CHAPTER-II

EXPERIMENTAL WORK

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Cationic and anionic dyes when mixed together, in presence of a suitable solvent, form a dye complex salt. Various such dye complex salts have been reported by Kusabayshi et. al.[1] The study of such dye complex salts formed between cationic dyes like malachite green (MG), Crystal Violet (CV), Methylene Blue (MB) and the anionic dyes like eosine (EO), enythrosine (ER), Rose Bengal (RB), Acid Rhodamine have been carried out to confirm molecular formula, by Seyewetz[2] and Plelet-Jolivet.[3] They have shown that the complex formed contains 2 moles of cationic part and one mole of anionic part.

In the present work, it was proposed to study effect of change of concentration and also solvent, on the absorption spectra of dye complex salt formed between Malachite Green (MG) and Rose Bengal (RB), which has been shown to be represented by molecular formula $MG_2 . RB$.[1]

The work was mainly conducted on following lines.

a) Purification of solvents and the dyes used i.e. malachite green and rose bengal.

b) Preparation of dye complex salt.

c) Preparation of sample solution for recording the absorption spectra.

d) Recording of Spectra.

2.1 : PURIFICATION OF SOLVENTS :-

The solvents selected were alcohols namely, methyl alcohol, ethyl alcohol, n-propyl alcohol, n-butyl alcohol, iso-propyl alcohol, t-butyl alcohol and iso-butyl alcohol.

All the solvents used were of SD's make A.R. grade solvents, except ethyl alcohol. Commercial absolute alcohol was used as ethyl alcohol. All the solvents were purified by fractional distillation method[4] and purity was confirmed as per specifications given in the `Indian Pharmacopia.[5] Complete glass distillation assembly was used for fractional distillation. The distilled water used, was double distilled.

2.2 : DISTILLATION PROCESS :-

METHYL ALCOHOL (METHANOL) :-

Methyl alcohol was first distilled and the fraction distilling in the range between 64.5^{o} C to 65.5^{o} C was collected. The fraction so collected was redistilled, by standing over type - 4A molecular sieve. The term molecular sieve applies to a group of dehydrated synthetic sodium and calcium aluminosilicate adsorbents called as eolite made by Union Carbide Company, U.S.A., incorporating uniformly sized holes or pores, which are able to accept the molecules smaller than the limiting dimension, the larger molecules do not diffuse into the lattice structure. The water molecules diffused into the pores are retained by adsorption. This redistilled methyl alcohol was further tested for purity. The methyl alcohol is generally associated with acetone and acid. Following method was used to confirm the absence of acidity and acetone.

a) Acidity -

A mixture of 5 ml purified methyl alcohol and 5 ml fresh distilled water was titrated against N/100 NaOH solution using phenolphthalein as indicator. Not more that 0.1 ml of N/100 NaOH was required for colour change colourless to pink, which indicated absence of any acid.

b) Acetone -

1 ml purified methyl alcohol was mixed with 19 ml distilled water and 2 ml of 1 % w/v solution of 2-nitrobenzaldehyde in 50 % ehtanol. To this mixture 1 ml 30 % w/v solution of NaOH was added and it was allowed to stand in dark for 15 minutes. No colour was developed, which indicated any absence of acetone.

ETHYL - ALCOHOL (ETHANOL)

Commercial absolute alcohol was first distilled by using complete glass distillation assembly. The fraction distilling in the range from 77.5° C to 78.5° C was collected. This distilled absolute alcohol was refluxed with freshly ignited and cooled calcium oxide for six hours, using double surface condenser and anhydrous calcium chloride guard tube. Then the mixture was kept overnight. The condenser was reassembled for downward distillation via splash head adaptor and connected to receiving flask, using $CaCl_2$ guard tube. Discarding first 20 ml. of distillate, fraction distilling in the range from 77.5° C to 78.5° C was collected. This was further purified by Lund and Bjerrum[4] method for preparation of super dry alcohol. The purified alcohol was further tested for absence of aldehyde by following method[5]

Test for Aldehyde -

25 ml of purified alcohol was taken in flask and 75 ml of dinitrophenyl hydrazine was added to it. The mixture was heated on a water bath for 24 hrs. The alcohol was removed by distillation and the remaining portion of reaction mixture was diluted to 200 ml using 2 % v/v solution of H_2SO_4. On keeping the solution for 24 hrs, no crystals were produced, which indicated the absence of aldehyde.

n - PROPYL ALCOHOL (1-PROPANOL) -

n-propyl alcohol was first fractionally distilled, twice using anhydrous K_2CO_3 as drying agent in complete glass distillation unit. The fraction distilling in the range from 96.5° C to 97.5° C was collected. The distilled alcohol was redistilled by Lund and Bejrrum 4 method for preperation of super dry alcohol. Discarding first 20 ml of distillate, the fraction distilling in the range from 96.5°C to 97.5°C was collected and used.

ISO-PROPYL ALCOHOL (2-PROPANOL) -

Iso-propyl alcohol was first subjected to removal of peroxide and further purified by following method.

a) Removal of peroxide -

Iso-propyl alcohol generally contains peroxide as impurity and hence it is essential to remove it before use.

The alcohol was first refluxed for an half hour with Tin (II) chloride in the proportion of 1.5 % (15 g. per lit of alcohol). Then the cooled portion was tested for presence of peroxide.

0.5 ml of 2-propanol was mixed with 1 ml 10 % KI solution, 0.5 ml dilute (1:5) HCl and one drop of starch solution. No blue colour was developed within one minute, which indicated the absence of peroxide.

b) Distillation of alcohol -

The alcohol free from peroxide was then refluxed for 4 hrs with calcium oxide and then fractionally distilled. The fraction distilling in the range from 82°C to 83°C was collected, after dischording first 20 ml of distillate. This was again redistilled.

Distillation of Higher Alcohols :-

The other alcohols used were, n-butyl alcohol, iso-butyl alcohol, sec.-butyl alcohol and t-butyl alcohol. They were purified by drying with unhydrous K_2CO_3 and fractional distillation. After discarding first 20 ml of distillate, the fractions of respective alcohol were collected within the temperature range given below and further redistilled.

a) n-Butyl alcohol (1-butanol) : 116.5° C to 118° C

b) Sec-Butyl alcohol (2-butanol) : 99° C to 100° C

C) iso-Butyl alcohol (2-methyl,Propan-1-Ol) : 106.5° TO 107.5°

d) t-Butyl alcohol (2-Methyl, propan-2-ol) : 81.5°C to 82.5°

2.3 : PURIFICATION OF DYES

SD's make certified grade malachite green and A.R. grade rose bengal were used for experimental work. Both the dyes were purified by recrystallization from pure absolute alcohol, twice before use. The purity was confirmed by melting point determination and absorption spectra of the aqueous dye solution.

The melting range for malachite green was found to be 112^{o} - 114^{o} C.

The λ max for aqueous solution was observed at 423 nm. and 618 nm. These observations are in close agreement with those reported earlier [6] which are 420 nm and 618 nm.

For rose bengal, absorption spectra of aqueous solution gave absorption peak at 544 nm.

These observations are in close agreement with those reported by Gurr [6], which is shown to be at 545 nm in aqueous solution.

2.4 : PREPARATION OF DYE COMPLEX SALT :-

The dye complex salt between malachite green and rose bengal was prepared by the method given by Kusabayashi et. al.[1]

In the preparation of dye complex salt double distilled water was used. 100 ml of 0.02 M aqueous solution of malachite green was mixed with 100 ml of 0.01 M aqueous solution of rose bengal. The mixture was stirred for half an hr., using magnetic stirrer. After half an hr. the dye complex salt with purple colour was seperated in the form of precipitate. The precipitate was filtered, washed with double distilled water, till colourless filtrate was obtained. It was dried at 100°C and then cooled and recrystallized twice from pure absolute alcohol. The purity was confirmed by determination of melting point. The observed melting point 155°C is in well agreement with that reported by Kusabayashi et. al.[1] The composition of complex salt has been suggested as MG_2 R.B. by them.[1]

2.5 : PREPARATION OF STOCK SOLUTION :-

A stock solution of MG_2 R.B. complex was prepared with a concentration of 2×10^{-3} M, by dissolving 0.3421 gms. of complex (2×10^{-4}M) in 100 ml of alcohol under investigation. For each type of alcohol, under study, this stock solution was prepared and calculated volume of this solution was used for further experimental work.

2.6 : RECORDING OF SPECTRA :-

The absorption spectra of samples were recorded on Shimadzu, UV-160 and Hitachi-300, UV/VIS/NIR - Spectrophotometers. the wave length range selected for the study was from 400 nm to 800 nm. In order to minimize the experimental errors following precaution were taken.

i) The final volume of sample solution was kept at 10 ml for all the samples prepared for recording.

ii) The volume of stock solution required was taken by automatic delivery pipette and same pipette was used throughout the experiment.

iii) All the 10 ml volumetric flasks were calibrated before use with one 10 ml corning A - class pipette.

iv) The instrumental parameters were selected so as to get maximum absorption and similar absorption spectra of same sample, on both the instruments, and the parameters selected were kept constant throughout the experiment.

v) The path length of the absorbing media was same for both the instruments.

2.7 : STUDY OF EFFECT OF CHANGE OF CONCENTRATION ON ABSORPTION SPECTRA

The concentration range selected for study was 4 x 10^{-5}M to 4 x 10^{-4}M. For each alcohol solution, absorption spectra of dye complex salt were recorded in this concentration range. The calculated volume of 2 x 10^{-3}M stock solution in respective alcohol was diluted with same alcohol to a final volume of 10 ml. The absorption spectra of all solutions were recorded under similar conditions of instrumental parameters and at 30°C temperuture, in the wave length range from 400 nm to 800nm.

2.8 : STUDY OF THE EFFECT OF CHANGE OF SOLVENT :-

To study the effect of solvent change on absorption spectra, calculated volume of 2 x 10^{-3}M stock solution of dye complex salt in respective alcohol was diluted to 10 ml using respective alcohol. The absorption spectra of each sample was recorded under similar conditions of instrumental parameters, at 30°C temperature and between wavelength range of 400 nm - 800 nm.

2.9 : INSTRUMENTS

For recording absorption spectra of dye complex salt solution, Shimadzu-UV-160 and Hitachi - 300 (UV/VIS/NIR) spectrophotometers were used. The working principles and the recording facilities available are same for both the instruments, with some minor differences like printing, absorption scale range ..... etc.

SHIMADZU-UV-160-SPECTROPHOTOMETER :-

The main features of this instrument are as below.

i) It is provided with a wavelength drive system with CPU - control using sine bar to realize high speed wave - length scanning.

ii) It is all in one type spectrophotometer with CRT and printer incarporated in the unit. This gives absorption spectra sample along with a print of

λ max values for peaks and valleyes.

iii) Back-up mode parameters are provided so as to enable single action operation.

iv) Easy data processing for obtained spectrum is available by conversation with CRT.

The photometric system consists of a deuterium lamp and tungston, halogen lamp as a source of light to cover UV and visible range from 200 nm to 1100 nm.

The light emitted by the light source either beuterium lamp or halogen lamp is reflected by a mirror and falls into the monochromator slit. The monochromator used is a 900 line/mm aberration - corrected concave halographic grating which allows to realize a monochromator of simple construction and little aberration. The beam from monochromator passes sequentially through slit, stray light rejection filter, mirror and then falls on beam splitter mirror to split into two beams. One of the beams passes through sample cell and other through reference cell, to fall into detectors which are photodiodes and the signals are fed to CRT printer.

The microcomputor (CPU) plays the central role of operation, controlling lamp lighting, lamp charging, filter charging, wavelength scan, CRT displya, key board, printer and other components. The printer used is, Fujitsu's - 280 dot parellel head printer.

The signals from sample side beam and reference side beam are received by two separate photodiode detector and both signals are logarithmically converted and difference is calculated by difference amplifier. The instrument can be operated within wave length range from 200 nm to 1100 nm and absorption range with optical density range from -2.5 to +2.5 arbitrary units. The recording rates can be set only either at fast rate or slow rate.

In present experimental work following operating conditions were used.

i) Slit width : 2 nm
ii) Wave length range : 400nm to 800nm
iii) Scan speed : slow
iv) Absorption range : -2.5 to +2.5 Abs.
v) Recording mode : SEQ (continuous)

HITACHI-330-UV/VIS/NIR-SPECTROPHOTOMETER :-

The working principles, optical systems and the recording facilities are almost similar to those in Shimadzu-UV-160 spectrophotometer. Only difference is that the detector used is photomultipier tube instead of photodiode and it can print the absorption spectra only but it cannot gives print out of λmax. values. However, it has some more advantages as

1) The wave-length range is wide, from 185 nm to 2600 nm., with UV/VIS recording between 185 nm to 878 nm and

NIR recording between 878 nm to 2600 nm.

2) The absorption range is from 0-6, optical density, in arbitrary units.

3) The wave length accuracy is ± 0.2 nm and wave length repeatability is ±0.1 nm.

4) The scan speed can be adjusted from 0.25, 0.50, 0.75, ... 100 nm per minute, in 1003 steps and wavelength expansion scale can be adjusted from 01, 02, ...200 nm per cm.

5) The scan speed is adjustable from 60nm/min. onwards, with a chart format of 20nm/cm.

6) The slit width can be automatically adjusted in the range from 0.1 to 0.6 nm for UV/VIS region.

7) The photometric accuracy is very high as +0.002 for 0-0.5 Abs and ± 0.004 for 0.5-1 Abs.

Following operating conditions were selected for recording absorption spectra of samples under study, for Hitachi-300 spectrophotometer. It was observed that, under these operating conditions, for the same solution, the absorption spectra recorded on Hitachi spectrophotometer are matching with those recorded on Shimadzu, UV-160 spectrophotometer with respect to λ max values and absorption intensities.

1) Slit : 2nm

2) Wavelength range : 400nm to 800nm

3) Scan speed : 60nm/min.

4) Absorption Scale : 0 - 1 Abs.

5) Chart format : 20nm/cm.

6) Recording mode : SEQ (continuous)

7) Response : 1 Sec.

8) Lamp Select : 350 Auto

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