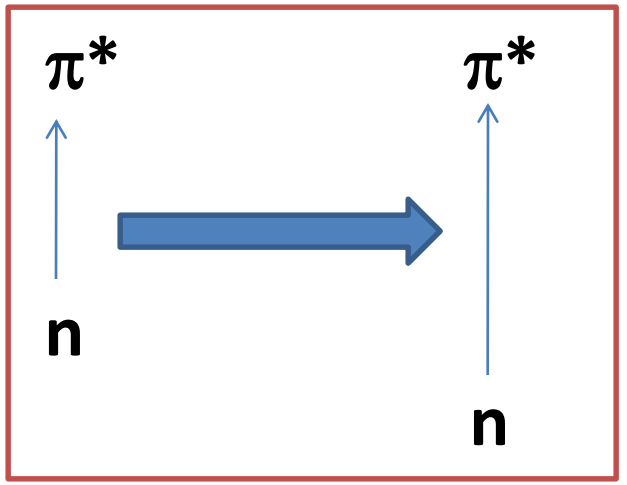
Result: Acetone in H2O vs. Cyclohexane



Explanation

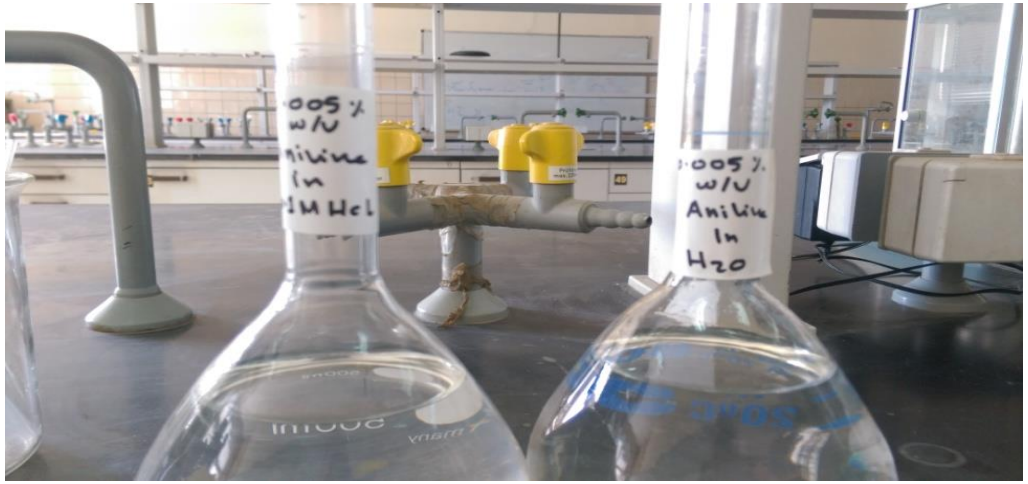
- Acetone form hydrogen bond with water which decrease the energy level of the **n-electrons** so the energy difference between n and π* will be higher, therefore, n-π* transition will need more energy (hence lower lambda) leading to a blue shift.

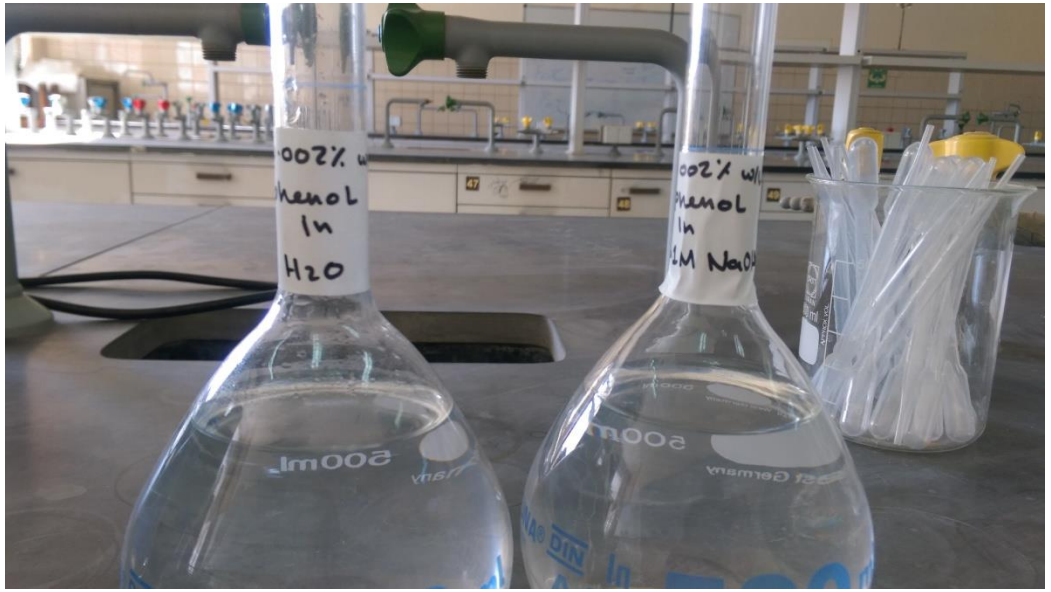




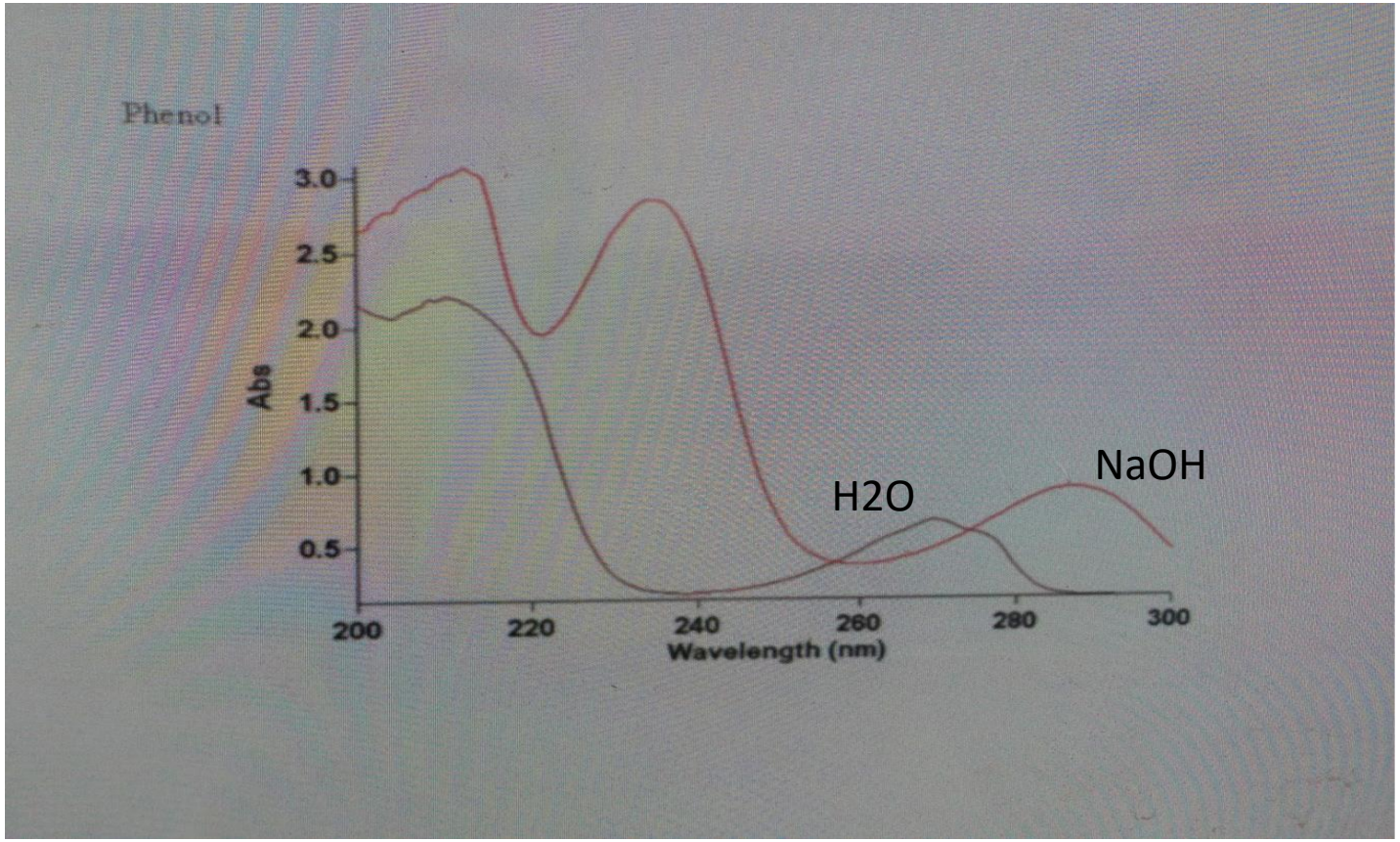
Procedure

- **2. Effect of pH on UV absorbance**
- a. Prepare the following pairs of solutions:
- Aniline in H_2O (0.005% v/v) and aniline in 0.1M HCl (0.005% v/v)
- Phenol in H_2O (0.002% v/v) and aniline in 0.1M NaOH(0.002% v/v)
- b. Scan (obtain UV spectrum) each solution between 200-300nm.



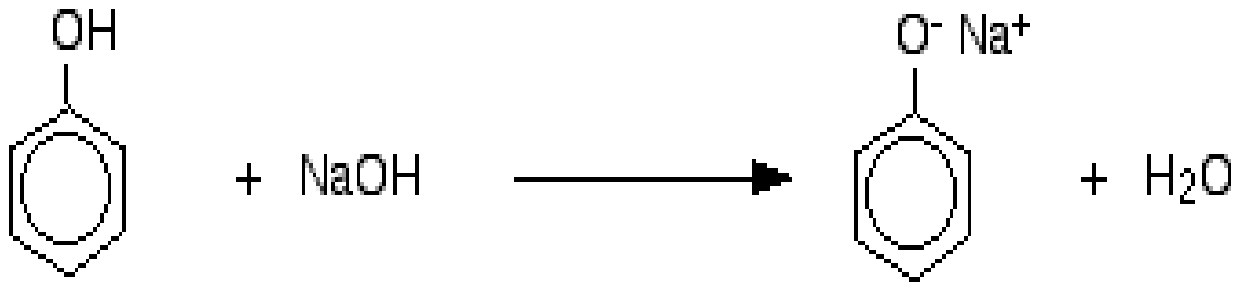


Result: Phenol in NaOH Vs. H2O



Explanation

- Phenol will be ionized in NaOH producing the negatively charged phenoxide ion which is a stronger chromophore than phenol so it will absorb light at a higher intensity (**Hyperchromic shift**).

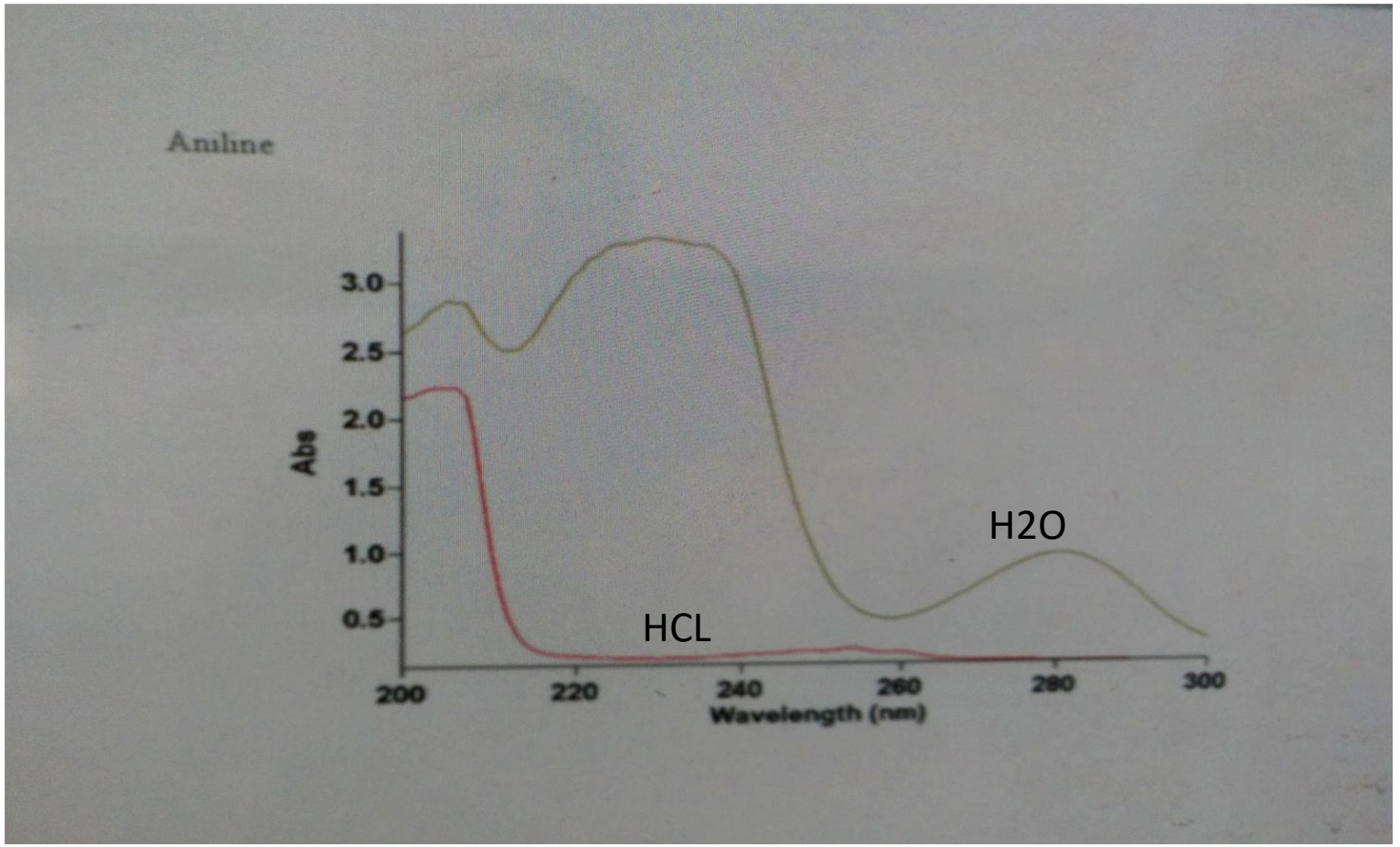


sodium phenoxide

Home work

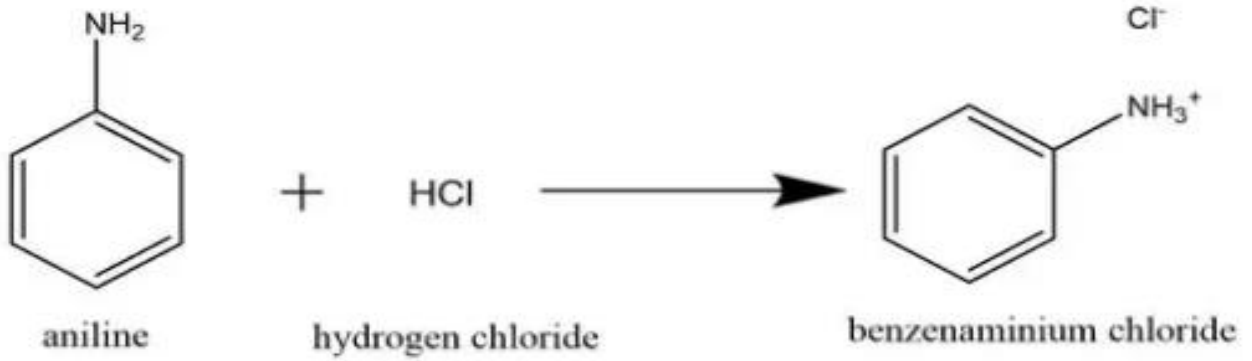
Can you see any other shifts in the phenol spectrum?? If yes what are they and what is your justification ?

Result: Aniline in H_2O Vs. HCL



Explanation

- Aniline will be ionized in HCL producing the positively charged benzenaminium ion which is a weaker chromophore than aniline so it will absorb light at a lower intensity (**Hyporchromic shift**).

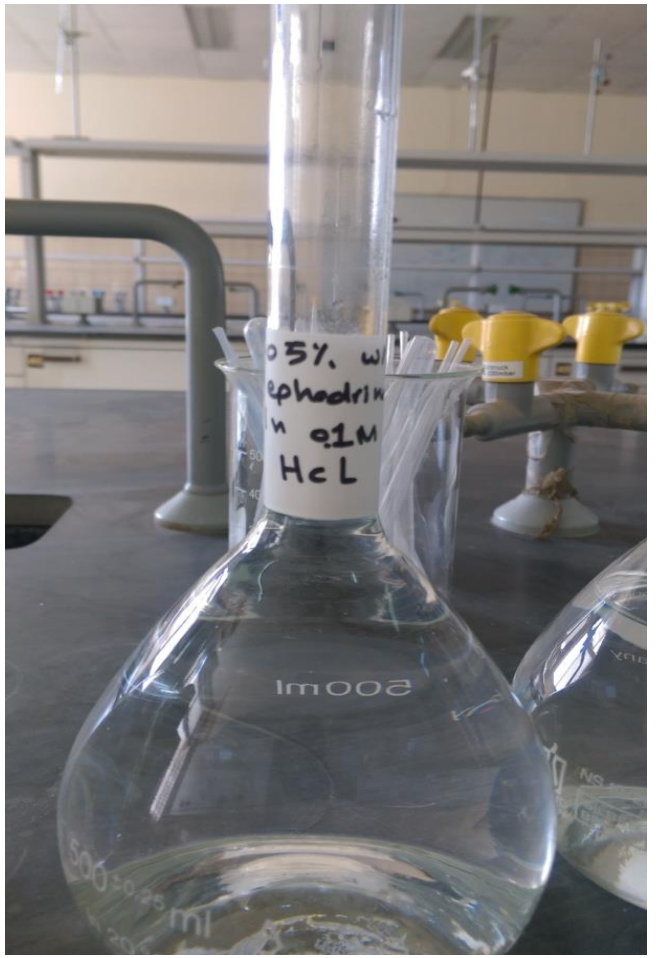


Home work

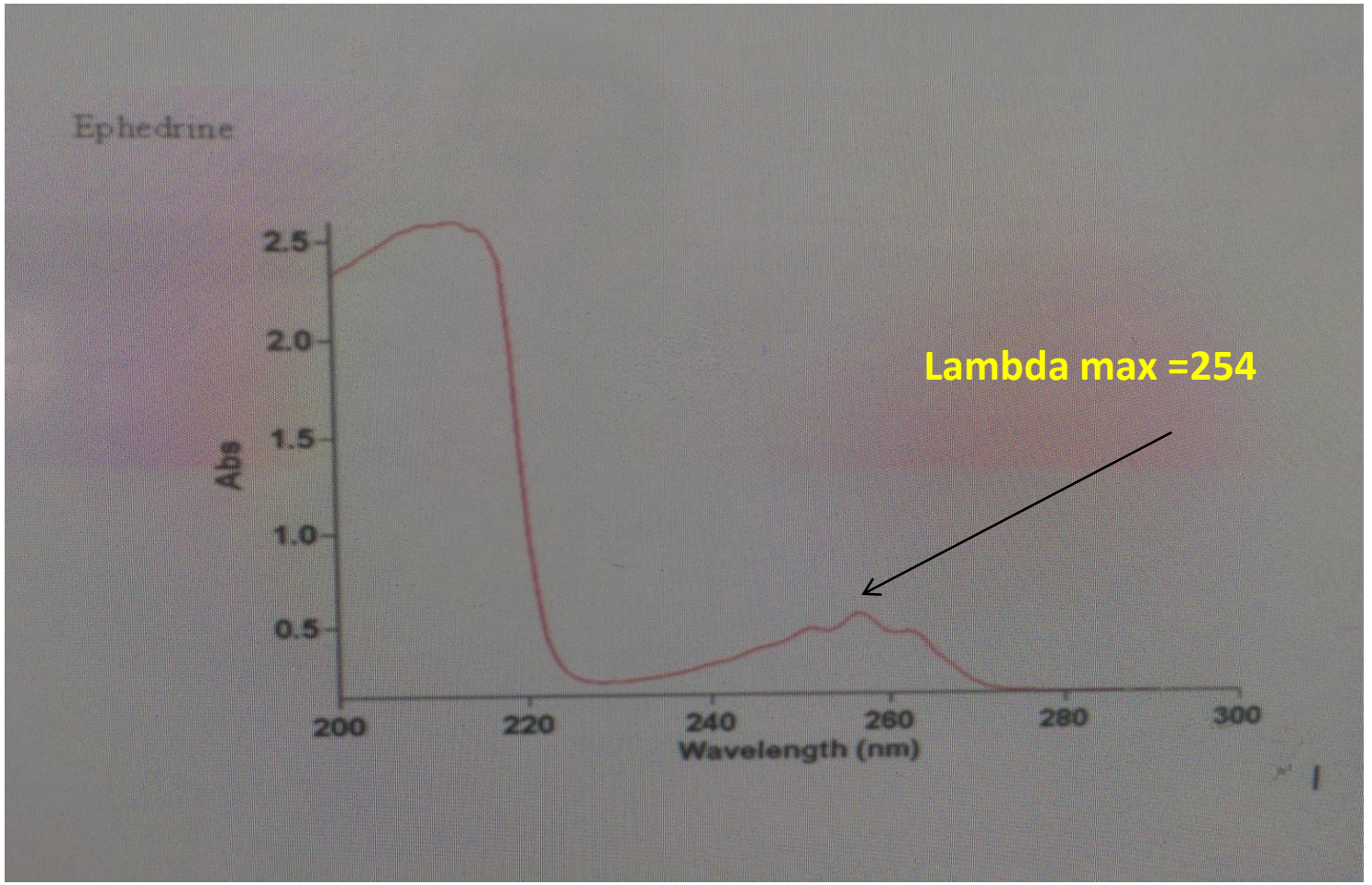
Can you see any other shifts in the Aniline spectrum?? If yes what are they and what is your justification ?

Procedure

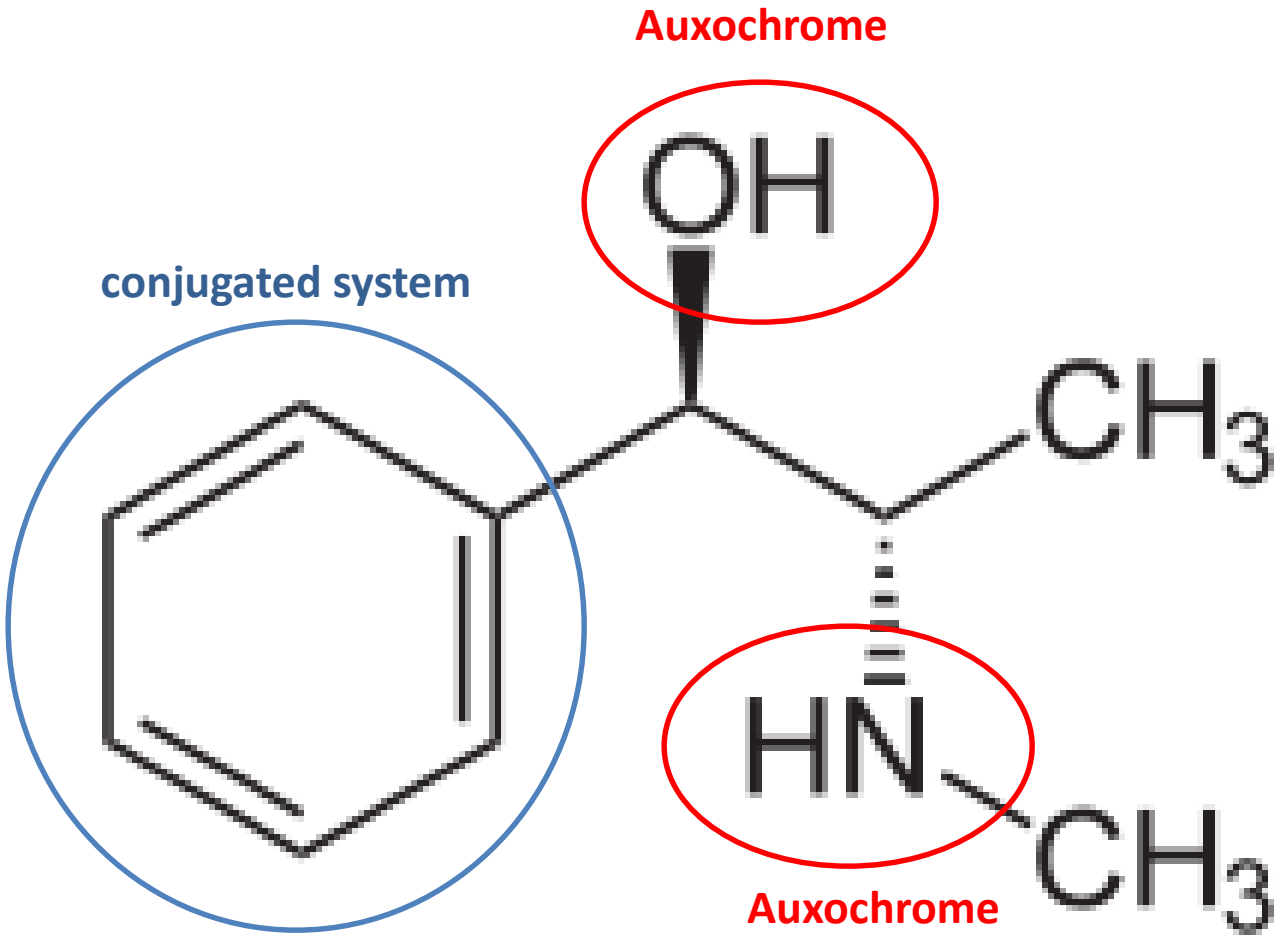
- **3. Effect of auxochrome on UV absorbance**
- a. Prepare (0.05% w/v) solution of ephedrine in 0.1M HCl and scan the solution between 200-300nm
- b. Compare the spectra of ephedrine to that of benzene.



Result: Ephedrine in HCL



Explanation



Ephedrine has the same lambda max of benzene. Since Auxochrome (-OH, -NH) are not directly attached to the conjugated system (benzene ring).

Compare the lambda max of aniline and phenol to that of ephedrine. What can you guess?

- **Well done! You did a great job reaching this slide ^_^**