KLE Technological University

Department of Chemistry

Unit III - Chapter 7 Instrumental Methods of Measurement

Basic Principles of Instrumentations

In this lesson we will discuss about the principles of Instrumentation followed by the various instrumental techniques. Further we will know about the advantages and limitations of instrumental analysis.

Introduction

Chemical analysis includes the use of instrumentation to solve an analytical problem.

The use of instrumentation has now become a part of chemical analysis and is applied for all areas of pure and applied science. Any single instrument could not solve an analytical problem; instead, several instrumental techniques are required to solve the problem to a maximum extent. Hence instrumentation plays an important role in the production and evaluation of new products and in the protection of consumers and the environment.

Principles of Instrumentation

In Instrumental analysis, a physical property of a substance is measured to determine its chemical composition. Analysis may be biochemical, analytical, inorganic, organic and physical. Whatever may be the type of analysis, the goal of the analysis is to provide information about the composition of the sample. This is called the quantitative analysis. This analysis is being done using instruments. The choice of the instrument depends on the property measured.

The basic principle of an instrument used for chemical analysis is as follows. It converts chemical information to a form that is observable. The instrument thus helps in (1) Generation of a signal (2) Transformation of a signal to one of a different nature (transducer) (3) Amplification of the signal. However all the steps are not incorporated in all the instruments.

Selection of Instrumentation

Depending on the property to be analyzed, the analytical method could be selected.

During the selection of the method, the concentration of the sample and accuracy needed must be taken into consideration.

Concepts of Instrumental Analysis

The Concept of Instrumental analysis reviews the various advantages and limitations of the same. It is essential to understand about the advantages and the limitations of every Instrumental analysis. We will see some of them here.

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Advantages of Instrumental Analysis

- Even small quantity of samples will be sufficient for the analysis.
- Results obtained are reliable.
- Complex samples can also be analyzed.
- The rate of determination is fast.

Limitations of Instrumental Analysis

- The accuracy and sensitivity depend on the instrument. It will vary with the instrument.
- The cost of the equipment is quite higher.
- The range of concentration that would be measured is limited.
- For every instrument, handling is suggested only after training.
- Suitable space is needed.
- There must be no fluctuation in the power supply.

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Electro analytical Methods

1. Principle, methodology and applications of Potentiometer

To determine the concentration of the Ferrous Ammonium Sulphate (FAS) by measuring EMF of the galvanic cell

Apparatus: Potentiometer, 100ml & 250ml beakers, burette, pipette, Platinum-Calomel electrode assembly, test tubes, etc.

Chemicals: Ferrous ammonium sulphate ($FeSO_4$. $(NH_4)_2SO_4.6H_2O$), Potassium dichromate solution ($K_2Cr_2O_7$), 2N sulphuric acid.

Theory: Potentiometric measurements are used in pollution monitoring by determining the concentration of cyanides, iodides, chlorides and nitrates in effluents & natural waters; in agriculture by determining the concentration of nitrates , chlorides, cyanides & ammonium ions in soil & fertilizers; in determining fluoride in drinking water; in determining calcium in dairy products & beer ; in determining potassium in fruit juices & wine making.

The potentiometric titration is a titration, in which the end point is detected by measuring the change in potential of a suitable electrode (which responds to the change in concentration) during the titration. The electrode which responds to the change in concentration of the ions in the solution is called the indicator electrode (Pt electrode). The indicator electrode is combined with a reference electrode (Calomel electrode), whose potential does not change during the titration. This electrode assembly forms a cell and the e.m.f. of the so formed cell is measured during the titration. The e.m.f. of the cell increases gradually in the beginning, increases rapidly nearer to the equivalence point and then onwards the increase in the e.m.f. is minimal. The equivalence point or the end point is obtained by plotting $\Delta E / \Delta V$ against volume of dichromate solution added.

The titration of FAS (Mohr's salt) solution with $K_2Cr_2O_7$ in presence of H_2SO_4 is a redox titration.

$$6Fe^{2+} \rightarrow 6Fe^{3+} + 6e$$

$$Cr_2O_7^{2-} + 14H^+ + 6e \rightarrow 2Cr^{3+} + 7H_2O$$

$$6Fe^{2+} + Cr_2O_7^{2-} + 14H^+ \rightarrow 6Fe^{3+}\ 2Cr^{3+} + 7H_2O$$

$$3Fe^{2+} + Cr^{6+} \rightarrow 3Fe^{3+} + Cr^{3+}$$

Reduced form — Oxidized form

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The presence of oxidised form (Fe^{3+}) and reduced form ($Fe2^{+}$) of the same substance in a solution gives rise to the formation of an oxidation-reduction electrode, developing an electrode potential, which can be picked up by dipping a Pt wire. Therefore, (Pt / Fe^{3+}, Fe^{2+}) is the indicator electrode, whose electrode potential is given by,

$$E = E^{o} + \frac{0.0591}{n} \log \frac{[\text{Oxidised form}]}{[\text{Reduced form}]} \quad \text{----------›(I)}$$

It is combined with a reference electrode (calomel electrode) and the e.m.f. of the cell is measured. During the titration [Fe^{3+}] goes on increasing and [Fe^{2+}] goes on decreasing as $K_2Cr_2O_7$ solution is added continuously with a gradual change in the potential. Nearer to the equivalence point the ratio [Fe^{3+}] / [Fe^{2+}] increases rapidly as [Fe^{2+}] decreases. Thus the e.m.f increases rapidly at the equivalence point according to equation (I).

When Fe^{2+} is completely converted into Fe^{3+}, by $K_2Cr_2O_7$; the electrode, Pt / Fe^{2+}, Fe^{3+} ceases to exist. But the presence of slight excess of $K_2Cr_2O_7$, brings in the existence of (Pt / Cr^{6+}, Cr^{3+}) electrode. Thus, after the equivalence point it is the e.m.f. of the (Pt / Cr^{6+}, Cr^{3+}) electrode, which is going to increase. In other words, the two factors which influence rapid increase in the e.m.f. at the equivalence point are- (i) the increase in the value of [Fe^{3+}] / [Fe^{2+}] & (ii) the change of electrode from Pt / Fe^{3+}, Fe^{2+} to Pt / Cr^{6+}, Cr^{3+} .

Calibration:

- Wash the electrodes with distilled water, wipe with filter paper and dip in the buffer solution of pH 4 and switch on the equipment.
- Set the equipment to the lab temperature.
- Press STBY & pH buttons and adjust the reading to 180mV using calibration knob.
- Release STBY button and Wash the electrodes with distilled water and wipe with filter paper.
- Dip the electrodes in the prepared FAS solution.
- Press STBY button and note down the reading for 0.0 ml of $K_2Cr_2O_7$ added.
- Carry out the titration as given in the procedure.

Procedure:

Pipette out the said volume of the given ferrous ammonium sulphate solution into a 100ml volumetric flask. Make the solution up to the mark using distilled water. Shake the solution thoroughly to make it homogeneous and transfer it into a clean 250ml beaker. Add two test tubes of 2N sulphuric acid. Dip the Platinum-Calomel electrode assembly into the solution in a such a way that the bulbs should get immersed in the solution and connect to the potentiometer. Note down the potential of the FAS solution, which corresponds to 0.0c.c. of potassium dichromate solution.

Fill the micro burette with standard potassium dichromate ($K_2Cr_2O_7$) solution and add 0.5c.c of this solution to FAS, **shake the solution well and wait for a while** (i.e. shake well for 30 sec. & wait for 30 sec.) and note down the potential. Calculate $\Delta E / \Delta V$ for each addition.

For the initial 3-4 additions, the $\Delta E / \Delta V$ values show small variations. The continued additions show a gradual increase in $\Delta E / \Delta V$ values, and when the increasing trend of $\Delta E / \Delta V$ value reaches around 40, add $K_2Cr_2O_7$ solution 0.1c.c by 0.1c.c till there is a sudden jump in the potential and also in $\Delta E / \Delta V$ value. Continue 0.1cc additions for 4 to 5 readings. Then onwards, add $K_2Cr_2O_7$ solution 0.5c.c by 0.5c.c for 4 to 5 additions when the potential varies only in third decimal.

Determine the end point by differential method i.e. by plotting a graph of $\Delta E / \Delta V$ against volume of potassium dichromate solution added, and calculate the normality of the FAS solution and hence the amount of FAS present in the solution.

Precautions:

1. Potentiometer should be calibrated before performing the experiment.
2. Stirring should be done after each addition of the titrant.
3. Electrodes should be handled carefully and immersed properly in the solution.

Record of Observations:

1. Solution in Beaker : A known volume of FAS diluted to 100ml. + 2 test tubes of 2N H_2SO_4

2. Solution in Micro burette : **0.4N** $K_2Cr_2O_7$ solution.

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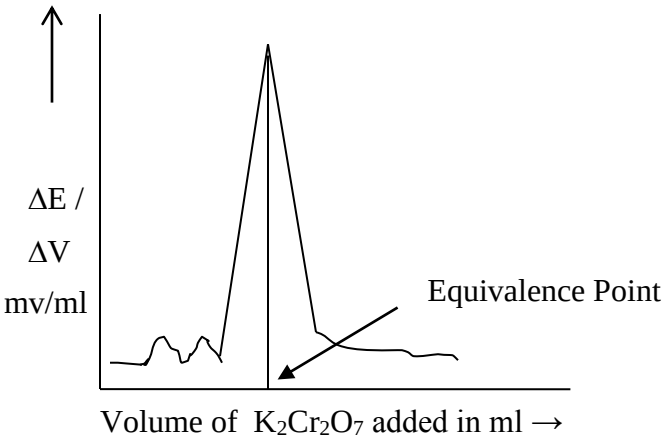
Nature of Graph:

$\Delta E / \Delta V$ Vs Volume of $K_2Cr_2O_7$ added



Tabulation:

Volume of $K_2Cr_2O_7$ added ml.	**EMF in mv**	**ΔE mv**	**ΔV ml.**	**$\Delta E / \Delta V$ mv/ml**
0.0 0.5 1.0 1.5 2.0 - - -		--	--	--

Calculation:

1. Normality of FAS solution = Normality of $K_2Cr_2O_7$ X Equivalence point (from graph)

Total volume of FAS taken

= 'x' **N**.

2. Amount of FAS in the given solution = '***x***' X Equivalent weight of FAS

= '***x***' X 392

= **'F' g./ lit.**

Applications of Potentiometric titrations

During the titration, the potential of the cell is measured after each addition of the solution. Time is allowed for the attainment of equilibrium. This methodology is useful for many titrations as followes.

Acid base titrations, Redox titrations, Precipitation titrations, Redox titrations and Precipitation titrations.

Advantages of Potentiometric titrations

1. The method is suitable for the analysis of dilute solutions too.
2. It could be applied for colored solutions also.
3. The interpretation of titration curves is easier.
4. The apparatus used is inexpensive, reliable and readily available.
5. Different components of different characteristic colour could be titrated at a same time.

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Opto analytical Methods

2. Principle, methodology and applications of Colorimeter

To determine the concentration and amount of Cu present in the copper pyrite ore solution by colorimetric method.

Apparatus: Colorimeter, colorimetric cells (Nesteller's tubes or Cuvettes), test-tube set, burretts.

Chemicals: Ore solution, Copper sulphate, Ammonium hydroxide solution.

Theory: Copper pyrite is a ore of Copper, which contains 34.5% of Copper, 30% of Iron and 35% of Sulphur. Copper is metallurgically extracted and used for the wide range of applications. 75% of the world's need for copper is supplied by this copper pyrite ore. In this experiment we determine the amount of copper present in the copper pyrite ore solution by colorimetric method.

Known quantity (10gm) of copper pyrite is dissolved in minimum quantity of 1:1 HNO_3 followed by the addition of Conc. H_2SO_4. This precipitates Fe in the form its sulphate. Sulphur is removed as its oxides. Filter the resulting solution to remove the $FeSO_4$. 10ml of the Filtrate is diluted to 100 ml. This solution needs neutralization which is carried out by adding pinch by pinch of solid $NaHCO_3$ till copper forms the precipitate of its carbonate which is a blue precipitate. The precipitate is dissolved in acetic acid till a clear blue solution is obtained. This clear blue solution is treated with NH_4OH solution to get blue colored complex solution. The amount of copper present in this comlex solution is determined graphically using the data from colorimetric analysis.

Colorimetric analysis depends upon the measurement of the amount of light absorbed by a coloured solution. When a monochromatic light of intensity (Io) is incident on a transparent medium, a part of the light is absorbed (Ia), a part of it is reflected (Ir) & the remaining part is transmitted (It).

i.e. **Io = Ia +Ir + It**

For a glass air interface, **Ir** is negligible.

Io = Ia + It

Now, Transmittance, T, is the ratio of intensity of transmitted light to the intensity of incident light.

i.e. T = It / Io

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According to **Beer's law**, Absorbance, A, or optical density, O.D, is the logarithm of ratio of the intensity of incident light to the intensity of transmitted light.

i.e. A = log Io / It

The relation between absorbance A, concentration C (mol/dm^3) and the path length *l* cm. is given by **Beer-Lambert's law**, which states that, ***"the intensity of a beam of monochromatic light decreases exponentially as the concentration of the absorbing substance and the thickness of the colorimetric cell increases arithmetically".***

A α C *l* i.e. **A = log Io / It = ε.C.*l***

Where ε is proportionality constant called as molar extinction co-efficient of the solution. Since ε & *l* are constants (i.e. by selecting colorimetric cells of uniform cross section).

Hence, **A α C**

i.e. the absorbance increases as the concentration of the solution increases. Thus, a plot of absorbance against concentration of Cu^{2+} gives a linear graph passing through the origin.

A series of solutions containing cupric ions is treated with aqueous ammonia (NH_4OH solution) to get deep-blue cuprammonium complex and is diluted to a definite volume by using distilled water.

$$Cu^{2+} + 4NH_3 \rightarrow [Cu(NH_3)_4]^{2+}$$

Deep-blue

The O.D. of each of these solutions is measured at 600nm since the complex shows maximum absorbance at this wavelength. Plot a graph of OD *vs*. concentration of Cu^{2+} ions.

Finally, an unknown volume of the Ore solution is treated with aqueous ammonia and diluted to the same volume as above and the OD of this solution is measured and the concentration is determined from the graph.

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Calibration:

- For every filter, calibrate the equipment to zero by taking blank solution in a colorimetric cell; then place the middle concentration of the series of the solutions prepared in a colorimetric cell (ie,4^{th} complex solution) and find the O.D. Amongst the filters, which gives max. O.D., will be the filter selected.
- After the selection of filter, fix that filter by calibrating to zero by taking blank solution in a colorimetric cell, and then place the respective complex solutions sequentially in the colorimetric cell and take the O.D's.

Procedure:

Clean the supplied series of test-tubes and colorimetric cells thoroughly 1-2 times with distilled water. Prepare a series of complex solutions & a blank solution by mixing Ore solution, H_2O & NH_4OH in eight different test tubes as shown in the tabular column.

(A) Selection of filter:

Take a colorimetric-cell, rinse it with complex solution from the 4^{th} test-tube (middle concentration solution) and take $2/3^{rd}$ of the complex solution in it. Take blank solution in another colorimetric cell, wipe its outside surface with filter paper and place in the colorimeter and adjust the knob in such a way that the colorimeter shows zero optical density. Take out the blank solution cell and place the complex solution cell. Note down the optical density. Similarly note down the optical density by changing the filter .Then select the filter which gives maximum O.D.

(B) Determination of Optical Density:

Fix the selected filter and place the colorimetric cell containing blank solution in the colorimeter and adjust the knob to zero O.D. Now take the prepared complex solution of the first test tube into a clean colorimetric cell and place in the colorimeter, observe the O.D. and note down. Similarly find the ODs of other series of complex solutions namely, from 2^{nd} test tube to 7^{th} test tube and unknown solution. For every series of complex solution, confirm zero O.D. by placing the colorimetric cell containing blank solution in the colorimeter.

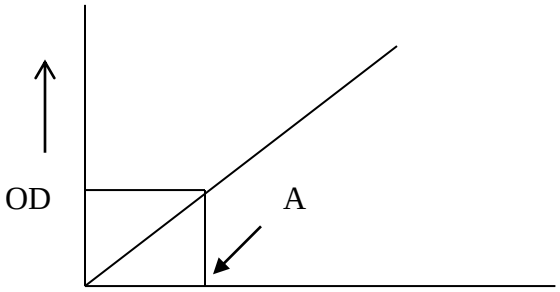
Plot a graph of optical density versus concentration of Cu^{2+} ions. From the graph, the concentration (A) of the complex solution is found out.

(C) Precautions:

1. The series of solutions should be prepared very accurately.
2. Cuvettes should be cleaned properly and must be wiped with filter paper.
3. Do no leave any finger marks on the cuvette.

Nature of Graph:

O.D. *Vs* Concentration of Cu^{+2} ions



Concentration of Cu^{+2} ions →

moles/dm^3

Record of observation:

(A) Selection of Filter:

Filter number with wavelength		Pure solvent
Filter number	**Wavelength in nm.**	
45	450	0.02
47	470	0.01
51	510	0.04
52	520	0.14
54	540	0.18
57	570	0.23
60	600	0.26
67	670	0.22

Conclusion: Filter number 60 with wavelength 600 nm is selected as it gives maximum optical density.

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Tabulation:

Determination of Optical Density:

Test tube No.	Volume of $CuSO_4$ in ml.	Volume of Water in ml.	Volume of NH_4OH in ml.	Total volume	Concentration of Cu^{+2} ions in moles/dm^3	Optical density
1	1	7	2	10	0.001	0.06
2	2	6	2	10	0.002	0.14
3	3	5	2	10	0.003	0.19
4	4	4	2	10	0.004	0.26
5	5	3	2	10	0.005	0.32
6	6	2	2	10	0.006	0.38
7	7	1	2	10	0.007	0.46
8 (Blank soln.)	0	8	2	10	0	0
Ore Complex Solution	--	--	--	--	To be determined graphically	0.17

Calculation:

1. Concentration of Cu^{2+} ions present in the unknown solution from graph =**A** moles /dm^3

3. Hence, the amount of Cu present in the unknown solution ='**A**' X Eq.Wt. of Cu

= '**A**' X 63.5

= **'C' g./dm^3**

Uses of Colorimeters:

Besides being valuable for basic research in chemistry laboratories, colorimeters have many practical applications. For instance, they are used to test for water quality, by screening for chemicals such as chlorine, fluoride, cyanide, dissolved oxygen, iron, molybdenum, zinc and hydrazine. They are also used to determine the concentrations of plant nutrients (such as phosphorus, nitrate and ammonia) in the soil or hemoglobin in the blood and to identify substandard and counterfeit drugs. In addition, they are used by the food industry and by manufacturers of paints and textiles.

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Spectral Methods of Analysis

3. DOUBLE BEAM ULTRAVIOLET SPECTROPHOTOMETER

A spectrophotometer is a device which detects the percentage transmittance of light radiation when light of certain intensity and frequency range is passed through the sample. Thus, the instrument compares the intensity of the transmitted light with that of the incident light.

The primary source of light is divided into two beams of equal intensity. Before dividing it into two beams, the incident radiation is dispersed with the help of a rotating prism. The various wave-lengths of a light source are separated with a prism and then selected by slits. This selected beam is monochromatic which is then divided into two beams of equal intensity.

One of the beams of selected monochromatic light is passed through the sample solution and the other beam of equal intensity is passed through the reference solvent.

The solvent and the solution of the sample may be contained in matched cells made of quartz or fused silica, which is transparent throughout the region under study.

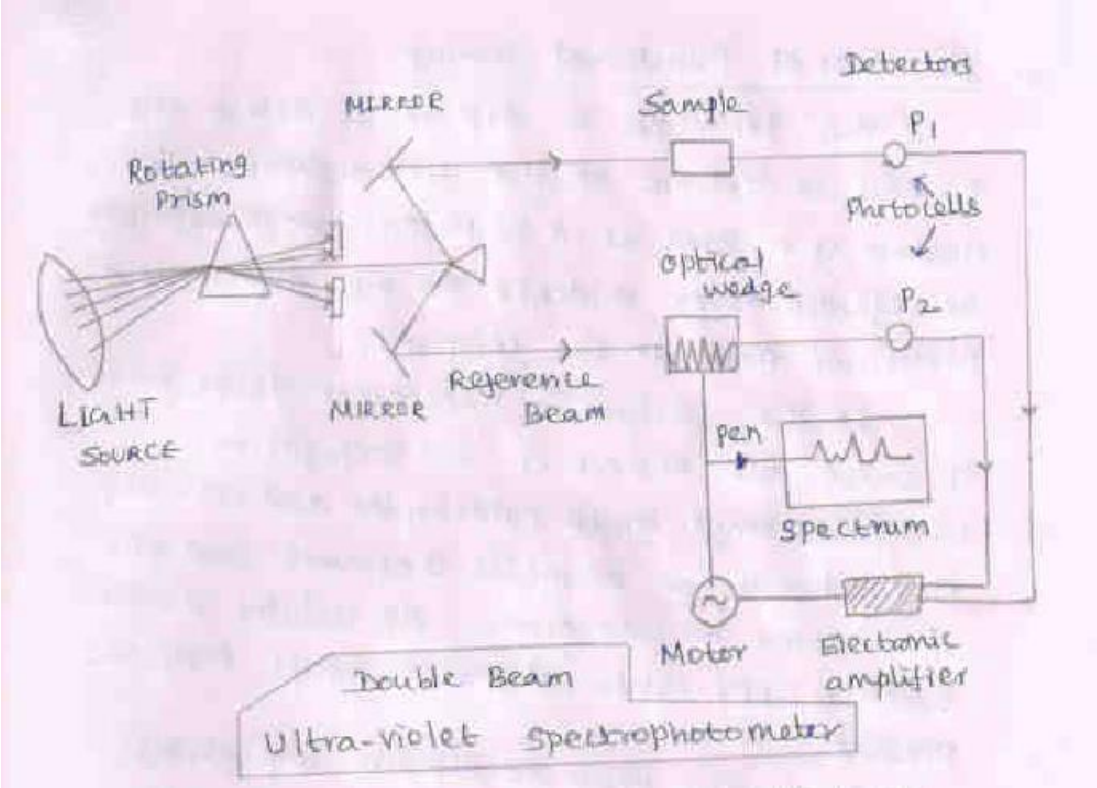
These cells are then exposed to the mono chromatic beams of equal intensity in the spectrometer.

When the sample absorbs light, its intensity is lowered. After passing through the sample and reference cells, the light beams are focused on to the detectors (P1 & P2). Thus, the photo cells P1 and P2 will receive an intense beam from the reference cell and a weak beam from the sample cell.

This results in the generation of pulsating or alternating currents which flow from the photoelectric cells to the electronic amplifier.

The amplifier is coupled to a small servomotor, which in turn is coupled to a pen recorder. Thus, it records the absorption bands automatically as a function of wavelength.

Note: Actually, the amplifier is coupled to a small servo meter which drives an optical wedge into the reference beam until the photo electric cell receives light of equal intensives from the sample as well as the reference beams.



The spectrum is usually plotted as absorbance, A ($\log_{10} I_O/ I$) against wavelength, λ (abscissa). The plot is often represented as Emax against wave length.

Applications of UV – Spectroscopy

Ultra violet spectroscopy has been mainly applied for the detection of functional groups (chromophore), the extent of conjugation, detection of poly nuclear compounds by comparison. Etc., some important applications are as follows:

1) **Detection of Functional Groups:**

This technique is applied to detect the presence or absence of the chromophore. The absence of a band at a particular wavelength may be regarded as an evidence for the absence of a particular group in the compound.

If the spectrum is transparent above 200mμ, it shows the absence of

i. conjugation
ii. a carbonyl group (aldehydes and ketones)
iii. Benzene or aromatic compounds and also
iv. Bromo or iodo atoms

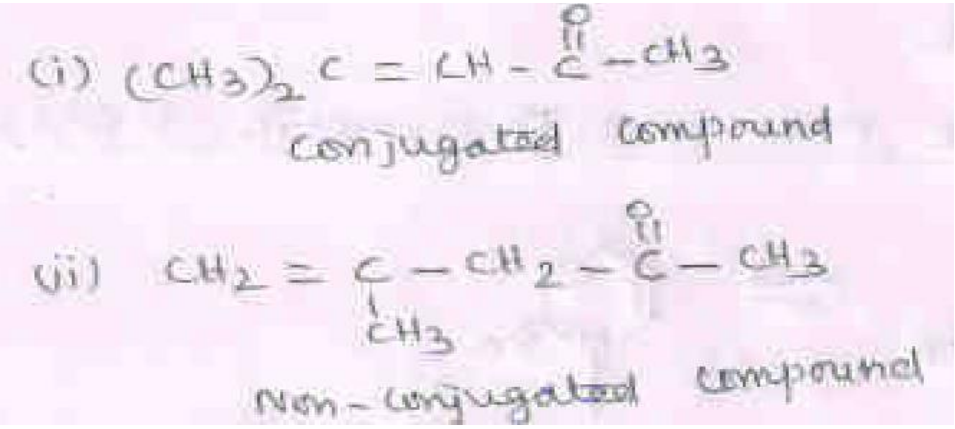
An isolated double bond or some other atoms or groups may be present.
It means that no definite conclusions can be drawn if the molecule absorbs below 200 mμ.

1. **Extent of conjugation:**

The extent of conjugation in polyenes R – $(CH = CH)_n$ –R can be estimated. Addition in unsaturation with the increase in the number of double bonds (increase in the value of n) shifts the absorption to longer wavelength. It is found that the absorption occurs in the visible region, i.e. at about 420 mμ, is n=8 in the polyene. Such an alkene appears coloured to the human eye.

2. **Distinction in conjugated and non-conjugated compounds:**

It also distinguishes between a conjugated and a non-conjugated compound. The following isomers can be readily distinguished since one is conjugated and the other is not.



The forbidden $n \rightarrow \pi^x$ band for the carbonyl group in the compound (i) will appear at longer wave – length compared to that for the compound and in compound (ii), the alkyl substitution in an alkene causes a bathochromic (red) shift.

4) **Identification of an unknown compound**

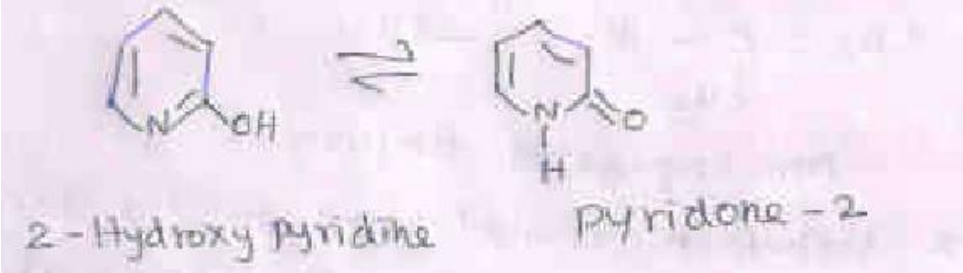
An unknown compound can be identified by comparing its spectrum with the known spectra. If the two spectra coincide, the two compounds must be identical. If the two spectra do not coincide, then the expected structure is different from the known compound.

5) Examination of poly nuclear hydro carbons:

Benzene and poly nuclear hydrocarbons have characteristic spectra in the ultra – violet and visible region.

Thus, the identification of the poly nuclear hydro – carbons can be made by comparison with the spectra of known poly nuclear compounds. The presence of substituents on the ring, generally, shifts the absorption maximum to longer wave length.

6) Preference over two tautomeric forms:



If a molecule exists in two tautomeric forms, preference of one over the other can be detected by ultra – violet spectroscopy. Consider 2 – hydroxy pyridine which exists in equilibrium with its tautomeric form, pyridone – 2.

The spectra of these two compounds were found to favour pyridone – 2 which is an α, β - unsaturated ketone and clearly, the equilibrium is shifted towards the right i.e. pyridone – 2.

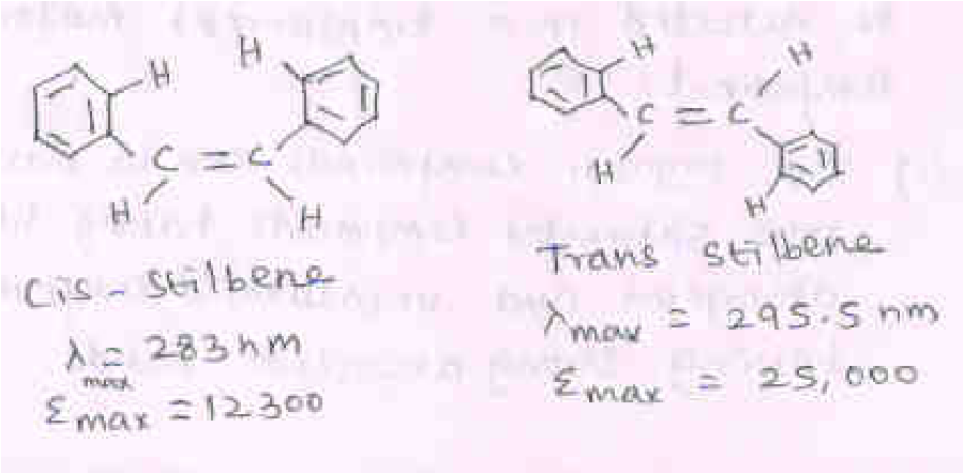
7) **Identification of a compound in different solvents:**

Sometimes, the structure of the compound changes with the change in the solvent.

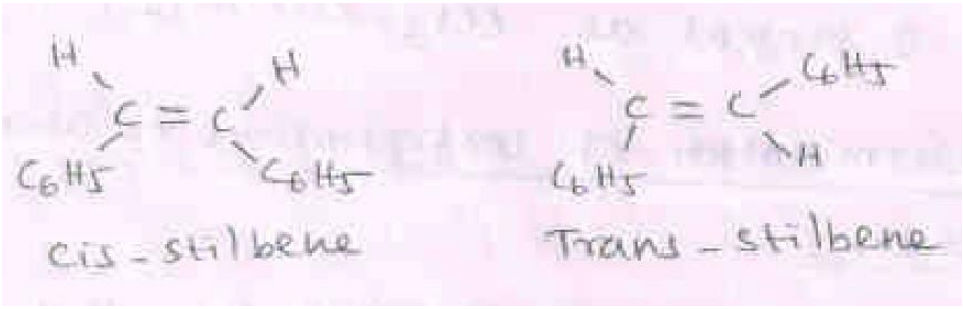
Chloral hydrate shows an absorption maximum at 290mμ in hexane while the absorption disappears in the aqueous solution. Clearly, the compound contains a carbonyl group in hexane solution and its structure is $CCl_3.CHO.H_2O$ where as in aqueous solution, it is present as $CCl_3.CH(OH)_2$

8) **Determination of configuration of geometrical isomers:**

The results of absorption show that cis-alkenes absorb at different wave-lengths as compared to their corresponding trans-isomers. The distinction becomes possible when one of the isomers is forced to be non-coplanar by steric hindrance. Thus, cis forms suffer distortion and absorption occurs at lower wavelength. For example, consider the spectra of Cis and trans stilbenes.



Out of Cis – and trans – stilbenes, a distortion in coplanarity in cis – stilbene is due to steric hindrance. This results in lowering the value of absorption maximum at lower extinction co-efficient. Thus, a band which appears at 295mμ Emax 25000 in trans stilbene has a value of 283mμ Emax 12300 in cis – stilbene.



9) Detection of impurities:

UV absorptions spectroscopy is one of the best methods for detecting impurities in the organic compounds. The main reasons for the superiority of this method are:

1. The bands due to impurities are very intense. For example, an impurity having an amount of 0.05% has an E value of 2000. Therefore, such an impurity can be detected in a transparent major component.
2. The organic compounds can be classified into saturated compounds having little absorption and unsaturated compounds having strong absorption bands.

The common impurity in cyclohexane is benezene. Its presence can be easily detected by its absorption at 255nm.

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In nylon manufacture, the starting materials like adiponitrile and hexamethylenediamine should be very pure. If these starting materials are not pure, the nylon obtained will be of very poor quality. The purity of these materials can be tested by UV absorption spectroscopy. Traces of unsaturated and aromatic impurities can be detected because the starting materials are transparent in the near ultraviolet region.

If a compound is being purified, the process should be continued until band due to impurity disappears completely.