4.1 Introduction

4.2 Random Sampling

4.3 Survey of Sanchi Manuscripts

4.3.1 Methodology Adopted

4.4 Observation Method

4.4.1 Methodology

4.5 Experimental Investigations

4.5.1 Background

4.6 Methodology

4.6.1 Manuscript samples, 4.6.2 Physical Test

4.6.2.1 Scanning Electron Microscopy (SEM)

4.6.3 Mechanical test

4.6.4 Chemical Test

4.6.4.1 X-Ray Diffraction, 4.6.4.2 FTIR Test; 4.6.4.3 Chemical treatment by EDTA disodium and alkaline hydrogen peroxide with silicon dioxide t

4.7 Conclusion
CHAPTER 4

METHODS AND METHODOLOGY

4.1 Introduction

Looking at the compendium of data required for the research work, various methods have been used for data collection and data generation. The research problem in question required data from diverse sources using different methods, such as, sampling, survey, interviews, observations and laboratory experimentation. Except random sampling technique which was used from secondary data source, other methods adopted were from primary data sources.

The survey method was necessary to collect some specific data from the select repositories to achieve some conclusions for the research work. It was important to make the parameters specific so that the fact can be recorded and utilized for further investigations. In the survey method, data were collected through questionnaire with precisely set parameters which were of objective type and easy to answer. The questionnaire method was followed by the interview method where a pre-set checklist of questions prepared and was asked to the owners of caretakers. The interview was necessary as some of the required information which have vast implication in the research work could be available only through interviews, not by questionnaire.

The survey method was followed by the observation method. In this method a preset checklist of parameters was prepared to observe and examine the collections of manuscripts preserved in the select repositories, on the spot, as per the parameters set. Along with the collections, the facilities of the repositories were also checked as per parameters. For this observation, along with 20 select repositories of Assam, 2 archives and 1 library in Germany were also selected. The motive behind the selection of archives in Germany was to observe the state
of the art technology employed for Egyptian, Greek, Arabic and Coptic collections preserved in those archives. The research work vehemently demanded such observations to be made on the spot to realise and understand facts and to document the evidences.

After conducting the survey and observation under the predetermined parameters, it was found that the data collected so far was not enough to achieve the objective set for the research work. Hence, it was inevitable to conduct few laboratory tests, without which it would not have been possible to achieve the final objective. These tests, namely physical test, mechanical test and chemical test were conducted in the sophisticated laboratories of the Indian Institute of Technology, Guwahati, fulfilling all administrative formalities. The methods employed in this research work have been schematically represented as given below.

![Schematic representation of methods applied for the research work](image)

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**Fig 4.1** Schematic representation of methodology
4.2 Random sampling

A pilot survey was conducted in Assam from 28th Nov. 2005 to 05 Dec. 2005 by the National Mission for Manuscripts, Govt. of India, in collaboration with the Sankardev Kalakhetra, Assam as a host representative of Ministry of Culture, Govt. of Assam. According to the report, Assam has 36,930 Manuscripts, most of which were sanchi manuscripts, spread over 4,973 repositories in 27 districts, excluding N.C. Hills. Taking this information in the backdrop, some 20 repositories belonging to 7 districts were selected randomly as data sources for the research work and to make survey, observations and interviews for the required parameters as summarized in in Table 4.1.

<table>
<thead>
<tr>
<th>Natural Occurrence</th>
<th>Sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Repositories in Assam: 4,973</td>
<td>Repositories opted (random): 20</td>
</tr>
<tr>
<td>Total Reported District: 27</td>
<td>District opted (random): 07</td>
</tr>
<tr>
<td>Total MSS (as per NMM record): 36,930</td>
<td>Manuscripts opted (random): 3970</td>
</tr>
<tr>
<td>(including 3970 manuscripts, both sanchi and others, of 20 repositories)</td>
<td>(out of these 1853 sanchi manuscripts taken for examination)</td>
</tr>
</tbody>
</table>

Table 4.1 Summary of Random sampling

4.3 Survey of sanchi manuscripts

The data collection from 20 randomly selected repositories has been done through two methods, namely questionnaire method and personal interviews to the manuscript owners and caretakers.

4.3.1 Methodology adopted

Simple survey methods have been used during the survey of manuscript collection in repositories for required data relating to various parameters. Two methods have been used for this research work as the following:
Methodical questionnaires have been prepared with all the required parameters with the objective to incorporate all the necessary data sources. There are three parts in the questionnaire, part-I related to the address and identification of the repositories, Part-II related to various parameters of collections of the repositories and the part-III is related to the preservation aspect of the manuscripts and repositories.

Total questionnaire distributed: 20

Category to whom communicated: Repository Caretakers/owners

Questionnaire Part-I: Item (Sl. No. 1-8) for identification and communication

Questionnaire Part-II: Parameters for status of collections (Sl. No. 1-5)

Questionnaire Part-III: Parameters for status of preservation (Sl. No. 1-12)

List of Parameters and Repositories: Details has been provided in the Appendix-II & III)

The analysis of the data collected with the help of various parameters provided in the questionnaire and the subsequent result have been made available in the Chapter 5.
ii. Interviews

All factual data are not retrievable through questionnaire. That is why the queries for interviewee were prepared in such a way that the real facts come out through discussions and understanding. Some of the interviews were recorded through digital camera and mobile phone with prior and proper permission. Photograph were also made whenever felt necessary with due permission. The statistics for interview are as follows:

- Total Interviewee: 20
- Total queries made: 07
- Category to whom interviewed: Repository caretakers and owners

iii. Check List of queries prepared for Interviewee

1. A brief history of the repository and manuscript collection.
2. Any initiative taken for preservation of manuscripts so far by any organisation.
3. Whether any awareness programme or training programme was attended?
4. Any other sources of knowledge for manuscript preservation.
5. Environmental status of the locality and its effect on manuscripts.
6. Housekeeping operations.
7. Status of natural calamities like rain, flood, fire, earthquake, etc.

The analysis of the data collected with the help of above queries and the subsequent result have been made available in the Chapter 5.

4.4 Observation method

Data can be distinguished from what interviewees speak during interviews and what they write in questionnaires. That is why in this research work, observation method has been applied with the expectation to gather more confirmed data.
from the surveys conducted on the repositories and also few archives visited in Germany.

4.4.1 Methodology

A check-list of 5 main parameters and 32 sub-parameters related to preservation of manuscripts were prepared as shown in the Table 4.2. These parameters were observed and examined in the 20 repositories selected for survey in Assam, and also in 2 archives and 1 library in Germany. These observations were extremely important to find out the extent of preservation practices, amenities and methodologies employed by the repositories to prevent physical, mechanical and chemical damages of the manuscripts.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sub-parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Use of Cloth &amp; Mount Board Box</td>
<td>i. Colour; ii. Type (cotton/silk); iii. Starch free/ Starched;</td>
</tr>
<tr>
<td>2. Status of Storage</td>
<td>i. Use of Wooden Plank; ii. Use of Wooden Box; iii. Use of Iron Box; iv. Use of Wooden Rack; v. Use of Iron Rack; vi. Use of Wooden Almirah; vii. Use of Steel Almirah; viii. Use of Hard Board; ix. Use of Mount Board (Acid Free);</td>
</tr>
<tr>
<td>3. Indigenous Traditions of Preservation Practices</td>
<td>i. Use of Smoke; ii. Sun shade Dry; iii. Use of Lime Stone; viii. Use of Camphor</td>
</tr>
<tr>
<td>4. Use of indigenous plant Materials</td>
<td>i. Use of Mustered oil; ii. Resining; iii. Use of Neem Leaves; iv. Use of Tobacco Leaves; v. Use of Turmeric; vi. Use of other plant materials</td>
</tr>
</tbody>
</table>
5. Modern Preservation Practices

| i. Use of chemicals for pest control |
| ii. Fumigators |
| iii. Use of Cleaning Agents |
| iv. Restorative Process |
| v. Flaking Arrest |
| vi. Use of Temperature Controller/AC/Thermostat |
| vii. Humidity measuring and control (Dehumidifier/RH controller) |
| viii. Light levels measuring and control |
| ix. Ventilation |
| xi. pH |
| xii. HVAC system |

Table 4.2 Check List for Observation

The results and analysis of the observation method applied in this research work have been provided in the chapter 5.

4.5 Experimental investigations

4.5.1 Background

Deterioration of wood origin manuscripts is a crucial problem for curators, preservators and librarians. To enable wood origin manuscripts to persist for longer time, it is usually preserved in an environment that arrests microbial activity. Such special conditions may allow manuscripts to survive for centuries but, in the absence of such ideal condition, some physical and chemical modification takes place which causes degradation of the manuscripts.

Biological agents such as bacteria, antinomycetes and fungi decompose cellulose content of manuscripts. The most severe deterioration occurs in indoor environments like sacred places, museum and repositories by cellulolytic fungi (Montgut, et al., 1991). Sanchi manuscripts are composed mainly of cellulose, as it is of wood origin. Cellulose is constituted by carbon, hydrogen and oxygen consisting of glucose units linked together in long chains. During biodegradation process of a wood origin manuscript, cellulose turns into glucose molecule. The degradation of cellulose into glucose molecules is caused by some fungi.
possessing a system of extracellular and intracellular enzymes known as cellulases (Selby, 1968). These fungi possess remarkable cellulose dissolving capacity which may cause staining or discolouration, fading, loss of strength, extensibility of manuscripts (Sagar, 1987; Gallo, 1985).

The fungi which have been reported by many studies having high celluloytic activity belong to the genera Aspergillus, Alternaria, Fusarium, Memnoniella, Penicillium, Scopulariopsis, Stachybotrys, Stemphylium, Trichoderma and Chaetomium (Gallo, 1985). Lal (1968) reported some high enzymatic fungi viz., A. niger, Chaetomium globosum, Fusarium solani, Memnoniella echinata, Scopulariopsis brevicaulis, Trichoderma harzianum, T. kanningi, Trichothecium roseum. Aspergillus strains i.e, A. fumigatus, A. terreus and A. niger were found to be more stable on higher temperature and wider pH range. Aspergillus niger and Trichoderma harzianum were tested to be the fungi with most cellulolytic activity.

The problem of biodeterioration is prominent in tropical and subtropical regions like northeast region of India where high temperature and high relative humidity favour proliferation of fungi. Aspergillus niger enzymes were stable up to 70° C and most active at 30° C, which is the common temperature of North-East India. Aspergillus niger showed equal activity at all pH (0-14) range however many other fungi like Trichoderma harzianum showed maximum activity of enzymes was in pH range 4.0-6.0 after which it decreased. The favourable conditions for growth of such fungi are lack of ventilation in the repositories, acidic pH level, high relative humidity and high temperature. The congenial environmental conditions required for fungal growth as reported by Guiliani and Nugari (1993) are 65% relative humidity and temperature $\geq 20 ^{\circ} C$.

It was therefore understood that the process of manuscript deterioration is a complex development. All the three factors viz., environmental, biological and chemical factors of deterioration are interlinked and act together. But, one thing is for sure, that whatever be the cause of deterioration, the affected element is the cellulose part of the manuscript. So, the study of cellulose would likely reveal the exact cause of deterioration, which might lead to the investigation for a solution.
of preservation technique. And, to study cellulose content of a wood origin sanchi manuscript, laboratory experiments like scanning electron microscopy, X-ray diffraction, Fourier transformed infrared spectroscopy along with few mechanical tests for stress and strain is a must to perform.

Aranyanak (1995) stated that scanning electron microscopy (SEM) is quick and reliable method for identification of fungal growth on cellulosic materials. Beside identification of fungal growth, scanning electron microscope also provides images of three dimensional microstructure of cellulose fibre and other morphology at a higher magnification of 500–100000 X. Therefore, to observe the fungal growth in cellulose and to understand surface morphology of sanchi manuscript, scanning electron microscopy was conducted.

The Fourier transformed infrared spectroscopy (FTIR) analysis of wood origin sample is a fast method to identify the structural and chemical changes induced by various common fungal degradation. (Pandey and Pitman, 2003). To observe the structural and chemical changes in sanchi manuscript, the FTIR analysis was conducted in this research work.

The wood decaying fungi seriously reduce strength of the manuscript by metabolizing the cellulose fraction of it which actually provides strength to manuscript. For example, brown-rot fungi may reduce mechanical properties in excess of 10% before a measurable weight loss is observed and before decay is visible. When weight loss reaches 5% to 10%, mechanical properties are reduced from 20% to 80%. Decay has the greatest effect on toughness, impact bending, and work to maximum load in bending, the least effect on shear and hardness, and an intermediate effect on other properties. Hence, to keep the strength of the manuscript intact, it is important to take adequate measures in two aspects, one, prevent decay before it occurs, and secondly, control budding decay by remedial measures. Therefore, measurement of strength of wood origin manuscript provides clues for possible decay due to metabolic activities of fungi on cellulose fraction. Hence mechanical tests to measure strain-stress, toughness, etc., of sanchi manuscript were necessary in this research work.
Crystallinity is an important feature of wood. It has an important effect on the physical, mechanical, and chemical properties of cellulose fibres related to structure of wood material, chemical composition and also physico-mechanical properties viz., Young’s modulus, dimensional stability, density, and hardness. The study of crystallinity of wood origin manuscript can be done by using X-ray diffraction (XRD) technique. X-ray diffraction (XRD) detects the interference pattern created when X-rays encounter the regularly spaced crystalline cellulose planes in wood manuscript. Hence, X-ray diffraction (XRD) test were conducted on sanchi manuscript.

To reach the objective of this research work, i.e. to initiate an integrated preservation system, analysis of the intrinsic nature and chronological degradation of physical or morphological characteristics along with chemical properties of Sanchi manuscripts belonging to different time-periods, was essential. Without knowing the cause of degradation, any preventive measure is fruitless. All the above studies, suggest that the cellulose degradation of manuscript is the prime cause of deterioration. Therefore the commonly applied experiments viz., the Scanning Electron Microscopy (SEM), Fourier Transformed Infrared Spectroscopy (FTIR) and Powder X-Ray Diffraction Test (Powder-XRD) have been used to study cellulose component of these wood-based manuscripts. Again, the mechanical properties such as stress, strain, young’s modulus, toughness and tensile strength of sanchi manuscripts, various tests such as, Toughness test, Stress-Strain Test using a Universal Testing Machine (UTM) are complementary to the above tests. Therefore, all the above tests herein were necessary to understand the cause and mechanisms of biodegradation of sanchi manuscripts and hence were conducted for useful inferences on the preservation of sanchi manuscripts. The methodology is described in the following sections.

Again, when the main causes of deterioration of sanchi manuscripts have been identified using the above mentioned tests, there was a need to identify the
probable remedies against the causes. As biodeterioration is an irreversible process, the remedies cannot ensure regrowth of the damaged cellulose, but eradication of fungus from the infested sanchi manuscripts and consolidation of the cellulose fibres is possible. This could be done by chemical treatment on the infested sanchi manuscripts. Treatment with EDTA disodium and alkaline hydrogen peroxide yielded better result on infested manuscripts (Yulin and Yuansheng, 2005).

4.6 Methodology

4.6.1 Manuscript samples

To determine the properties of sanchi materials belonging to 5 (five) different time periods, 5 (five) different samples of sanchi manuscripts viz., freshly prepared manuscripts, 100 years old, 200 years old, 300 years old and 400 years old manuscripts were taken for various experiments as shown in Photo 4.1. The age of the samples have been determined on the basis of the colophon, a written statement provided at the beginning of the manuscript text showing the specific date and time of its preparation in literary form.

It was expected that the manuscripts of different time period would show different characteristics while testing and would produce a chronological sequence of degradation (if any).

The samples have been used to conduct study on the microbial degradation of cellulose component as well as the morphological properties of Sanchi manuscript samples using Scanning Electron Microscope (SEM).

For studying the mechanical properties, Universal Testing Machine (UTM) has been used. The dimensions of the manuscript specimens obtained from the manuscript samples are provided in Table 4.3.
In case of X-ray diffraction (XRD) and Fourier Transformed Infrared (FTIR) spectroscopy analysis, the samples were powdered in a ball mill pulveriser so that the intrinsic properties of the samples could be conserved during the pulverization which is important to get accurate results during X-ray diffraction and spectroscopy analysis.

A Ball Mill Pulveriser (Insmart make) was used to make powder from the samples of various ages. The sun dried samples were cut into small pieces of about 5mm x 2mm and weighed in a weight-balance (Sartorius make) and milled in the pulveriser with the help of zirconium balls, so that a minimum amount of 5g powder could be extracted from each sample. Depending on the hardness of the samples the pulveriser was allowed to rotate at different speed for different time durations. The Table 4.3 below shows the weight of the manuscript pieces, rotational speed and time of the pulveriser for each sample. Photo 4.3 shows the various process of milling the pieces of the manuscript samples.

The table 4.3 showing the specification of manuscript specimen used in ball mill pulverizer

<table>
<thead>
<tr>
<th>Samples</th>
<th>Dimension</th>
<th>Weight</th>
<th>Time taken</th>
<th>Speed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimen 1</td>
<td>21 x 6</td>
<td>08</td>
<td>900</td>
<td>150</td>
</tr>
<tr>
<td>Specimen 2</td>
<td>21 x 6</td>
<td>7.73</td>
<td>675</td>
<td>190</td>
</tr>
<tr>
<td>Specimen 3</td>
<td>21 x 6</td>
<td>7.65</td>
<td>675</td>
<td>190</td>
</tr>
<tr>
<td>Specimen 4</td>
<td>21 x 6</td>
<td>7.40</td>
<td>450</td>
<td>190</td>
</tr>
<tr>
<td>Specimen 5</td>
<td>21 x 6</td>
<td>7.30</td>
<td>450</td>
<td>190</td>
</tr>
</tbody>
</table>

Table 4.3 Specification of manuscript specimen used in ball mill pulverizer
a. Fresh sanchi MSS (Sample 1)  
b. 100 yrs old sanchi MSS (Sample 2)  
c. 200 yrs old sanchi MSS (Sample 3)  
d. 300 yrs old sanchi MSS (Sample 4)  
e. 400 yrs old sanchi MSS (Sample 5)  

Photo. 4.1  Sanchi manuscript samples considered in the study
4.6.2 Physical Test

4.6.2.1 Scanning Electron Microscopy (SEM)

Wood cell walls are comprised of three major chemical components namely cellulose, lignin and hemicelluloses. In simplified terms the cellulose forms a skeletal matrix that is surrounded and encrusted by the hemicelluloses and lignin. Cellulose is composed of glucose units that are organized into chains. These chains are arranged in microfibrils, which are parallel groups of about 36 cellulose chains and considered as the smallest building element of cellulose.
Cellulose micro-fibrils (aggregated fibrils 10–20 nm in diameter) are visible using Scanning Electron Microscope (Ek et al. 2009).

Observations on wood origin manuscript's macro- and microstructure as well as fibre morphology along with microbial degradation are primarily conducted using a variety of microscopic methods. For studies at higher magnification (e.g. 500–100000 X) that gives a three dimensional view of manuscripts, the scanning Electron Microscope (SEM) is routinely used to give details of the ultra structural architecture of individual cells and wall layers as well as degradation of cellulose structure and infection of fungi as stated above.

To observe and analyze the microbial infection and morphological properties of the sanchi manuscript samples of various time periods, Leo 1430vp Scanning Electron Microscope (SEM) was used with acceleration voltage 10 kV and with electron beam capacity of 300 mA (Photo 4.3). A 5mm square piece each of all the manuscript samples were attached to the metal stub in the Scanning Electron Microscope one at a time. To improve the conductivity of the samples and the quality of the SEM images, the samples were coated with a 200° A of very thin layer of gold–palladium alloy using a covering SEM device (SEM BALTEC MED 020). Same procedure was repeated for all the samples. The result and analysis of the images retrieved from the SEM have been given in the chapter 5.
4.6.3 Mechanical Test

The mechanical properties of manuscripts of wood origin are its ability to resist applied or external forces. The mechanical properties of sanchi manuscript have been observed by means of special testing apparatus in the laboratory called Universal Testing Machine. The mechanical properties of sanchi manuscript considered in research work are: (i) Modulus of Elasticity (ii) Tensile Strength (iii) Toughness and (iv) Breaking Elongation. In connection with these, relevant concepts have been illustrated. Study of the mechanical properties of a material is concerned with its behaviour in relation to stresses and strains, and the factors affecting this behaviour.

The tensile stress on a material is defined as the force per unit area as the material is stretched (Fig. 4.2). The cross-sectional area may change if the material deforms as it is stretched, so the area used in the calculation is the original undeformed cross-sectional area $A_0$. The unit of stress is Pascal (Pa).

\[
\text{Stress} = \frac{\text{Force}}{A_0} \quad (4.1)
\]

If sufficient external force is applied the natural shape and size of the body changes. This distortion or deformation of the material is known as the strain.
The strain is a measure of the change in length of the sample as shown in Fig. 4.3.

Every stress produces a corresponding strain, and within the elastic limit, the strain is directly proportional to the stress producing it. The strain is a unitless number.

\[
\text{Strain} = \frac{(L - L_o)}{L_o} \quad (4.2)
\]

As the stress increases there is a corresponding increase in the strain. This ratio may be graphically shown by means of a diagram or curve plotted with the increments of load or stress as ordinates (Y axis values) and the increments of strain as abscissa (X axis values). This is known as the stress-strain curve as shown in Fig. 4.4.
The breaking-elongation is the ultimate strain on a sample when it breaks. This usually is expressed as a percentage (%). Fig. 4.5 shows the breaking-elongation on a stress-strain curve.

If successively larger loads are applied to an object and then removed it will be found that, at first the body completely regains its original form upon release from the stress in other words, the body is elastic. Eventually a point will be reached where the recovery of the specimen is incomplete. This point is known as the elastic limit, which may be defined as the limit beyond which it is impossible to carry the distortion of a body without producing a permanent alteration in shape. The elastic limit of a material under is determined from the stress-strain diagram. It is the point in the line where the stress-strain curve begins to curve prominently.

Fig. 4.5  Breaking-elongation on an object

Fig. 4.6  Stress-strain curve showing elastic limit

113
Modulus of elasticity or Young’s Modulus ($Y$) is the ratio of stress to strain. It is the slope of a stress-strain curve. Stress-strain curves often are not straight-line plots, indicating that the modulus is changing with the amount of strain. In this case the initial slope within the elastic limit of the stress-strain curve is usually considered as the modulus of elasticity.

$$Y_{\text{elastic-limit}} = \frac{\text{Stress}}{\text{Strain}} \quad (4.3)$$

The toughness of an object is the area under a stress-strain curve till the ultimate strain at which the material breaks. The toughness is a measure of the energy a sample can absorb before it breaks.

![Stress-strain curve showing toughness](image)

**Fig. 4.7** Stress-strain curve showing toughness

To observe and analyse the mechanical properties such as stress, strain, toughness and tensile strength of sanchi manuscripts, the experiments were performed on an Universal Testing Machine (Instron) under axial loading. A gauge length of 50 mm was considered at the central section of the specimen of
length 150 mm and width 50 mm, to study the stress-strain properties as well as obtain the breaking elongation of the specimen as shown in Fig. 4.8.

![Cross-section of the manuscript specimen](image)

*All measurements are in mm.

Fig. 4.8

The specimens were carefully positioned at the centre of the cross-head with its end faces exactly perpendicular to the longitudinal axis to get accurate results. To ensure adequate grip, a length of 50 mm was left at both ends for clamping the manuscript specimen ((Photo 4.4(a)-4.4(d)). The experiments were conducted at a constant crosshead speed of 2mm/min. The stress-strain data were obtained for each specimen from the automatic computerized chart recorder with the help of software called testXpert software inbuilt in the machine. Using recorded data, stress-strain graphs were prepared to study the variation of mechanical properties of the five manuscript samples. Details of result and analysis have been provided lucidly in Chapter 5.
4.6.4 Chemical Test

4.6.4.1 X-Ray Diffraction

X-ray diffraction (XRD) is based on the concept of formation of an interference pattern by incident X-rays when they encounter a regularly spaced matrix of cellulose. In case of wood, this process is used to determine the average width of the cellulose microcrystal, the percent of crystalline cellulose within the wood, and can be used to examine the changes in these parameters during microbial degradation. Understanding of the mechanisms and effects of wood degradation
through X-ray diffraction may enhance knowledge of the decay processes and development of more effective preservation techniques.

Wood cell walls are composed of a matrix of cellulose, hemicelluloses, lignin, pectin, proteins, and other trace materials. Among wood components only cellulose is crystalline; the other polymers are non-crystalline. The free hydroxyl groups present in the cellulose macromolecules are associated in a number of intramolecular and intermolecular hydrogen bonds, which may give rise to various ordered crystalline arrangements.

A single chain of cellulose can be formed up to 10,000 glucose subunits. These chains are arranged in microfibrils, which are parallel groups of about 36 cellulose chains that are wound around the wood cell aligned at an angle parallel with the fibre axis (Zabel and Morrell, 1992). The microfibrils as shown in Fig. 4.9 consist of 60-70% crystalline cellulose and 30-40% amorphous cellulose surrounded by a hemicellulose and lignin matrix (Ek et al., 2009). Crystallinity is an important property of woody materials. The crystallinity of wood is defined as the weight fraction of crystalline material (crystalline cellulose) in wood (i.e. weight of the crystalline cellulose divided by total weight of wood). The crystalline portions are formed when the cellulose chains that comprises the microfibrils bind together to form hydrogen bonds, creating a regularly patterned structure (O'Sullivan 1997). Approximately 30% of wood weight is cellulose in its crystalline form (Andersson et al., 2003). The amorphous portions of the microfibril, which lack the hydrogen bonding and regular structure found in crystalline cellulose, disintegrate these cellulose crystallites. Crystalline cellulose, which has a regularly patterned structure, can also be decomposed by fungi and some bacteria. Changes in wood crystallinity during decay can be used as an indicator for microbial degradation of sanchi manuscripts.

Crystallinity has an important effect on the physical, mechanical, and chemical properties of cellulose fibers related to structure, and chemical composition and also the physico-mechanical properties viz., Young's Modulus, dimensional
stability, density, and hardness of wood origin materials. For example, Young’s modulus, tensile strength, alpha-cellulose content, dimensional stability, density, and hardness increase with increasing crystallinity, while moisture regain, chemical reactivity, swelling, and flexibility decrease. Therefore, the study of crystallinity is important for understanding the ultra-structure and composition of wood-based materials (Jiang et al., 2007).

Fig. 4.9 The internal structure of cellulose fibres (Insel, 2006)

The study of crystallinity of sanchi manuscript can be done by using X-ray diffraction (XRD) technique. X-ray diffraction (XRD) technique, which detects the interference pattern created when X-rays encounter the regularly spaced crystalline cellulose planes, has been used as a rapid, non-destructive method for observing the crystalline portion of wood materials, and is one of the primary tools used in the determination of the conformation and structure of cellulose microfibrils (Lichtenberger et al., 1999).

The interference pattern created by the crystalline cellulose is used to determine the distance between the crystalline planes, the width of the microfibrils, and the overall percent of the wood that is in a crystalline state. The distance between the crystal planes can be determined by Eq. 4.4.

\[ d \sin \theta = \lambda m \]  

(4.4)
where, \( d \) represents the distance between the crystal planes, \( \theta \) (theta) is the angle between the plane and the diffracted or incident beam (Fig. 4.10), \( \lambda \) is the wavelength of the X-rays, and \( m \) is an integer.

The XRD analysis of powdered samples of 5 sanchi manuscript was performed on a Bruker D2 phaser diffractometer equipped with a Kristalloflex 760 sealed-tube copper anode generator, operated at 40 kV and 40 mA, and on a two-dimensional position sensitive wire-grid detector (Bruker AXS) pressured with xenon gas (Photo 4.5). The powdered specimens of sanchi manuscripts of various time periods which were previously collected from the Ball Mill Pulveriser were pressed into the shape of a tablet with rectangular dimensions of 15 \( \times \) 20 mm with a thickness of 1 mm. The specimen was measured in a 2\( \theta \) range between 10° to 80°. The X-ray diffractometer was operated at a voltage of 40 kV with a current density of 30 mA with diffracted intensity of Cu Ka radiation (\( \lambda = 0.1542 \) nm) and scan speed of 0.05°/s.
The crystallinity was evaluated in terms of crystallinity index from the Peak-height method as developed by Segal et al. (1962). Crystallinity index was calculated from the height ratio between the intensity of the crystalline portions to the total intensity of the sample. First, crystallinity index was calculated from the height ratio between the intensity of the crystalline peak ($I_{002} - I_{AM}$) and total intensity ($I_{002}$) after subtraction of the background signal measured without cellulose as illustrated in Fig. 4.11.

![Fig. 4.11 Schematic showing Peak-height method for calculating crystallinity index (Park et al., 2010)](image)
The formula for crystallinity index, CrI, is adapted from (Segal et al., 1962) as shown in Eq.4.5.

\[
\text{CrI} \, (\%) = \left( \frac{I_{002} - I_{AM}}{I_{002}} \right) \times 100
\]  

(4.5)

where, \(I_{002}\) is the maximum intensity of the 002 diffraction peak and \(I_{AM}\) is the minimum intensity of a peak representing the amorphous intensity. The results and analysis of X-Ray Diffraction Test has been described in the chapter 5.

### 4.6.4.2 Fourier Transform Infrared Spectroscopy (FTIR) Test

Several types of fungal wood decay are documented including white-rot, brown-rot and soft-rot (Blanchette, 1995). The Brown-rot fungi decay structural carbohydrates, with limited lignin degradation resulting in an increase in lignin composition. Worrall et al. (1997); Enoki et al., (1998). White-rot fungi generally decay all structural cell wall constituents at a similar rate, resulting in homogeneous cell wall decay. Some studies have reported that hemicellulose and lignin may be decomposed resulting in defibrillation through dissolution of the middle lamella. (Blanchette et al. (1995). Changes in wood chemistry resulting from these fungal decay types have been studied directly FTIR (Faix et al. (1991); Korner et al. (1992). FTIR analysis of wood samples is a fast method to identify structural and chemical changes induced by various degradation mechanisms since minimal sample preparation is required and very small quantities of wood can be analysed (a few milligrams). The powdered specimens of sanchi manuscripts of various time periods which were previously collected using Ball Mill Pulveriser were used as sample.

The powdered sample of the manuscript (2mg) and dried KBr (350 mg) were placed in an agate mortar and mixed properly and pulverized to obtain an uniform composition. The mixture was dried at 60°C for 4 hours and then poured into a tabletting mould to form transparent tablets. The infrared spectra were recorded by using a Nicolet IS-10 Fourier transform Infrared Spectrophotometer (FTIR) as shown in Photo 4.6.
The spectrophotometer was set to a resolution of $4\text{cm}^{-1}$ over a range of 4000-4000 cm$^{-1}$ with 100 scans per sample. Heights of peaks were measured from the baseline constructed by connecting the lowest data points on either side of the peak. A vertical line is then drawn from top of the peak to the X-axis as shown in Fig. 4.12. The portion of the line between the top of the peak and the baseline represents the corrected peak height (Pandey, 2005, Dobrică et al., 2008).
The measured spectra found for each sanchi manuscript sample were referred with a standard Infra-red Spectrum table for wood (Table 4.4) for determining the possible constituents of the wood-based samples corresponding to a particular absorption wavenumbers.

Spectra of the different aged manuscript samples show the characteristic bands of lignin and carbohydrates. The lignin content was determined from the C=C band intensity. Changes in FTIR band intensity and position is a good indicator of the chemical and structural changes of decayed wood. Wood degradation are commonly induced by fungal attack, bacterial attack, UV/Visible light irradiation or accelerated weathering.

Decrease in lignin content confirms the degree of microbial degradation. Fungal degradation results in a reduction of cellulose content therefore in an increase of relative amount of lignin.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Characteristic absorption wave numbers (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose I</td>
<td>663,1425</td>
</tr>
<tr>
<td>Xylan(Hemicellulose)</td>
<td>890,1735</td>
</tr>
<tr>
<td>Glucomannan</td>
<td>768,805,870</td>
</tr>
<tr>
<td>Lignin</td>
<td>1270,1505,1595,1670</td>
</tr>
<tr>
<td>Lignin with CH2 symmetrical bending</td>
<td>1460</td>
</tr>
<tr>
<td>Cellulose and Hemicellulose with CH bending</td>
<td>1372</td>
</tr>
<tr>
<td>Cellulose and Hemicellulose with C-O-C stretching and vibration</td>
<td>1163</td>
</tr>
<tr>
<td>Cellulose and Hemicellulose with C=O stretching and vibration</td>
<td>1058-1060</td>
</tr>
<tr>
<td>O-H stretch for amines, amides and hydroxyl compound</td>
<td>3300–3400</td>
</tr>
<tr>
<td>C-H stretch for hydrocarbon</td>
<td>2800–3000</td>
</tr>
<tr>
<td>Carbonyl group with C-O deformation for esters, amides, ketones</td>
<td>1100.00</td>
</tr>
<tr>
<td>Carbonyl group with C-O stretch</td>
<td>1319.62</td>
</tr>
<tr>
<td>Alkyl groups with C-H deformation on bonds of alkyl groups</td>
<td>896.92, 1056.05</td>
</tr>
<tr>
<td>Methyl groups with C-H deformation</td>
<td>560.30</td>
</tr>
</tbody>
</table>

Table 4.4  Infrared spectrum of wood (Marchessault, 1962)
4.6.4.3 Chemical treatment by EDTA disodium and alkaline hydrogen peroxide with silicon dioxide

EDTA disodium (Ethlenediaminetetraacetic acid, $\text{C}_{10}\text{H}_{16}\text{N}_{2}\text{O}_{8}$) is a cleaning agent and has the ability to dissolve metallic ions deposited as dirt on the surface of sanchi manuscripts. Moreover, EDTA disodium has the ability to soften up the brittle part of the manuscript and thereby consolidate the cellulose to a substantial extent.

EDTA disodium shows very less acidity (1.782) and a high pH value of 12 indicating is alkalinity to restore wood origin manuscripts.

The reasons for selecting EDTA disodium for this test due to the following properties:

- It exhibits low toxicity $2.0 - 2.2 \text{ g/kg}$.
- A chelating agent with fewer environmental pollution implications.
- It is fully biodegradable.
- It can be used as a decalcifying agent.
- It is used as a chelating agent preventing clumping of cells grown in liquid suspension, or detaching adherent cells.
- It is slime dispersant, and has been found to be highly effective in reducing microbial infestations.
- It is a food-grade preservative or stabilizer to prevent catalytic oxidative discoloration, which is catalyzed by metal ions.
- It is mainly used to sequester metal ions in aqueous solution. In the textile industry, it prevents metal ion impurities from modifying colours of dyed products.
Alkaline hydrogen peroxide (H₂O₂) solution acts as a belching agents and an established industrial process for bleaching for cellulosic materials. It increases the brightness of cellulose. The pH is alkaline and ranges from 11.75. Again, alkaline hydrogen peroxide is useful for removing stains of pigmentation without damaging the cellulose contents of the manuscript samples.

Silicon dioxide (SiO₂) has the following important properties which can be used for manuscript cleaning while making a solution with Alkaline hydrogen peroxide (H₂O₂)

- It is a Food grade substance i.e. non toxic
- Flow agent in powdered food
- Hygroscopic compound to absorb excess water
- It has Insect control properties
- It helps in filtration process.
- It is used as an abrasive for removing tooth plaque.
- It has light defusing property and natural absorbent property.
- It is also acts as a good dehumidifying agent.

Combination of these two chemical treatments on sanchi manuscript samples has shown very good result which may help preserving sanchi manuscripts for a longer time.

From tests like SEM, Stress-Strain test, XRD and FTIR, it has been ensured that the samples of sanchi manuscript had biodegradation. This degradation is mainly due to infestation of fungus. It is known to all that the biodeterioration is an irreversible process. It is not at all possible to re build the damaged cellulose, but eradication of fungus from the infested sanchi manuscripts and consolidation of the cellulose fibres is possible. Treatment with EDTA disodium and alkaline hydrogen peroxide may provide better result as these two solutions are proven bleaching and cleaning agents against infested cellulose.
i. Softening with the EDTA disodium

The samples of the infested sanchi manuscripts (Photo. 4.7) lost its usual natural elasticity and became hard, brittle with deposition of metal ions on the surface and with patch of stains from infestation of fungus. The idea to use EDTA disodium was to softening the surface of the manuscripts samples so that the metallic ions and stains could be removed.

Procedure:

- 20 gm of EDTA powder was dissolved with 400 ml of distilled water, stirred and the 5% EDTA disodium solution was made.
- 100 ml each of 5% EDTA disodium solution was taken in 4 beakers.
- 4 samples of infested sanchi manuscripts of different time periods, viz., 100 years old, 200 years old, 300 years old and 400 years old were taken for experiment.
- Samples were submerged in to the 5% EDTA disodium solution kept in 4 beakers for 24 hours for observation. (Photo 4.8)

The result has been analysed in the chapter 4.
Samples of infested Sanchi manuscripts of different time periods are treated with 5% EDTA disodium solution.

### ii. Alkaline Hydrogen peroxide and Silicon dioxide

As the Sanchi manuscript material is stronger than the paper and ink used generally hydrophobic, a solution of alkaline hydrogen peroxide and silicon dioxide may be used in liquid form as a bleaching agent.

**Procedure:**

- 50 mg of Silicon dioxide was mixed with 400 ml of 30% alkaline hydrogen peroxide to make a bleaching solution.
- 100 ml each of the solution were taken in 4 petri-dishes.
- Each sample treated with EDTA disodium were submerged in the bleaching solution one by one for one minute and observed.
- The samples were then covered with acid free blotting paper and kept pressed with heavy metal object till dried up.

The results have been analysed in the chapter 5.
4.7 Conclusions

The present study entailed data collection and analysis from different methods, such as, sampling, survey, interviews, observations and laboratory experimentation. Except random sampling technique which was used from secondary data source, other methods adopted were from primary data sources. The survey method informed us about the state of prevalent preservation techniques in the selected repositories of Assam, based on circulation of questionnaires. The questionnaire method was followed by the interview method where a pre-set checklist of questions prepared and was asked to the owners of caretakers.

The survey method was followed by the observation method. In this method a preset checklist of parameters was prepared to observe and examine the collections of manuscripts preserved in the 20 selected repositories of Assam, 2 archives and 1 library in Germany. The motive behind the selection of archives in Germany was to observe the state of the art technology employed for Egyptian, Greek, Arabic and Coptic collections preserved in those archives. The research work vehemently demanded such observations to be made on the spot to realise and understand facts and to document the evidences. Finally, the data collected regarding the state of preservation of manuscripts and causes of their deterioration were applied for understanding the intrinsic properties of manuscripts belonging to different time periods, through a series of physical, mechanical and chemical test. The physical test conducted was Scanning electron microscopy which focused on the surface morphology and type of microbial infestation. The mechanical tests suggested the loss of strength in manuscripts and the chemical tests hinted on the loss of crystallinity and cellulose degradation.

Treatment with EDTA disodium and alkaline hydrogen peroxide are proven bleaching and cleaning agents without harming the cellulose part of the manuscripts and expected to yield better result on eradication of fungus and also
to help to bring flexibility of the rigid surface and consolidation of the cellulose fibres of the treated sanchi manuscripts.