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IDENTIFICATION OF FIBRES AND SIZING OF TIMBUKTU MANUSCRIPTS USING POLARISED LIGHT MICROSCOPY AND FOURIER TRANSFORM INFRARED SPECTROSCOPY

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Abstract

The aim of this work was to determine the material composition as well as the optical characteristics of the fibres of the Timbuktu manuscripts. Analysis of the fibres was done using Polarized Light Microscopy (PLM) and sizing determination was done using Fourier Transform InfraRed (FTIR) spectroscopy. PLM was done with ordinary light, plane polarised light and cross polarised light as well. FTIR measurements were done in reflection mode by use of Attenuated Total Reflection (ATR). The sample fibres were observed to be colorless with joints, surface irregularities fractures and swollen tissues. The retardation color was found to be yellow, with a birefringence of 0.060. These optical properties are consistent with hemp fibres. The total crystallinity index of 0.2 and lateral order index of 0.35 were deduced for the sample fibre. FTIR results showed that the sizing on the manuscripts were kaolin, gelatin and clay. These results provide useful insight in planning for the conservation of the Timbuktu manuscripts.

Keywords: Polarised Light Microscopy, Fourier Transform Infrared spectroscopy, Timbuktu, sizing, cellulose fibres.

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1 INTRODUCTION

There are more than 700 000 manuscripts in public and private libraries in Timbuktu that are in different stages of degradation. Most of them are very brittle, both on the inked and un-inked sections of the manuscripts. This entire African intellectual legacy is on the verge of being lost. Long periods of exposure to harsh environmental conditions have left the manuscripts in a brittle state. Conservators are faced with a difficult task of how to conserve manuscripts whose ink and paper composition are not yet fully known. A quick Abbey pen test on the manuscripts has shown that they are extremely acidic, with a pH of less than 5 (Lojewska et al 2005). Their brittleness therefore may be due to acid hydrolysis on the fibre and filler material or sizing of the manuscripts. The nature of the fibres and sizing of the Timbuktu manuscripts has not yet been studied in all the literature surveyed. This paper therefore provides an analysis of the fibres and sizing of the Timbuktu manuscripts. The identification of the nature of the fibre and sizing material of the manuscript samples can be done using both Polarised Light Microscopy (PLM) and Fourier Transform Infrared Spectroscopy (FTIR). The degradation mechanism that is taking place in the manuscripts depend to some extent on the fibre and sizing material that make up the manuscripts.

The most common constituents of cellulosic paper that are found in ancient manuscripts are cellulose, hemicellulose and lignin (Garside and Wyeth 2003). These fibres have optical characteristics that can be used to uniquely identify them. Many transparent solids are optically isotropic, meaning that the index of refraction is equal in all directions throughout the crystalline lattice. Many polymers, including cellulose, and a wide variety of both organic and inorganic compounds are isotropic (Wood et al 1977; Stoiber 1994). A careful observation of the morphology, size, surface texture, extinction angle and sign of elongation together with the determination of refractive index and birefringence can be used to uniquely identify the nature of the natural fibres of the manuscripts. These optical properties can then be compared with those in literature for positive identification.

Spectroscopic techniques are often used to determine the chemical composition of material. One of the most popular spectroscopic techniques is FTIR because it requires little or no sample preparation. Cellulose has FTIR absorption peaks at 1365 cm^{-1} and 1315cm^{-1} assigned to δ(C-H) and 1155 cm^{-1} assigned to the asymmetric ring breathing as well as 1105cm^{-1} associated with the ʋ(C-O-C) glycosidic bonds (Edwards et al 1997). The FTIR absorption peak at 808$cm^{-1}$is associated with hemicellulose and the C=C in-plane aromatic vibrations at 1505cm^{-1} and 1595cm^{-1} are assigned to lignin. Most cellulosic material consists of crystalline and amorphous domains whose proportions depend on both history and source of the material. The physical properties, chemical behaviour and reactivity of cellulose depend on the arrangement of the cellulose molecules with respect to each other and with respect to fibre axis. It has also been shown that the higher the accessibility (and the lower the crystallinity) of cellulose, the higher the susceptibility to deterioration (Manso and Carvalho 2009). Most of the reactants can penetrate only the amorphous regions and it is only in these regions with a low level of order that the reactions take place leaving the crystalline regions unaffected. This therefore means that in order to check the degradation potential of the Timbuktu manuscripts, it is necessary to check the crystallinity of the cellulose that forms the matrix of the paper sample. This can be done by determining the Total Crystallinity and Lateral Order indices. The Total Crystallinity Index (TCI) is inferred from the ratio of the integrals of the FTIR band at 1370 cm^{-1} to that at 2900 cm^{-1} (Klemm et al 2001; Ivanov et al 1961). Lateral Order Index (LOI) is the ratio of the integrals of the FTIR band at 1420 cm^{-1} to that at 898 cm^{-1}. The two bands from which LOI is obtained are sensitive to the amount of crystalline versus amorphous structure in the cellulose. These bands broaden in a more disordered structure. Thus the smaller the LOI, the more ordered the cellulose structure. FTIR absorption band in the region 3600-3100 cm^{-1} is due to O-H stretching vibrations. The peaks characteristic of the hydrogen bonds from amorphous cellulose become sharper and are of lower intensity compared to the crystalline cellulose. In the amorphous cellulose, the peak is shifted to higher wave numbers. The band at 2900 cm^{-1} corresponds to the C-H stretching vibrations. The shift of this band to higher wave numbers also confirms the presence of amorphous cellulose. The shift is normally accompanied by a strong decrease in the intensity of this band. In addition, the intensity of the FTIR absorption band at 1430 cm^{-1} due to the CH_2 bending vibrations decreases due to reduction in crystallinity. This band is also called the crystallinity band. The absorption band at 898 cm^{-1} is called the amorphous band. This band is assigned to the C-O-C stretching at the β-(1-4)-glycosidic linkages. The intensity of this band increases in amorphous samples (Strlič and Kolar 1995).

Sizing refers to the process of imparting to paper some degree of resistance to the absorption or penetration of liquids. In paper production, sizing exists in two forms namely surface and internal sizing. Surface sizing entails application of chemicals to the surface of the paper after it has been formed rather than adding chemicals to the wet pulp. Internal sizing is used to describe the practice of adding chemicals to the aqueous slurry that contain cellulose fibres to improve paper hydrophobicity (Hubbe 2007). The sizing used gives an indication of when the manuscript or paper was made. The sizing used was different over time as knowledge and paper manufacturing technology changed. The identification of the sizing material in the manuscript can therefore be used to determine the technology used to make the manuscript and hence the period during which the manuscript was made. Over time the sizing material tend to react with environmental elements resulting in the production of degradation by-products. The degradation by-products in turn affect the stability of the manuscripts.

2 MATERIALS AND METHODS

2.1 Optical Characteristics

2.1.1 Sample Preparation

A glass dropper was used to transfer a small drop of the refractive index liquid of 1.55 onto the center of a cleaned microscope slide. The dropper was wiped on the edge of the refractive index bottle several times before quickly touching the dropper on the slide. This was done to ensure that only the minimum amount of the liquid was transferred to the microscope slide. The sample was placed on another pre-cleaned microscope slide and the slide holding the sample was put under the stereo microscope. Using a small steel needle, a single fibre was scratched off the paper sample and transferred onto the refractive index liquid drop previously put on the centre of the first slide. The surface tension helped wash the fibre off as the needle was withdrawn from the liquid. This process was repeated until a representative amount of fibre was transferred to refractive index liquid drop on the first slide. A pre-cleaned cover glass fragment was then used to cover the refractive index fluid droplet that contained the fibre samples. There was enough liquid to completely fill the area beneath the cover glass. The excess liquid was absorbed using a membrane filter fragment. The prepared slide was then placed onto the Olympus BX51 polarised light microscope stage and focused on a single fibre ready for observation.

2.1.2 Observation Using Ordinary Light

Morphology was the only property that was determined using ordinary light. The prepared slide was placed onto the microscope stage and focused on a single fibre. The general shape of the fibre was recorded together with a brief description of surface markings, texture, inclusions and surface roughness. Many fibres have characteristic morphology which can be recognized on sight. The observed characteristics were then compared with those documented in literature. The absorption colour was also recorded under transmitted light.

2.1.3 Observations Using Plane Polarised Light

Two fibre properties were determined using plane polarised light namely, pleochroism and refractive index. Pleochroism is a change in colour of a fibre relative to the vibration direction of the plane polarised light. Coloured fibres that are anisotropic (have more than one refractive index) exhibit different degrees of light absorption in different directions within the fibre resulting in the fibre showing different colours or different intensities of colour, when rotated in plane-polarised light. Ordinary light will not work for microscopic specimens such as fibres, because light vibrates in all directions and therefore the fibre will show the composite of all effects. This composite effect is not useful for sample analysis. For observing transparent coloured fibres, the stage was rotated at least 90^0. Alternatively, the polariser was rotated instead of the stage and the changes in colour or intensity were observed. The changes were recorded in a fibre identification characteristics sheet. Since fibres are elongated, the colour changes seen are related to the shape. If the fibre length was oriented east/west, that is, parallel to the polariser vibration direction, the observed colour was characteristic of the fibre length (Dell 2011). The stage was then rotated 90^0 so that the fibre was now oriented north/south, the colour of the fibre colour was also observed to change. The width was now parallel to the polariser vibration direction and the colour observed was now characteristic if the fibre width. Thus, two colours are recorded in the column under pleochroism. Fibres can also be uniquely identified by their refractive indices together with other optical properties. Solids may have more than one refractive index namely α, β and ɣ depending on the orientation of the fibre to the plane of polarization of the light. By definition, α is the lowest refractive index and ɣ is the highest. It should be noted that β being the intermediate can be closer to α or closer to ɣ. The determination of the refractive index of a solid is based on the premise that if the solid is placed in a liquid of equal refractive index, the solid will be invisible in that liquid. Thus, a repeated careful choice of refractive index liquids will result in the exact determination of the refractive index of a given fibre. This determination is done at a standard wavelength of 589 nm. In most cases however, it is sufficient just to know whether the refractive index of the fibre is greater than or lower than the medium in which it is mounted. This is done by using Becke lines. Becke line is a halo that forms around a particle because of using axial illumination achieved by closing the aperture diaphragm of the microscope. An orange interference filter (wavelength of 589 nm) was placed over the light exit port of the microscope so that all measurements were done at one wavelength. The Becke line halo is best produced with high numerical aperture objectives, usually with 40x/0.65 objective when a 589 nm filter is in place. The fibre image was brought into focus under these conditions. The fine adjustment was then suddenly turned in such a way that one was focused above the best focus. During this operation the Becke line will be seen to move either into the fibre or into the medium in which the fibre is mounted. On focusing above the best focus like this, the Becke line always moves towards whichever medium has the higher index. If one focuses below the best focus, the opposite effect occurs. The refractive index of the fibre was recorded as either greater than or less than the refractive index of the medium. The fibres were correctly oriented with respect to the polariser vibration direction.

2.1.4 Observations Using Crossed Polarisers

Three optical fibre properties were determined using crossed polarisers namely birefringence, sign of elongation and extinction angle. Birefringence was determined using the Michel Levy colour chart (Dell 2011). This required measuring the fibre thickness and estimation of the retardation using the colour chart. The fibre was rotated to a position of maximum brightness. At that position, the thickness of the fibre was measured directly by using the Stream particle characterization software. The retardation was obtained by matching colour at the point where the thickness is determined with the colour on the Michel Levy chart. The Michel Levy chart is actually a graphical solution to the birefringence equation up to the limit of 50µm of thickness. Going to the Michel Levy chart, one entered the thickness, moved along that thickness line to the right until one got to the retardation colour that the fibre was showing. There will be a diagonal line at the intersection of the thickness and retardation. Following the diagonal line up to the right, the birefringence can be read off. The sign of elongation refers to the orientation of high and low refractive indices relative to the direction of elongation of the fibre. A fibre which has high refractive index parallel to its length has positive sign of elongation and is described as ‘length slow’. Fibres which have low refractive indices parallel to their length have negative sign of elongation and are described as ‘length fast’. The sign of elongation was determined by use of a compensator. A compensator is a device with a known fixed retardation and vibration direction used to determine the amount of retardation of an anisotropic substance. The most common compensators are the first order red (530-550nm), quarter-wave (137-147 nm) and the Sernamont compensator. A fibre whose sign of elongation was to be determined was oriented 2 o’clock / 8 o’clock in the field of view (45^o to the cross hair) and viewed under crossed polarisers. The colour was noted and recorded. A quarter-wave compensator (137 nm) was inserted and the colour changes noted again. The compensator’s retardation of 137nm can either add to or subtract from the observed fibre colour. The retardations are subtractive when slow is on fast or fast is on slow. The colours go down the scale towards the lower retardation values on the Michel Levy chart. On the other hand, if the slow is on slow or fast side is aligned with the fast side the retardation will be additive. This will mean going up the scale on the Michel Levy chart. The compensator was inserted into the beam path at 45^o to the vibration direction of the polariser when it is at position 0^o. The compensator’s slow direction is 2 o’clock/8 o’clock. By aligning the fibre at 45^o to the eye piece cross hairs, the fibre length was made to be parallel to the compensator slow direction. Thus, if the fibre’s colour goes down on the Michel levy colour chart then it has negative sign of elongation. If the fibre’s colour goes up the chart, then it has positive sign of elongation.

In order to determine the fibre extinction angle, the slide was moved until the fibre was under the crosshairs. By rotating the stage, the fibre was lined up with either the north-south or east-west crosshair. This aligned the length of the fibre to the crosshair. If the fibre cannot be seen at this position, then it is at its extinction angle and the internal vibration directions lie parallel to the length of the fibre. This corresponds to the polariser and analyser vibration directions and the extinction angle is 0°. The extinction direction was then recorded as either parallel or perpendicular to the polariser direction.

2.2 FTIR MEASUREMENTS

The FTIR measurements were done in reflection mode by use of Attenuated Total Reflection (ATR). ATR is a reflection technique where the Infrared (IR) beam is directed through the internal reflection element (IRE) with a high index of refraction. The method requires no sample preparation. The IR light is totally reflected internally off the back surface which is in contact with the sample as shown in figure 2.1 below.

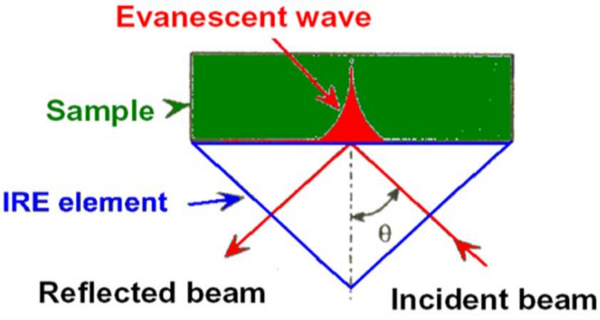

Fig.1. Single bounce attenuated total reflection (Setnička 2020)

The sample must have a lower index of refraction than the IRE to achieve total internal reflection.

Upon reflection at the IRE/sample interface, the IR light penetrates the sample to a small degree and the IR data from the sample is obtained. This evanescent wave that penetrates the sample decays exponentially. The depth of penetration is determined according to Setnička (2020). ATR has been used by Cain and Kalansky (1983) to determine gelatine size in paper samples.

A ZnSe ATR crystal was used on a Bruker FTIR machine. This crystal has a refractive index of 2.4 at wave number of 1000cm^{-1} and a cutoff spectral range of 520cm^{-1}. The penetration depth for this crystal is stated as 2μm meaning that it is a surface analysis instrument. The crystal was pressed against the sample to ensure that there is sufficient contact between the crystal and sample. The angle of incidence of the incoming IR for the ATR crystal is greater than the critical angle to ensure total internal reflection. This method is fairly routine, and the results are highly reproducible due to the controlled presentation of the sample to the IR radiation by ATR crystal. FTIR spectrum was collected for analysis for the Timbuktu manuscript sample and the results are presented in the following section.

3. RESULTS AND DISCUSSION

3.1 Optical Properties of the Fibres

The samples were mounted in a liquid whose refractive index was 1.55. It was observed by analysing the movement of the Becke lines that the refractive index of the fibre parallel to the fibre length ($n_{\parallel}$) was greater than 1.55. The refractive index of the fibre perpendicular to the fibre length ($n_{\perp}$) was less than 1.55. It was also observed that the fibre showed colour pleochroism and positive sign of elongation. Table 1 below shows a summary of the optical properties under both plane and cross polarised light.

Optical properties of sample investigated showing morphology, thickness at point of measurement (t, in μm), Retardation colour (Γ), Birefringence (Δn) fibre type, refractive index parallel to fibre length ($n_{\parallel}$) and refractive index perpendicular to fibre length ($n_{\perp}$).

TABLE 1

Sample name	Morphology	*t* *(μm)*	Retardation Colour	Δn	Fibre type	$n_{\parallel}$	$n_{\perp}$
Timbuktu	Joints, colourless, with surface irregularities, fractures and swollen tissues.	6.89	Brown, yellow	0.060	Hemp	>1.55	<1.55

The fibre type was deduced by direct comparison with the morphologies of known fibres (Cargille Chemical Microscopy set) as shown in Fig. 2(b).

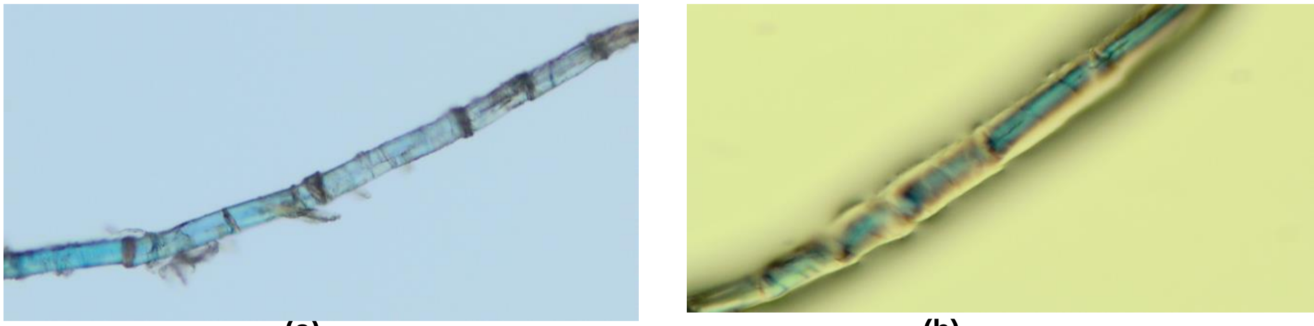
(a) (b)

Fig. 2. Micrograph of the fibre from (a) Timbuktu manuscript sample and (b) hemp reference fibre.

The fibre sample was observed to be colourless with surface irregularities. It also had joints, fractures and swollen tissues. The birefringence of fibres from the Timbuktu sample was found to be 0.060. These fibre properties are consistent with hemp as shown in Fig. 2 (a) and (b).

3.2 Fibre FTIR Results

The FTIR spectrum of the analysed sample is shown in Fig. 3.

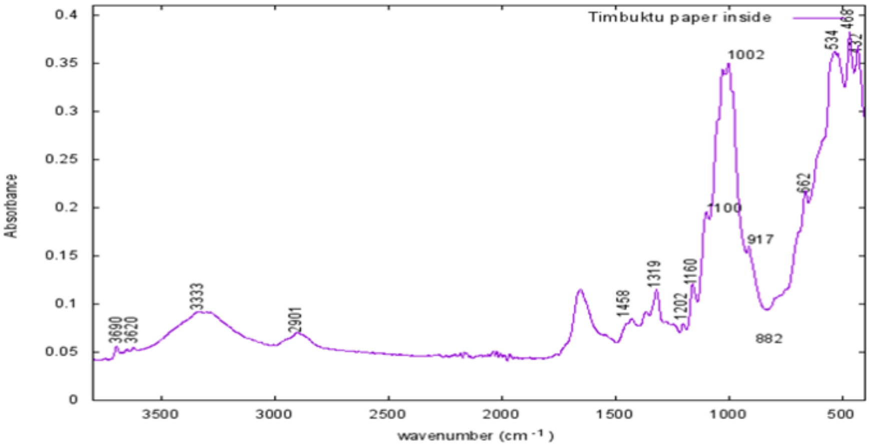

Fig.3. spectrum of the Timbuktu manuscript sample

The Timbuktu manuscript sample had absorption peaks at 1365 cm^{-1},1315 cm^{-1},1155 cm^{-1} and 1105 cm^{-1}. These absorption bands are associated with cellulose. The broad peak in the region 3600-3100 cm^{-1} is due to the O-H stretching vibrations and gives considerable information about the hydrogen bonds in the cellulose. The Timbuktu sample has a very broad and strong absorption in this region. This band is also shifted to higher wavenumbers peaking around 3333 cm^{-1}. This shows that the Timbuktu sample has a significant region that is made up of crystalline cellulose (Klemm et al 2001; Ivanov et al 1961). The Total Crystallinity Index (TCI) of the sample was also calculated in order to determine the susceptibility of cellulose to degradation agents. This index is the ratio of the integrals of the FTIR band at 1372 cm^{-1} to that at 2900 cm^{-1}. The integrals were taken over the ranges 1390 – 1339 cm^{-1} and 2959 – 2830 cm^{-1} for the bands at 1372 cm^{-1} and 2900 cm^{-1} respectively as shown in table. 2 below.

TABLE 2

Measurement of Crystallinity Indices TCI and LOI.

Sample name	I_{1372} (1339-1390) cm^{-1}	I_{2900} (2830-2959) cm^{-1}	TCI	I_{1420} (1458-1407) cm^{-1}	I_{1372} (917-882) cm^{-1}	LOI
Timbuktu	0.528	0.106	0.200	0.103	0.295	0.35

The Timbuktu sample has a value of Total Crystallinity Index (TCI) of 0.2. This means that about 20% of the cellulose making up the Timbuktu sample is crystalline. The TCI result seem to agree with the observed spectral modifications of the O-H stretching band. The value of the calculated Lateral Order Index (LOI) is shown in the last column of Table 2. This index is the ratio of the integrals of the FTIR band at 1420 cm^{-1} to that at 898 cm^{-1}. The integrals were taken over the ranges 1407 – 1458 cm^{-1} and 882 – 917 cm^{-1} for the bands at 1420 cm^{-1} and 898 cm^{-1} respectively. The two bands from which LOI is obtained are sensitive to the amount of crystalline versus amorphous structure in the cellulose. The smaller the LOI, the more ordered the cellulose structure. The sample has an LOI value of 0.35. LOI reflects the ordered regions perpendicular to the chain direction.

3.3 Sizing and Fillers

Table 3 below shows a summary of the filler material detected in the sample. There is strong indication that the sample has gelatine. This is because the sample has FTIR absorption bands of amide I and amide II bands at 1550 cm-1 and 1650 cm-1 respectively associated with gelatine. There is no evidence of starch which has absorption bands at 1148 cm-1, 995 cm-1, 933 cm-1 and 851 cm-1. There is evidence of clay in the sample shown by the doublet at 3690 cm-1 and 3620 cm-1 together with sharp peaks at 910 and 1000 cm-1. The sample also has absorption bands at 470 cm-1 and 524 cm-1. These are the characteristic absorption bands of kaolin.

A summary of filler material detected in the sample under investigation. The detected fillers are marked by a tick (✓) together with their observed absorption bands in cm^{-1}.

TABLE 3

Sample	Filler Absorption Peaks (cm^{-1})												
	Kaolin				Calcium Carbonate			Gelatine		Clay			
	1032	1004	524	470	1415	874	710	1650	1550	3690	3620	1000	910
Timbuktu	✓	✓	✓	✓			✓	✓	✓	✓	✓	✓	✓

It is highly improbable that the sample has calcium carbonate because only characteristic peak 710 cm-1 was observed. The other two characteristic absorption peaks could not be observed. Such an interpretation has merit because the presence of $CaCO_3$ could have neutralised the acidity of this sample.

4 CONCLUSION

The morphology as shown in table 2, birefringence and refractive index of the fibre showed that the fibre is most likely hemp. This was confirmed by direct comparison with a sample of hemp reference fibre from Cargille Chemical Microscopy set as shown if figure 2.

The sample fibre also showed FTIR characteristic absorption peaks of cellulose with Total Crystallinity Index of 0.2 and Lateral Order Index of 0.35. The Timbuktu sample also showed evidence of kaolin, gelatine and clay as the sizing material. Knowledge of composition of the manuscript will greatly assist conservators in formulating conservation strategies for these manuscripts.

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