CHAPTER-3

EXPERIMENTAL
3. EXPERIMENTAL

Experimental section is divided into two parts:

1. Extraction of Natural Dye
2. Synthesis of Indigo using Microwave irradiation

Section I includes the selection of dyes on the basis of color and methods to extract these dyes from the dye source. Section II describes the synthesis of indigo and derivatives of indigo using microwave irradiation.

Extraction of Natural Dyes

The dyes selected in this study and their extraction from their sources are given as follows:

3.1 DYES

1. Sappan Wood

   Family: Caesalpinia
   Parts Used: Wood

Sappan Wood is commonly called as ‘Patang Wood’. The wood is orange red, hard, very heavy (wt. 1073 Kg/m³, air dry), Straight-grained with a fine and even texture. It has tendency to split and warp. The wood is reported to contain a glucoside containing β-amyrin, glucose and three amino acids, alanine, aspartic acid, glycine, proline, valine, leucine, threonin. Free sugars like lactose, galactose, 2-deoxyribose and glucose are present. The wood has the inner part, the lumen filled with the dye substance along with tannin, tannic acid, gallic acid and a
special flavonoid called Brasiline. The coloring matter Brasiline \((C_{16}H_{14}O_5)\) is nearly colorless and freely soluble in water, alcohol and ether, gets oxidised to Brasilein \((C_{16}H_{12}O_5)\), Brasiline gives red rose color with orange fluorescence in water. On exposure to air, Brasiline quickly absorbs oxygen and gets converted to Brasilein which is brown in color. The former is obtained as minute dark crystals, which have a metallic, grey lusture on drying. When crushed they become brown red powder. This powder is freely soluble in hot water. The solution has a pale orange yellowish pink color. Further oxidation breaks the Brasiline into inactive \(\beta\)-resorclyc acid \((2,4\text{-dihydroxy benzene-1-carboxylic acid})\) and 3,7-dihydroxy-chromone.

\[
\text{Brasiline} \rightarrow \text{Brasilein}
\]

The wood also contains several aromatic compounds apart from Brasiline, they are sappanchalcone, caesalpin \(P\) \((C_{16}H_{12}O_5)\), protosappanin B and eight other compounds, which are biosynthetically related to Brasiline. It also contains three new homo-isoflanonoids viz. 7-hydroxy-3-(4-hydroxybenzylidine)-chroman-4-one, 3,7-dihydroxy-
3(4-hydroxybenzyl)-chroman-4-one, and 3,4,7-trihydroxy-3(4-hydroxybenzyl)-chroman, sappanol and episappanol. All these compounds are very closely related, but Brasiline, is the main colorant in the wood needed for the dyeing property.

**Source:** Sappan wood was procured Alps Industries, Ghaziabad.

### 2. Eucalyptus

**Family:** Myrtaceae  
**Part used:** Bark

Eucalyptus grows on desert, swampland, valleys and mountains. This genus, which includes 450 species, forms the preponderant vegetation and most valuable timber producing trees. Eucalyptus trees characterized by their leathery, whitish leaves, their ragged bark, shredded periodically and have a peculiar aroma.

The important compounds found in Eucalyptus bark are eridictyol, naringerin, quercetin, rhamnazin, rhamnetin, taxifolin apart from tannins of which some are colorant.
Source: The Eucalyptus barks were collected from the premises of Indian Institute of technology Kanpur, because of easy availability.

3. Cineraria

Family: Asteriaceae

Part Used: Flower

Cineraria is a seasonal flower. Cineraria has conspicuous daisy like flower, about 2- inches, are held above large dark green foliage. Flower colors include pink, magenta, purple, white, dark blue and bicolor. These potted annuals like bright, light and cool temperature, with soil consistently moist, but not water logged to prolong bloom. These are herbaceous perennials that are often grown as annuals. There are three basic types: the large flowered, the dwarf and the intermediate strains growing 1 foot tall and having a compact, free flowing growth. They all come in wide range of colors. There are two main types of the tall and small flowered cinerarias. The most popular is the star flowered, which produces an abundance of small flowers in blue, pink,
salmon, white and other hues. We have chosen deep purple variety of cinerarias for our study.

Cineraria flower were collected during the flowering season, i.e. from February- April and used immediately.

Source: The cineraria flowers were collected from the premises of IIT Kanpur, because of easy availability and great abundance as potted and as garden plant.

4. **Canna**

Family: Cannaceae

Part used: Flower

Canna belongs to the family Cannaceae, it is half hardy summer rhizome. Canna is enjoying resurgence in popularity as a prime summer garden plant. They are divided into three groups: tall green leveled and dwarf varieties. The blooms are upto 5 inches (12.5 cm) and leaves are very decorative. Canna flower blooms longer if dead flowers are removed. This plant grows in full sun in a rich amended soil. Require ample of watering and fertilizer for full bloom. All the hybrid varieties can be grown in normal garden situations and gives ample flower yielding good amount of natural colorant. The other advantage of the canna plant is that it is self-propagating.
The flower chosen for the study is of the deep red color variety, this is a hybrid grown from a very complex breed that involves several species.

**Source:** Canna flowers were collected from the premises of IIT Kanpur, because of easy availability and great abundance.

5. **Lac Dye**

Lac is a material of animal origin being secreted by a tiny insect Kema lacca (Kerr), which thrives on certain host plants such as Pallas, Kusum or Ber.

Lac contains water-soluble red dye laccic acid, alkali and spirit soluble yellow dye, erythrolaccin. Laccaic acid has a basic structure of anthraquinoid type. It is a mixture of 5 closely related compounds of anthraquinone origin.

![Laccaic Acid Structure]

Laccaic acid-

- A \((R=CH_2NHCOCH_3)\)
- B \((R=CH_2OH)\)
- C \((R=CH(NH_2)COOH)\)
- E \((R=CH_2NH_2)\)
The general formula of Laccaic acid is $C_{262}H_{19-26}N_{0-1}O_{12}$. The structure elucidated for Laccaic acid A, C and E show the presence of one nitrogen atom. Laccaic acid C is an amino acid derivative, while laccaic acid-D is a carboxylic acid derivative. Laccaic acid B is an alcoholic derivative. The 3-hydroxy group on the anthraquinoid skeleton is responsible for chelation with the metal ion of the mordant. Actually Laccaic acid, which is responsible for dyeing, is a hydroxy anthraquinone carboxylic acid.

Lac dye is acidic in nature and is generally present as its sodium potassium salts, which are completely soluble in cold water, on
the other hand pure dye is soluble in boiling water. Pretreatment of the fabric with acetic acid and formic acid makes lac dye and fabric's compatibility very good. Lac dye has no affinity for attachment. Thus can be used as mordant dye with prior treatment to fabric.

**Source:** Lac dye was procured from Indian Lac Research Institute, Namkum, Ranchi.

### 3.2 CHEMICALS AND REAGENTS USED

- Methanol, L. R. grade (Sd- fine-chemicals ltd.)
- Acetic acid, L.R. grade (Qualigens)
- Sodium Chloride, L.R. grade (Qualigens)
- Alum, L.R. grade (Qualigens)
- Borax, L.R. grade (Nice chemicals)
- Ferrous sulphate, L.R. grade (Nice chemicals)
- Stannous chloride, L.R. grade (Nice chemicals)
- Stannic Chloride, A.R. grade (Spectrochem)
- Tannic Acid, L.R. grade (Nice chemicals)
- Silica gel for chromatography (Acme), For column silica gel used was of 100-200 mesh.

Distilled water was used in all the process.
3.3 METHODS OF EXTRACTION

Dyes were extracted by four methods:

i) Aqueous extraction:
ii) Solvent extraction
iii) Sonicator extraction
iv) Supercritical fluid extraction

3.3.1 Aqueous Extraction: To obtain the dye in aqueous extract the wood shavings or flowers are crushed and dipped into water. Overnight soaking gives the desired shade. Dye from Sappan wood, Cineraria, Canna and Eucalyptus were obtained with this method.
In case of Eucalyptus, however a little modification of dye preparation was carried out, dye was prepared by boiling bark sample (5-10gms) with water (100ml) for an hour. After that it was left to cool down. This solution was evaporated to dryness in an oven and used for dyeing later on or the solution was used for dyeing immediately. The dried dye has long shelf life, does not show any deterioration in color content even after several months.

3.3.2 Solvent Extraction: In the case of Sappan wood, soxhlet was used to extract the dye in organic solvent. The solvent used was methanol. Wood shavings were refluxed in methanol till it discharged color. The process took 5-6 hours. The decoction was used immediately as the Sappan wood has the tendency to oxidize. This is the only example of soxhlet extraction in our study.

3.3.3 Sonicator Extraction: Sonicator was used for the first time for the extraction of dye. We used this for the Sappan wood and Cineraria flower. The dye extraction took only one hour.

The sonicator used is of 20KHz frequency, which is found to be suitable for introducing cavitation. High-energy ultrasonic wave causes cavitation. When bath is irradiated with high-energy ultrasonic cavitation occurs which releases considerable amount of energy and collapse of the bubbles. This increases with the surface tension at the bubble interface and decreases with the vapor pressure of the liquid. Since the aqueous extraction bath has water, which has comparatively
high surface tension, it is very effective medium for cavitation. Interestingly cavitation in alcohol solution (methanolic extract of dye in our case) is considerably high because of increase in vapor pressure. Thus the dye is extracted with least amount of exposure to air in a much efficient manner.

**Extraction of lac Dye:** Lac dye is extracted from crude raw material, stic lac, which is harvested from the Lac host plants. Indian Lac Research Institute, Ranchi has developed a special technique to recover lac dye. Even Indian Standard specification IS: 12921-1990 has been made available by BIS.

0.5 gm of powdered lac dye was weighed in 100ml beaker. To it 50 ml of distilled water was added and heated on a water bath up to 90°C for 10 minutes. The clear solution was decanted into a measuring flask. 50ml of distilled water was added again followed by heating on water bath at same temperature. The clear solution was decanted. This procedure is repeated till whole of the dye goes into solution. The solution was allowed to cool down at room temperature. The solution was filtered; stock prepared with this solution can be stored for at least four days.

3.3.4 **Supercritical extraction:** Supercritical fluid extraction (SCFE) is a further advancement over the extraction method making significant step over the use of conventional solvent extraction technology because SFE uses CO₂ as the bulk extraction media, it has an advantage that CO₂ is non-hazardous,
significantly less hazardous chemicals are used in the extraction process and minimal waste is generated in its application.

SUPERCRITICAL CO$_2$ EXTRACTOR
SCFE is a two step process, which uses a dense gas as solvent usually carbon dioxide above its critical temperature (31°C) and critical pressure (74 bar) for extraction. The natural product is powered and charged into the extractor. Carbon dioxide is introduced to the extractor through a high-pressure pump (100-350 bar). The extract charged carbon dioxide is sent to a separator (60-120 bar) via a pressure reduction value. At reduced temperature and pressure conditions the extract precipitates out in the separator. The extract free carbon dioxide stream is recycled for effective extraction of all the dye material from the natural product.

Supercritical Fluid Extraction is superior over the traditional solvent extraction of natural dyes because it uses a clean, safe, inexpensive, nonflammable, nontoxic, environmentally friendly, nonpolluting solvent-carbon dioxide (CO₂). Secondly, the energy costs associated with SCFE are lower than the conventional techniques.

Indian Institute of Technology Powai, Mumbai, has provided the facility for this extraction.

3.4 EQUIPMENT USED FOR ANALYSIS AND THEIR PRINCIPLE

1) Microwave:
Model Make: MIS 1200 mega, Milestone

Microwaves are waves of energy, not heat. Microwave exists between IR radiation and radio waves on the electromagnetic spectrum (a frequency range of 300-300,00MHz). They consist of an electric field and a magnetic field perpendicular to the electric field. The electric
field is what is responsible for energy transfer between the microwave source and the object being irradiated.

Microwave travel directly to the solution. In a microwave oven, a magnetron converts ordinary electrical energy into microwaves, which are electromagnetic waves, just like radio and TV waves, but have shorter wavelength and higher frequency. The microwaves enter the oven cavity through a wave-guide. A fan like device called the field stirrer or microwave stirrer helps distribute the microwaves evenly throughout the oven chamber.

The amount of microwave energy absorbed by a given specimen depends upon many factors. Among these are the size of the load, its orientation with respect to the waves, and the dielectric and the thermal properties of the material.

Microwave energy can affect molecules in two ways. The first effect is on dipole rotation. When microwave energy passes through a sample, the molecules of the sample having dipole moments will try to align themselves with it. The more polar the compound, the stronger the interaction with the electric field. The amount of energy transferred, the loss tangent, is a function of both the dipole moment and the dielectric constant, but this is not a linear function. The energy transfer is more efficient when the molecules are able to relax quickly, the most efficient transfer occurs when the relaxation time matches the frequency of the microwave energy. Large molecules, such as polymers, take longer to relax and receive energy. However, once the temperature of a polymer increases, it can absorb more energy
efficiently. Conversely, small molecules can relax quickly than the resonating microwave energy. As small molecules (such as water) absorb microwave energy, they move farther away from the resonance frequency and absorb less energy as they heat.

The second effect is ionic conduction. In the presence of an electric field, ionic species will migrate in one direction or the other depending on the electric field. During this migration, energy is transferred from the electric fields causing ionic interaction that speed up the heating of a solution. Ionic conduction increases with the temperature allowing ionic solution to become stronger absorbers of microwave energy as they are heated. It is this principle, which is used in the synthesis of organic molecule.

2) **Sonicator:**  

   **Model Make:** Julabo-SRO5  

The idea of utilizing ultrasound energy in dyeing cotton with direct and reactive dyes is not new one, however it has been used for the first time for the natural dyes by us. The sonicator used is of 20KHz frequency which is found to be suitable for introducing cavitation. High energy ultrasonic waves cause cavitation. When dye bath is irradiated with high energy ultrasonic cavitation occurs which releases considerable amount of energy and collapse of the bubbles. This increases with the surface tension at the bubble interface and decreases with the vapor pressure of the liquid. Since the aqueous dye bath has water, which has comparatively high surface tension, it is very effective medium for
cavitation. Interestingly cavitation in alcohol solution (methanolic extract of dye in our case) is considerably high because of increase in vapor pressure. Here the later plays a dominant role. We thus were interested in looking into this aspect of dyeing cotton.

3) Ultraviolet and Visible Absorption Spectroscopy:

Model Make: Perkin Elmer, Lambda 400

Ultraviolet and Visible (UV-VIS) absorption spectroscopy is the measurement of the attenuation of a beam of light after it passes through a sample or after reflection from a sample surface. Absorption measurement can be at single wavelength or over an extended spectral range. Ultraviolet and visible light are energetic enough to promote outer electrons to higher energy levels, and UV-Vis spectroscopy is usually applied to molecules or inorganic complexes in solution. The concentration of an analyte in solution can be determined by measuring the absorbance at some wavelength and applying Beer-Lambert law.

The Beer-Lambert law is the linear relationship between absorbance and concentration of an absorbing species. The general Beer Lambert law is usually written as

\[ A = a \cdot \lambda \cdot b \cdot c \]

Where A is the measured absorbance, a(\lambda) is a wavelength-dependent absorptivity coefficient, b is the path length, and c is the analyte
concentration. When working in concentration units of molarity, the Beer-Lambert law is written as:

$$A = e \cdot b \cdot c$$

Where $e$ is the wavelength-dependent molar absorptivity coefficient with units of $\text{M}^{-1} \text{cm}^{-1}$. Strong absorptions in molecules will have $e$ values of $10^4$ to $10^5 \text{M}^{-1} \text{cm}^{-1}$.

Since the UV-Vis range spans the range of human visual capacity of approximately 200-750nm, UV-Vis spectroscopy is useful to characterize the absorption, transmission and reflectivity of a variety of technological important materials.

4) **Fourier Transform Infra Red (IR) Spectroscopy:**

**Model make:** Bruker, Vector 22

The IR absorption spectroscopy is based on the absorption of infra red radiation by molecules and is most widely used for the identification of the organic compounds. The atoms in molecules vibrate constantly in a variety of stretching and bending motions. The different types of motion are called vibrational modes. Atoms that are connected by covalent bonds can stretch or bend at natural resonance frequencies, which depend on the strength or stiffness of the bonds.

$$\begin{align*}
\text{C-C} & \quad 1200 \text{cm}^{-1} \\
\text{C=O} & \quad 1650 \text{cm}^{-1} \\
\text{C≡C} & \quad 2150 \text{cm}^{-1}
\end{align*}$$

The double and triple bonds are stronger than a single bond and have correspondingly higher energies of vibration. Similarly, stretching
modes have higher energies than bending modes for the same atoms. These vibrational modes can be excited to higher energy states, which cause the atoms to vibrate with greater amplitude that is a greater displacement from their average position. Vibrations can be excited by increasing the temperature or by absorption of photons of the appropriate energy. The energies of the vibrational modes are quantised, can be excited only with discrete amounts of energy. A photon that has the same energy as vibration is said to be in resonance with that vibration and can be absorbed.

Infrared radiation, typically 4,000-400 cm⁻¹, is in the energy range that can excite molecular vibrations to higher vibrational energy levels. When an IR photon of the same energy as a vibrational mode passes by a molecule, the molecule can absorb that IR photon. The energy of the photon is converted to a greater vibrational amplitude. Eventually this energy is transferred to the surroundings, e.g., by collision with solvent molecules, resulting in an increase in the temperature of the sample.

Since different functional groups in molecule vibrate at distinctly different frequencies, IR spectra are useful for qualitative identification of molecular compounds.
5) **Gas chromatography-Mass spectra:**

*Model make:* Finnigan, Fison

Gas chromatography is a process by which a mixture is separated into its constituents by moving gas phase passing over a stationary sorbent. The technique is thus similar to liquid-liquid chromatography except that the mobile liquid phase is replaced by a moving gas phase. Gas chromatography is divided into two major categories: gas liquid chromatography (GLC), where a separation occurs by partitioning a sample between a mobile gas phase and a thin layer of non volatile liquid coated on an inert support, and gas-solid chromatography (GSC), which employs a solid of large surface area as the stationary phase.

GC-MS has a mass detector attached to it, which measures the fragmentation peaks of the particular compound as they elute in the column. Helium is the best carrier gas to use in conjunction with this type of detector since its thermal conductivity is much higher than any other gas; on safety grounds as well helium is preferred because of its inertness.

Facility for all the above mentioned equipment was availed from FEAT (Facility for Ecological and Analytical Testing), I.I.T. Kanpur.

6) **Xenoster:**

This is used to test the light fastness of the dyed fabric.

7) **Wash Wheel:**

*Model Make:* Thermolab
This is used to test the washing fastness of the dyed fabric.

8) **Perspirometer:**
   
   Model Make: Sashmira
   
   This instrument is used for the testing of perspiration fastness of the dyed fabric.

9) **Crock meter:**
   
   Model Make: Ravindra Engg.
   
   This instrument is used for testing the rubbing fastness of the dyed fabric.
   
   All the testing of the fastness properties of the dyed fabrics were carried out at Textiles Committee, Kanpur.

### 3.5 PREPARATION OF FABRIC TO BE DYED

3.5.1 **Specification of the fabric:**

Fiber content: cotton

Weave: Plain weight per unit area

Fabric count per inches, warp:30

Weft: 20
3.5.2 **Physical characteristic of cotton:**

- Fiber length (mm) 15-50
- Elementry cell (mm) 5-50
- Degree of polymerization 2500-5000
- Diameter (um) 11-22
- Fineness (Denier) 1.2-3.5
- Specific gravity 1.54
- Elongation at break (%) 6-8
- Moisture Region (%) 8
- Absorption (%) 25-27
- Volume Swelling (%) 40

3.5.3 **Chemical composition of cotton fiber:**

- Cellulose 88.0 %
- Hemi-cellulose ---
- Lignin ----
- Waxes 0.5%
- Pechns 1.0%
- Mineral matter 1-2%
Preparation of fabric before dyeing:

Before dyeing the material must be free of impurity as far as possible. The fabric is soaked in mild soap solution and washed properly so that no starch or very little starch is left on the cloth. It is dried. It is then treated with 0.4% (owf) tannic acid. The fabric should be dipped in tannic acid for at least 4-5 hours. This cloth is now used for dyeing depending up on the type of mordanting that has to be subsequently carried on.

3.5.4 Dyeing: As mentioned earlier while working with Natural Dyes, mordanting is mandatory. Mordanting of the textile material is to improve the color yield in the development of shade and to help fixation of the colorant molecule to the substrate as a link, in effect, mordanting also improves the fastness during subsequent processing, shades and the final product. There are three types of mordanting:

- **Pre-mordanting:** Cloth is treated with mordant (4% owf) and kept on water bath at 50°C for one hour before dyeing.
- **Post-mordanting:** Cloth is treated with mordant after dyeing.
- **Simultaneous Mordanting:** Both dye and mordant are used in the same bath at the same temperature together.
There is a separate method for the lac dyeing provided by the BIS, according to this method 2% of the dye solution is prepared by boiling the dye with water (100 Parts) and Borax (1part) and filtered through cloth. The dye solution is mixed with common salt solution and the solution is made just acidic with few drops of acetic acid. The pH should be between 6.0-6.5. The fabric is dipped in the sonicator and is run for one hour. Mordant (2%) is added to obtain desired shades. Although for synthetic fiber the pH recommended is 4.5 but since we wanted to dye cotton also in the same dye bath after terycot fabric dyeing, we kept the pH at 6.0-6.5. Acidic pH is maintained throughout the process of dyeing as alkaline pH causes decomposition of the lac dye.
Synthesis of Indigo using Microwave irradiation

This section includes synthesis of indigo and its derivatives using microwave irradiation. We have synthesized two important Indigoid dyes in the laboratory by using Microwave energy. This novel transformation offers environmentally clean methodology with very high yield. The reaction time has been brought down from hours to minute. The reaction temperature is lower when microwave irradiation is used as compared to the conventional method.

CHEMICALS AND REAGENTS USED

- Acetone, L.R. grade (S.d. Fine chemicals)
- Sodium hydroxide, L.R. grade (S.d. Fine chemicals)
- Nitrobenzaldehyde, A.R grade (Nice chemicals)
- Benzoyl Chloride, A.R grade (Nice chemicals)
- Nitrobenzene, A.R grade (Nice chemicals)
- Bromine, A.R grade (Nice chemicals)
- Copper powder (S.d. Fine chemicals)

PROCEDURE

**Indigo:** Acetone (2ml) and 1m NaOH (1ml) are sequentially added to ortho-nitrobenzaldehyde (1.208 gm, 8mmol) and the mixture is subjected to the Microwave irradiation a Teflon vessel at 80 watt power and 2.45 GHz for 10 minutes. 1ml of 1MNaOH was added, and
MW irradiation was carried for another 10 minutes. At the end of exposure to microwave, the reaction mixture was cooled to room temperature. The product was then left for air oxidation of the indoxyl moiety for 4 hours under basic conditions. The crude product was recrystallized from acetone to yield 77% of indigo. An identical mixture was refluxed for 40 hours using conventional heating (oil bath) and subjected to identical work up and purification to give 40% of the same product, some polar product was also formed in the controlled experiment which could not be identified.

**Indigo Yellow 3G:** A mixture of Indigo (0.262 gm, 1m mol) and benzoyl chloride (0.181 gm, 2m mol) in nitrobenzene (2ml) in presence of copper catalyst (0.010 gm) was subjected to microwave irradiation a Teflon vessel at 80 watt power and 2.45 GHz for 10 minutes. Aqueous work up followed by extraction with ethyl acetate gave the product in 65% yield along with 20% starting material and 15% of a very polar compound was obtained. An identical mixture was refluxed for 36 hours using conventional heating (oil bath) and subjected to identical work-up and purification to give 45% of the same product and unreacted starting material was also recovered in the controlled experiment.

**Tyrian Blue:** A mixture of Indigo (0.262 gm, 1m mol) and bromine (0.11ml, 2mmol) in nitrobenzene was added drop wise and heated in microwave in a Teflon vessel at 80 watt power and 2.45 GHz for 10
minutes. Aqueous work up followed by extraction with ethyl acetate led to the 5,5' dibromindigo product obtained in 69% yield and the rest of the starting material was recovered. An identical mixture was refluxed for 20 hours using conventional heating (oil bath) and subjected to identical work up and purification to give 35% of the desired product along with tri and tetra brominated products in the controlled experiment.
Scheme 1