2. Materials and Methods

2.1. Materials

This chapter provides a detailed description of the materials and procedures used in the study. These include the sources of the raw materials required to prepare *Sāncipāt*, old *Sāncipāt* samples, the traditional methods of preparation and conservation of *Sāncipāt* and woodcarvings, traditional mineral and herbal pigments as well as all other raw-materials ingredients required for painting of woodcarvings. The chapter also describes the chemicals and analytical methods used in this study.

Sānci bark: *Sānci* bark was obtained from Mr. Debajit Gogoi, a *Sānci* grower from Golaghat, Assam, who grows *Sānci* tree as a crop for extracting a valuable perfume oil from its stem. The *Sānci* bark strips of about 2m long, 15cm wide and 3mm thick were collected from a 17-years old *Sānci* tree (Figure. 2.1).

Figure. 2.1: *Bholā Sānci* tree (a, b) *Bholā Sānci* tree after removal of bark (c, d).

Sāncipāt: A valuable collection of century-old *Sāncipāt* manuscripts was generously gifted by Mr. Rupam Kumar Sarma from Bam Beseria, Tezpur, Assam. The collection included both well-preserved and damaged manuscripts, which had suffered from insect, fungus, water, or humidity damage. These manuscripts were then utilized for a range of nondestructive and destructive experiments (Figure. 2.2).

Mineral and Herbal Pigments: Three mineral pigments, *viz.*, *Hengul* (cinnabar, vermillion), *Hāitāl* (yellow orpiment), *Khārimāti* (clay), *Tutia* (blue vitriol,

$CuSO_4.5H_2O$), and an herbal pigment, *viz.*, *Nīl* (Indigo) were obtained from Kamakhya Bhandar, Nagaon, Assam (Figure. 2.3). *Lā* (lac), a hardened natural resinous sap collected from some trees which is used for varnishing, was purchased from local market.

Figure. 2.2: (a) Some damaged manuscripts with another manuscript in good condition, (b) Devi Mahatmya (Markendeya Puran) Saka 1769, received from Mr. Rupam Kumar Sarma from Bam Beseria, Tezpur, Assam.

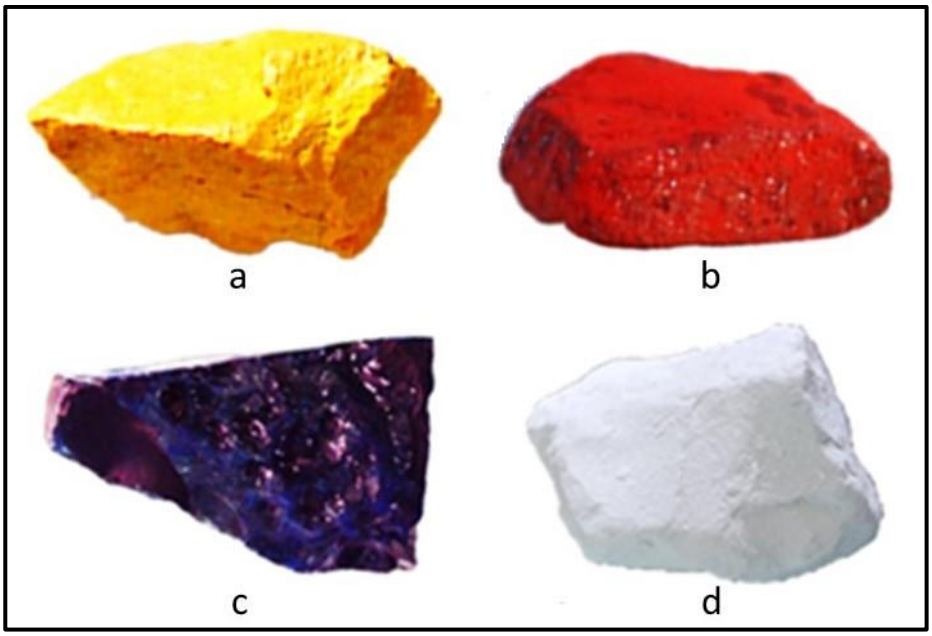

Figure. 2.3: Pieces of (a) *Hāitāl*, (b) *Hengul*, (c) Nīl and (d) Khārimāti.

Other natural materials: Seed of *Konibih* (*Croton tiglium*) and Ghila (a hard fruit with red color, Nicker bean, *Entada scandens*) were collected from local markets for the preparation of *Sāncipāt* (Figure. 1.2). Chalkuwari (Aloe-vera, *Aloe barbadensis*) was

collected from Tezpur University campus (Figure. 1.2). A thin coat of a fine paste of a skinned fatty pulse, called *Matimah* (*Phaseolus radiatus*) were collected from a local grocery shop. Sawdust required for restoration of woodcarvings was collected from the local timber industry.

Adhesive: Bael (wood apple) is collected from the university campus for extraction of bael gum used is conservation of *Sāncipāt* and preparation of different shades of pigments. Bael gum is reported to be a mixed polysaccharide containing 71% d-galactose, 12.5% l-arabinose, 6.5% l-rhamnose and 7% d-galacturonic acid [163]. A synthetic adhesive, Fevicol, a polysaccharide-based glue, obtained from local market was also used for restoring damaged woodcarving and for comparison purposes (Figure. 2.4).

Figure. 2.4: Bael (wood apple) collected from Tezpur University campus.

Reagents and Chemicals: Analytical grade isopropyl alcohol, Ethanol (absolute 99.9%), cetrimide and thymol (2-isopropyl-5-methyl phenol) were *Sigma-Aldrich products*. The chemicals, i.e., sodium hydroxide (NaOH) extra pure AR 98% (SRL), hydrogen peroxide, (H_2O_2) 30% (Emplura), hydrochloric acid (HCl) 35% (Emplura), sulphuric acid (H_2SO_4) 98% (Merck), polyvinyl alcohol (PVA) hot (GS Chemical Testing Lab and Allied Industries, M.W. 125000 $gmol^{-1}$), Zirconium Oxychloride Octahydrate extra pure ($ZrOCl_2.8H_2O$) 99.5% (SRL), Polyvinyl Alcohol Hot, Degree of Polymerization 1700-

1800 (GS Chemical Testing Lab & Allied Industries), Ethanol, absolute 99.9%, and distilled water were used as received without further purification.

Potato dextrose broth (PDB) and Agar were purchased from Himedia, Mumbai. Distilled water was used to prepare sodium hydroxide and copper sulphate solutions used in degumming process. Tap water was used in the preparation process of *Sāncipāt.* The base agar was created for an antibacterial investigation utilizing a product that was purchased from Merk, Mumbai. The culture medium for all strains and potato dextrose agar for fungal strains, both of which were obtained from Himedia Laboratories in Mumbai. As a positive control for the antibacterial test, 10 g/ml of antibiotic and an antimycotic solution was used, and 5g/mL of fluconazole was used for the antifungal test. From Mumbai's Himedia laboratories, both were purchased.

Solvent used: Double distilled and deionized (Milli-Q) water with pH 6.6, were used for all instrumental process. Other common solvents like molecular biology grade ethanol, supplied by Himedia Laboratories, Mumbai, isopropyl alcohol, AR grade, procured from *Sigma-Aldrich* were used without further purification. Sodium hydroxide (NaOH) extra pure AR 98% (SRL), hydrogen peroxide, (H_2O_2) 30% (Emplura), hydrochloric acid (HCl) 35% (Emplura), sulphuric acid (H_2SO_4) 98% (Merck), Laboratory grade tetrahydrofuran (THF) (RANKEN) were also used as solvent.

2.2. Methods

2.2.1. Preparation of Sāncipāt

Sāncipāt were prepared by both traditionally and simplified process. Traditional processes are already mentioned in the section 1.2.4.1. The simplified process is given below,

A 10-15-year-old, instead of a 15–20-year-old traditionally chosen, *Sānci* tree gives a thin *Sānci* bark which is suitable for making *Sāncipāt* manuscripts. The *Sānci* bark is cleaned and cut into convenient pieces in traditional way and then partially degummed by boiling in presence of Tutia for an hour instead of soaking in water overnight in presence of Tutia [8]. The pieces are then dried partially using hot-air oven and mild hot-pressing using an iron instead of sundried. A thin *Mātimāh* (fatty pulse) paste is then applied on the piece to smoothen the surface by filling any wrinkles or hair cracks present. It is then dried again using hot-air oven and mild hot-pressing [8]. Next, a thin coating of *Hāitāl* is applied on

the piece followed by drying and smoothening by hot-pressing to get a thin *Sāncipāt* which is ready for manuscript writing and illustration. The freshly prepared *Sāncipāt* was to be physically strong, smooth, and glazing. The samples were powdered and dried in desiccator for 7 days prior to physicochemical analysis.

2.2.2. Preparation of solutions

0.1 M Tutia ($CuSO_4$) solution: To make 0.1M Tutia solution, 0.1g $CuSO_4.5H_2O$ is added in 100 ml distil water.

5% (w/v) aqueous cetrimide: To make 5% w/v cetrimide solution, 5g cetrimide is added in 100ml H_2O for 2hours.

5% (w/v) thymol: To make 5% w/v thymol solution, 5g cetrimide is added in 100ml 1:20 ethanol-water solution.

5% w/v PVA: To make 5% w/v PVA solution, 5g PVA is added in 100ml H_2O.

3% v/v H_2O_2: To make 3% v/v H_2O_2 solution, 10ml of 30% H_2O_2 added in 90ml H_2O.

0.5 M NaOH: To make 0.5 M NaOH solution, 2g NaOH is added in 100ml H_2O.

2.2.3. Preparation of shades of pigments with binders

A recipe of preparation of *Hengul*, *Hāitāl*, *Kharimāti* and *Nīl* as per the traditional method is briefed here. About 30g of each of *Hengul* or *Hāitāl* is ground in a hard granite mortar manually into fine powder. About 50mL of water is added to the pigment powder and ground again for about half an hour. Then the mixture is allowed to settle down for about 10 min. The highly insoluble pigment powder is settled down with scum appearing at the surface. The scum is removed by decanting to get cleaner powder. The pigment is ground again after replenishing the water. The process of removing scum is repeated until the scum appears on grinding and the particle size is reduced to approximately 5-10 and 27-32 μm. In case of *Kharimāti* and *Nīl*, the grinding is easy, and the removal of scum is done only once.

About 40 fresh and matured wood apples were cut cross-sectionally into two halves with a knife and the glue was squeezed out from around the seeds and collected in a ceramic bowl. About 20 mL of distilled water is added to 80 mL of pure glue obtained from Bael fruit and thoroughly mixed to get 100 mL of the natural glue, Bael gum. Various shades have been prepared by mixing the pigments in varying ratios together with a certain quantities of Bel gum as a natural binder (NB) and water as shown in Table 2.1. A wheat-based synthetic polysaccharide glue is also used as a synthetic binder (SB) in the place of the natural binder for comparison.

Table 2.1: Ratio of volumes of the pigments, binder and water in mL mixed to prepare paints of various shades with their average (of at least four measurements) particle size measured in Hagman scale. (-) indicates absence of the respective component in a particular shade.

Color	*Hengul*	*Hāitāl*	*Nīl*	*Kharimāti*	Bael gum	Water	Particle size (μm)
Red	1	-	-	-	1	-	7
Yellow	-	1	-	-	1	-	10
Blue	-	1	2	-	6	-	4
White	-	-	-	4	1	8	5
Brown	1	8	-	-	12	-	22
Green	-	6	1	-	9	-	32
Black	4	-	1	-	12	-	31

2.2.4. Degumming

The *Sāncipāt* pieces were soaked in 500 ml water in a plastic tray in presence of 0.1M *Tutia* ($CuSO_4$), 10 crushed seeds of *Konibih* (*Croton Triglium*) and 30g of crushed leaves of *Chalkuwari* (*Aloe Barbadensis)* for 12h for partial degumming.

2.2.5. Antimicrobial assay

For testing antifungal activity of *Sānci* bark and *Sāncipāt*, three filamentous fungi, *viz.*, *Aspergillus niger*, *Candida albicans* and *Fusarium oxysporum*, were sub-cultured. A standard procedure was used for antifungal activity test on *Sānci* strip at four different stages of the preparation [164] . The *Sānci* manuscript was cut into small pieces having the equal surface area for the experiment. For preparation of potato dextrose broth (PDB) solution, 5g of PDB was dissolved in 50 mL distilled water followed by autoclave at 121^oC and 15 pounds for 15 min. Moreover, potato dextrose plates were prepared with PDB and 1.8 g of agar. All the fungal strains were seeded to 50 mL of PDA containing conical flasks and incubated for 7 days at approximately 20^oC. For the antifungal test, 100 μL of fungal inoculums were spread carefully in the PDA plates and placed all small

pieces of the samples. The samples along with controls were incubated in static incubator and their growth were monitored systematically. Water was used as a negative control whereas antimycotic solution from Himedia at 10µg/mL were used as a positive control. All the experiments were performed in triplicates.

2.2.6. Extraction of cellulose

Cellulose was extracted from both old and new *Sāncipāt* using a standard method as mentioned below:

Extraction process of cellulose: The extraction method was adapted and modified from that developed by Fitriana et al., 2020 in accordance with TAPPI method T-429 and ASTM D-588 (Figure. 2.5) [165]. Two stages extraction of alkaline and peroxide treatments were conducted to extract cellulose from raw *Sānci* bark. First, the freshly prepared *Sānci* bark was pre-treated with 2% w/v NaOH solution at 100^{o}C for 5 hours until the structure of the bark becomes mushy and compromised. The fibers obtained were light yellow in colour which were washed several times to bring them to a neutral pH. The second stage involves bleaching the fibers with 3% v/v H_2O_2 solution at adjusted pH 9-10 which was adjusted with 0.5 M NaOH, temperature of 60^{o}C, and reaction time 60 minutes. The fibers were resuspended in 3% v/v H_2O_2 solution under the same conditions until the fibers turn white in colour. The obtained cellulose fibers were washed with distilled water and oven dried at temperature of 50^{o}C followed by weighing. Petri dish was used to store the cellulose fibers at room temperature in a desiccator.

2.2.6.1. Preparation of modified cellulose

New cellulose (NC) fibers obtained from *Sānci* were chemically modified according to the methodology of Mulinari et al. 2010 (Figure. 2.5) [166]. Accordingly, 2g of zirconium oxychloride were dissolved in 100 mL of aqueous hydrochloric acid solution (0.5 mol L^{-1}), in which 5g of cellulose fibers extracted from *Sānci* were immersed. The material was precipitated with 10 g of urea under heating, filtered, and exhaustively washed with distilled water for the complete removal of chloride ions (negative silver nitrate test was performed to confirm the above), followed by oven drying at 50^{o}C for 24 h. The resulting material was designated as *Sānci* Cell/ZrO_2.nH_2O or MC in shorter terminology.

2.2.6.2. Preparation of Neat PVA films

Neat PVA films were cast from aqueous solutions containing 5% w/v PVA in distilled water according to the methodology by Lim et al., 1994 (Figure. 2.6) [167]. PVA powder was slowly added in pre heated distilled water under vigorous stirring at rotational speed of 600 rpm for 1 hour, followed by heating at 80^{o}C for almost 3 hours until all the PVA are dissolved. The final mixture was degassed for 15 minutes using Rivotek Ultrasonic cleaner and the solution was cast on a petri dish until all the solvent was evaporated at room temperature for a period of 3 days. PVA films of thickness 1.00 mm were obtained. The films were stored in desiccators at ambient temperatures for at least 24 hours before they were studied. Mechanical properties, such as tensile strength, was examined using Universal Testing Machine (UTM). Repeated measurements were done to confirm the reproductivity, and the averaged results were presented.

2.2.6.3. Preparation of PVA/Cellulose film

PVA/cellulose fibers composites films were prepared according to the methodology followed by Frone et al., 2011(Figure. 2.6) [168]. Accordingly, an aqueous solution of PVA (5% w/v) was prepared by slowly adding the PVA powder in pre heated distilled water under vigorous stirring at rotational speed of 600 rpm for 1 hour, followed by heating at 80^{o}C for almost 3 hours until all the PVA are dissolved. 50 mL of the prepared PVA solution was mixed with 0.5g of cellulose fibers at 80^{o}C for another 3 hours with vigorous stirring at 700 rpm. The final mixture was degassed for 15 minutes using Rivotek Ultrasonic cleaner and cast on a petri dish. The films were kept for 3 days under laboratory conditions at 25°C and 60% RH until complete drying. Composite films with a thickness of 0.80 mm were obtained. The films were stored in desiccators at ambient temperatures for at least 24 hours before they were studied. Mechanical properties, such as tensile strength, was examined using Universal Testing Machine (UTM). Repeated measurements were done to confirm the reproductivity, and the averaged results were presented.

2.2.7. Extraction of lignin

Lignin was extracted from both old and new *Sāncipāt* using the method as mentioned given below:

Extraction process of lignin: Lignin was extracted as per the methodology described in TAPPI standard T222 (Figure. 2.5) [169]. Accordingly, small pieces of raw *Sānci* bark

were first taken in a beaker and 35% HCl (50ml) was added in small increments while stirring with a glass rod. The beaker was kept in an ice-cold water bath while adding the acid. Following that, 5 ml of 98% H_2SO_4 was added, and the mixture was stirred several times over a period of few hours and allowed to stand overnight at room temperature. After the fibers were dispersed, the mixture was diluted with 200 ml distilled water and boiled for 4 hours at 80^oC maintaining a constant volume by frequent addition of hot water. The solution was then allowed to stand overnight to allow the insoluble material (lignin) to settle. Without disturbing the precipitate, the solution was then decanted, centrifuged using Remi R-8C and washed with distilled water. The lignin collected was oven dried at 50^oC, weighed and stored in a petri dish in a desiccator.

2.2.7.1. Preparation of modified lignin

New lignin (NL) obtained from *Sānci* bark was chemically modified using the same method as that of cellulose modification (Figure. 2.5). The resulting material was designated as *Sānci Lig/* $ZrO_2.nH_2O$ or ML in shorter terminology.

Figure. 2.5: (OC) Old cellulose, (NC) new cellulose, (OL) old lignin, (NL) new lignin, (MC) modified cellulose and (ML) modified lignin.

2.2.7.2. Preparation of PVA/lignin film

For the preparation of PVA/Lignin composite films, simple solvent casing method was used as followed by Posoknistakul et al., 2020 [Figure. 2.6] [170]. The PVA (5% w/v) was dissolved in distilled water under vigorous stirring at 600 rpm, at 80^{o}C for almost 4 hours. To create the PVA-Lignin composite films, 50 mL of PVA solution were combined with an exact measurement of 0.5g of lignin samples. The homogenous mixture was transferred to a petri dish for evaporation and dried at room temperature for 3 days. The fabricated composite film was found to be 0.90 mm thick. The films were stored in desiccators at ambient temperatures for at least 24 hours before they were studied. Mechanical properties, such as tensile strength, was examined using Universal Testing Machine (UTM). Repeated measurements were done to confirm the reproductivity, and the averaged results were presented.

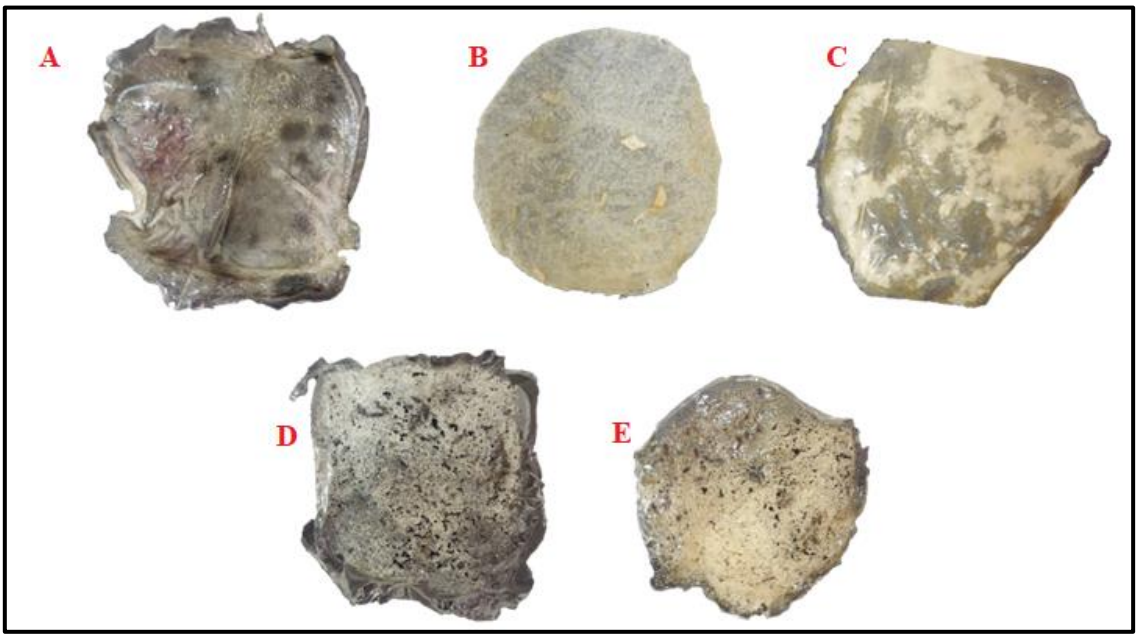

Figure. 2.6: (A) Neat PVA film, (B) PVA/New cellulose film, (C) PVA/Old cellulose film, (D) PVA/New lignin film and (E) PVA/Old lignin film.

2.2.8. Instrumental analysis

Physicochemical analysis: Hot-pressing with a BAJAJ DX2 light-weight 600W dry iron was used for rapid drying and smoothening of the Sānci bark after cleaning, after partial degumming and after application of fatty pulse. Hot air oven with Model No 101 (Sr No. 180217, Mfd. By Vindish Instruments Pvt. Ltd.) was used to dry different sample for

different experimental analysis. Digital weight balance, model ME204, Mettler Toledo, was used for weighing of all the samples.

All the cellulose and lignin samples were centrifuged using REMI R-8C centrifuge instrument with Resolution: 2.4A0 (FEI Company, USA) and at an accelerating voltage of 200 kV. All the Ultraviolet-visible (UV-visible) spectra of liquid samples were recorded on a Shimadju UV-2550 and the other solid UV- visible spectra were recorded on UV–vis diffuse reflectance spectroscopy (DRS) by Shimadzu, UV-2450. For the solid samples, the samples were mixed thoroughly in a mortar using Magnesium oxide as reference and a thin film was prepared through which the light was passed. UV-Visible spectroscopy is an analytical technique that measures the number of discrete wavelengths of UV or visible light that are absorbed by or transmitted through a sample in comparison to a reference or blank sample. This property is influenced by the sample composition, potentially providing information on what is in the sample and at what concentration. Perkin Elmer spectrophotometer model Frontier MIR FIR was used to record Fourier Transform Infrared (FTIR) Spectra with a resolution of 4 cm^{-1} and in the range of 400–4000 cm^{-1} by using standard KBr pellet method. To prepare KBr pellets, about 3 mg of dried samples were mixed thoroughly with 9 mg of spectroscopic grade KBr in an agate mortar and then pressed into a disc by subjecting compressive load. Fully dried powders of all the samples were considered for recording FT-IR spectra. IR spectroscopy is an absorption technique made possible because molecular vibration modes absorb specific frequencies of the electromagnetic spectrum at varying intensities. However, a change in electric dipole moment must occur during the vibration [171]. It is a rapid, non-destructive, time saving method that can detect a range of functional groups and is sensitive to changes in molecular structure [172]. FTIR provides information on the basis of chemical composition and physical state of the whole sample, detecting functional groups and characterizing covalent bonding information [172].

Powder- X-ray diffraction (p-XRD) was recorded on a Brucker AXS, D8 Focus X-ray diffractometer, Germany at a room temperature in Bragg-Brentano geometry with Cu-Kα radiation (λ= 0.154 nm) at 30 kV and 30 mA at a scanning rate of 0.05°/s in 2θ ranges from 10° to 70°. For the determination of peak position, no internal standard was used. The identification was made only by the use of International Centre for Diffraction Data Powder Diffraction Files database (PCPDF-WIN software). X-ray diffraction is a powerful non-destructive technique used in materials science that provides information on structures, phases, preferred crystal orientations (texture), and other structural parameters;

average grain size, crystallinity, strain, and crystal defects. The technique is often known as X-ray powder diffraction because the material being analyzed typically is a finely grounded down to a uniform state. The diffraction pattern obtained is the fingerprint of periodic atomic arrangements in a given material.

Raman spectrophotometer model Renishaw basis series with 514 lasers has been used to measure the Raman-active vibrational modes of the pigments. A desktop Enwave Optronics EZRaman-N Raman spectrophotometer in California that has a temperature-controlled 785 nm laser sources was used to record the Raman signals (variable output power up to 500 mW). Integration time was maintained at 10 s with an average of 5s. Prior to recording each spectrum baseline adjustments were made, and peak smoothing was done for Raman processing.

Thermal analysis has been performed on a thermal analyzer, model Shimazu TGA-50 was used where about 3–4 mg of samples was taken in a platinum crucible and heated from room temperature to 600ºC at a heating rate of 20ºC min^{-1} in presence of dynamic nitrogen atmosphere with a flow rate of 30 mL min^{-1}. Thermogravimetric Analysis (TGA) is the most widely used thermal analysis method that is based on measurement of mass loss of a material as a function of temperature [173]. It is an analytical technique used to determine a material's thermal stability and its fraction of volatile components by monitoring the weight change that occurs as a sample is heated at a constant rate in an inert gas atmosphere. Thermogravimetric kinetics may also be explored for insight into the reaction mechanisms of thermal (catalytic or non-catalytic) decomposition involved in the pyrolysis and combustion processes of different materials.

The differential scanning calorimetry (DSC) was done by DSC 60, Simadzu, Japan at 3ºC min^{-1} heating rate under the nitrogen flow rate of 30 mL min^{-1} from 50 to 100ºC. Differential Scanning Calorimetry (DSC) is a thermal analysis technique in which the heat flow into or out of a sample is measured as a function of temperature or time, while the sample is exposed to a controlled temperature program [174]**.** It is a very powerful technique to evaluate material properties such as glass transition temperature, melting, crystallization, specific heat capacity, cure process, purity, oxidation behavior, and thermal stability.

The molecular weights and polydispersity indexes of both cellulose and lignin were determined by gel permeation chromatography analysis (UV/V VISIBLE DETECTION-2489, REFRACTIVE INDEX DETECTOR-2414, HPLC PUMP-515, Waters Corporation, USA using EMPOWER-2 software) using THF as solvent.

The C, H and N contents were determined by 2400 SERIES 2, Perkin Elmer, USA. The SEM and EDX analysis of the samples was observed by scanning electron microscope (SEM) of model JSM-6390LV, JEOL software, Japan. CHN Elemental Analyzer provide means for the rapid determination of the amount (typically a weight percent) of carbon, hydrogen, and nitrogen in different compounds with accuracy and precision [175]. It is based on the principle of "Dumas method," using flash combustion of the sample to cause an instantaneous oxidation into simple compounds which are then detected with thermal conductivity detection or infrared spectroscopy. The hydrodynamic size and differential light scattering (DLS) measurements were ascertained using Micromeritics Nanoplus zeta/nano particle analyser by employing water as the medium. Dynamic light scattering (DLS) is based on the Brownian motion of dispersed particles. When particles are dispersed in a liquid they move randomly in all directions. The principle of Brownian motion is that particles are constantly colliding with solvent molecules [176]. These collisions cause a certain amount of energy to be transferred, which induces particle movement. One can determine the hydrodynamic diameter by measuring the speed of the particles where the relation between the speed of the particles and the particle size is given by the Stokes-Einstein equation. DLS provides information on the mean particle size as well as on particle size distribution. The polydispersity index (PDI) is given in order to describe the broadness of the particle size distribution. Samples were dispersed in water medium by sonicating them for a few hours in Rivotek Ultrasonic Cleaner.

An instrument called a Hegman gauge, also known as a grind gauge, grind gage, or grindometer is used to measure how finely a grind is made or to detect the presence of coarse particles and agglomeration in a dispersion (ASTM Test Method D-1210). Zeta potential and nano-particle size analyzer model Nanoplus-3 was used to measure the particle size and the zeta potential of the samples. A digital gloss meter, make S.C. Dey & Co., Kolkata, was used to compare the brightness of old *Sāncipāt* and woodcarvings with newly restored *Sāncipāt* and woodcarvings. The hiding power measurement was done using a Spectrophotometer, AGS, model x-rite Ci200UV.

Image analysis: The SEM and EDX analysis of the samples was observed by scanning electron microscope (SEM) of model JSM-6390LV, JEOL software, Japan at an accelerated voltage of 5−15 kV. A scanning electron microscope (SEM) projects and scans a focused stream of electrons over a surface to create magnified detailed images.

The electrons in the beam interact with the sample, thereby producing various signals that can be used to obtain information about the surface's topography and composition [177]. The signals that derive from electron-sample interactions reveal information about the sample including external morphology (texture), chemical composition, and crystalline structure and orientation of materials making up the sample [177]. The surface of the sample was platinum coated before SEM analysis. Energy dispersive X-ray spectroscopy (EDX) is an analytical method for analytical or chemical characterization of materials. EDX can be used for both qualitative and quantitative analysis, enabling users to identify both the type of elements that are present as well as the percentage of each element's concentration within the sample. The data generated by EDX analysis consist of spectra showing peaks corresponding to the elements making up the true composition of the sample being analyzed [178]**.** The samples were deposited on a brass holder and sputtered with platinum.

The TEM images were obtained on a TECNAI G2 20 S-TWIN, resolution: $2.4A^0$ (FEI Company, USA) and at an accelerating voltage of 200 kV. Samples were sonically dispersed in ethanol for few hours in Rivotek Ultrasonic Cleaner and deposited on a carbon-coated copper grid before examination. The transmission electron microscope is a very powerful tool for material science [179]. A high energy beam of electrons is shone through a very thin sample, and the interactions between the electrons and the atoms can be used to observe features such as the crystal structure and other fine details. The beam of electrons that passes through the specimen analyzes the internal structure of the specimen in the form of images [179].

Mechanical analysis: The tensile strength was measured with the help of Universal Testing Machine (UTM), Zwick Z010, Germany with a 10 kN load cell and crosshead speed of 40 mm/min. A universal Testing Machine or commonly known as UTM is a materials testing machine, is used to test the mechanical properties (tension, compression etc.) of a given test specimen by exerting tensile, compressive or transverse stresses [180]. The machine has been named so because of the wide range of tests it can perform over different kind of materials. Different tests like peel test, flexural test, tension test, bend test, friction test, spring test etc. can be performed with the help of UTM. These machines usually use a hydraulic cylinder to create the force. The applied force is determined by system pressure which can be accurately measured.

2.2.9. Color co-ordinate study by CIELAB-1986

All the Colorimetry measurements were performed for numerical characterization of color using a Ultrascan VIS colorimeter, Hunter at Associates laboratory Inc., USA. The light source was pulsed xenon (Xe) lamp. The instrument was calibrated well with standard white tile and black glass as specified by the manufacturer. Colorimeter method (Ultrascam VIS, Hunterlab, USA) was used to characterize the colors imparted by various compositions of the paints used in the study in terms of L*, a* and b* scales [181-183]. Lightness *vs.* darkness is indicated on L* scale. The range of 0-50 on the L scale reveals the darkness of the sample and the range of 51-100 on the L* scale means lightness of the sample. a* scale indicates redness vs greenness. The value of a* can be either positive or negative. The positive value of a* means redness and the negative value means greenness of the sample. Yellowness *vs.* blueness is indicated on the b* scale. The value of b* can be either positive or negative. Here yellowness is indicated by positive value and blueness indicated by negative value. The color characteristics of various paints have been prepared separately using both Bael gum and same amounts the commercial starch-based gum for comparison. The h indicates hue angle, and it is given by $h= \tan^{-1}(b^*/a^*)$. C* is for chroma/saturation and a positive C* indicates brightness while a negative C* indicates dullness. C* is given by $C^*= [(a^{*\wedge}2) + (b^{*\wedge}2)]^{1/2}$.