

Research Article

Chemical and Morphological Characterization of Crinis Carbonisatus

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Abstract

Crinis Carbonisatus, prepared by pyrolysis of human hair, is known as a traditional Chinese medicine used for increasing blood clotting and wound healing. Its uses have been explored in literature but no detailed structural study is yet reported. This work is aimed at studying the chemical and morphological variation of Crinis Carbonisatus under given heating conditions. Crinis Carbonisatus was obtained after pyrolyzed of human hair at 300 °C in a sealed ceramic pot. The obtained samples were characterized in terms of their physicochemical properties by scanning electron microscopy (SEM), Fourier Transform Infrared (FTIR) spectroscopy and X-ray Diffraction (XRD). Distinct morphology with nanoparticulate structure was observed on the SEM micrograph. FTIR spectroscopy of the samples revealed the presence of functional groups like -OH, -COO-, and -NH as well as methyl (-CH₃) and methylene (-CH₂-) groups. The nanoparticulate graphitic form was confirmed by XRD. It has been found that with the increase in pyrolysis time; the amorphous nature of the Crinis Carbonisatus materials increases while their particle size decreases.

Keywords: Crinis Carbonisatus, FTIR, Human hair, Pyrolysis, SEM, XRD

Introduction

Hair is a keratin filament having three major compartments; cortex medulla and cuticle. It is a characteristic feature of mammals and usually grows to cover a body part or the whole. The cuticle is the

outer covering of hair fibre composed of overlapping flattened cells (Bhushan, 2010; Dawber, 1996). The cortex having cortical cells, comprises the middle layer of the hair which contains macro fibrils of the keratin and matrix proteins. The medulla, the innermost layer has cells

with air spaces and amorphous materials. This layer is responsible for the hardness of hair filaments. Overall, hair fibres are keratinized dead cells (Swiff, 1977). Biomolecules of the hair include keratin (alpha and beta) protein, matrix protein, lipid, melanin and water. In addition to basic organic elements carbon, oxygen, hydrogen, nitrogen and sulphur these biomolecules of hair contain trace metals like zinc, magnesium, copper, cobalt, chromium, and nickel (Rouse, 2010). The concentration of some of these metals may vary with age and sex as well as colour (Henry, 1969). Matrix protein contains 20 to 50 amino acids which are structured in helical form. Serine, cystine, methionine, threonine, glutamic acid, citrulline, cysteine, proline, tryptophan, glycine, alanine, valine etc. are the major amino acids. Functional groups like carboxylic acid (-COOH) and amino (-NH₂) group are present as peptide (CONH) group along with carbonyl (=CO) and imino (NH) group in amino acids. Among these, cystine is the most abundant amino acid in the hair filaments. Cystine imparts disulphide linkage between two nearby helical chains of protein. The matrix proteins having helical chains that are responsible for disulphide bonding are termed keratin-associated proteins. Although other binding structures like salt bond and hydrogen bonding are also present between two helices, disulphide bonding has the most remarkable effect (Rogers, 2006; Schweizer, 2006).

Despite having the same composition, the thickness and morphology of hair can vary with the geographical origin of the people. Human hair of Asian, African and Caucasian origin has diameters of 100 µm, 80 µm, and 50 µm respectively. Also, the outer cuticle of hair from an Asian has a more folded structure than that of others (Bhushan, 2010). Moreover, even a single strand of hair has a different structure on its tip, middle and near scalp region. Diameter and cuticle deposition gradually decrease from root to tip. Cuticle may even be absent in the tip due to mechanical stress, friction and combing (Wei et al., 2005).

Human hair is not bio-degradable and is considered a waste. Burning of hair in an open environment generates harmful gases like ammonia, carbonyl sulphide, hydrogen sulphide, sulphur dioxide, phenols, nitriles, pyrrole and pyridines. This also imparts unpleasant and foul odours on the locality (Brehu & Spiridon, 2011; Gupta, 2014; Robbin, 2012). However, controlled heat treatment of such

municipal waste provides an alternative way to minimize pollution. Heating rate, temperature and quenching phenomenon decide the resultant products. Pyrolysis and gasification are used to produce carbon products from waste such as fuels, chemicals, and solvents (Chen et al., 2015; Sorum et al., 2001). Pyrolysis is a method of changing any organic matter to obtain an array of solid, liquid and gas products by controlled heating. Based on the temperature range, pyrolysis is of three types; low temperature (< 550 °C) pyrolysis, moderate temperature (550 °C to 800 °C) pyrolysis and high temperature (> 800 °C) pyrolysis. Char, gas and tar are the final product of pyrolysis. Char from the waste has a large surface area and porosity which can be used for the manufacturing of active carbon (Klass, 1988; Savova et al., 2001).

Pyrolysis of human hair yields a black, shiny solid product at low and moderate temperatures. The use of pyrolyzed hair was first reported by a Chinese herbalist Li Shi-Zhen in his book *Ben Cao Gang* in the 16th century as a medicine. It was named *Xue Yu Tan* and termed *Crinis Carbonisatus* in English (Rouse, 2010). This is still used in some parts of South Asian countries for fast relief as well as long-term recovery of wounds (Chaudhari et al., 2014; Saurez et al., 2002; Qian et al., 2014)

Chemically modified pyrolyzed hair has been reported in the literature. Guo et al. (2016) prepared samples at 200°C by hydrothermal treatment and reported the presence of carbon, nitrogen and oxygen by X-ray photoelectron spectroscopy. Fourier Transform Infrared (FTIR) results of the same work revealed the presence of carboxylic, alkyl, aryl, and amino functional groups (Altuntaş et al., 2019, Shaikh, 2009). Chaudhari et al. (2014), and Qian et al. (2014), reported the crystalline nature of the pyrolyzed sample at 800 °C as confirmed by XRD. The crystalline behaviour of the sample increased with an increase in carbonization temperature (Akhtar et al., 1997).

However, Altuntaş et al. (2020) found that the crystalline nature decreases with an increase in pyrolysis temperature when the hair sample is activated by ZnCl₂. Transmission Electron Microscopy (TEM) images of hydrothermally prepared carbonized samples showed a quantum dot of size 2-10 nm. However, TEM images of the pyrolyzed sample (800 °C) revealed the presence of the micro/mesoporous channel-like texture. While

investigating the same sample by Scanning Electron Microscopy (SEM), images displayed graphite-like carbon flakes structure with a high level of disorder. This heteroatom-doped carbon material had dimensions in the nanometer range with high surface area and better porosity (Shaikh, 2009). Due to these properties, it is useful for intensifying oxygen reduction reactions in fuel cells and increasing the electrochemical performance in supercapacitors (Chaudhari, 2014; Qian et al., 2014).

Rodriguez et al. (2020) obtained the hair char from the Tannery process at 300 °C, 350 °C, 400 °C and 450 °C and used this for the removal of drugs amoxicillin (AMOX) and diclofenac (DFC) from wastewater. It has been found that physicochemically processed char at 450 °C removes more than 90% of AMOX and more than 80% of DFC. Anom & Lombok (2022) studied the reaction kinetics in the pyrolysis of human hair waste and found the activation energy values 81.769×10^3 kJ/mol and 30.487×10^3 kJ/mol for unwashed and washed samples, respectively.

The works so far reported have been carried out on chemically modified pyrolyzed human hair. However, works related to the structure and properties of *Crinis Carbonisatus* have not been reported in the literature so far. This work is focused on the pyrolysis of hair under different heating conditions to obtain the *Crinis Carbonisatus* without any chemical modification and hence on the investigation of the physicochemical properties of the substance.

Materials and Methods

Materials

Black-coloured human hair was collected from the donor (a seventeen-year-old young girl). A hot air oven, muffle furnace, narrow mouth ceramics pot with lid, mortar and pestle were used to pyrolyzed hair. Chemicals used in this work were acetone, ethanol and distilled water which were purchased from Thermo Fisher Scientific, India, Ltd.

Characterization techniques

FTIR spectroscopy: To study the vibrational nature of different bonding and functional groups of the sample, we carried out FTIR spectroscopic

measurements. For this, we utilized the IRTracer-100 spectrometer of the Shimadzu Company with the serial number A217053.

SEM: SEM experiments were carried out for both fresh neat hairs as well as pyrolyzed samples to study their morphology using an FEI NanoSem 200 (FEI, Eindhoven/The Netherlands) at 2 kV and a working distance of approximately 9.5 mm.

XRD: XRD was done to study the morphology, structure and crystalline behaviour of the samples. This was done by D₂ phaser Bruker X-ray Diffractometer with 0.154 nm X-ray.

Sample preparation

The hair sample was washed with double distilled water and then soaked in a mixture of ethanol and acetone (1:1) for 24 hours to remove any dye if present. After the cleansing process hair sample was dried at 60 °C in a hot air oven and cut into small pieces of size about 3 mm.

The hair sample was then pyrolyzed using a muffle furnace at controlled conditions. At first narrow-mouth ceramic pot with a tight lid was collected from the local market. The pot was heated on the burner to remove gaseous matter if any inside the pot. Then, the dried sample was kept in the pot and the lid was sealed using cement. At first, the sample was prepared by a gradual increase of temperature up to 600 °C in the presence of oxygen. It was carefully observed and found that there was no gas evolution at 200 °C. So, the temperature was further increased and the fume started at 240 °C and stopped at 400 °C. When the sample was heated for 5 hours at 600 °C and then cooled gradually to room temperature, the resultant material was a white powder.

Therefore, it was decided to prepare a pyrolyzed sample at 300 °C to get sample properties like *Crinis Carbonisatus* as mentioned in Ben Cao Gang. Three different samples named PH-1, PH-2, and PH-24 were prepared at 300 °C with different time intervals. PH-1 was heated for 1 hour, PH-2 was heated for 2 hours and PH-24 was heated for 24 hours to study the structural variation with different time frames. Thus, we used a ceramic pot to prepare the samples, instead of the closed iron vessel used to prepare *Crinis Carbonisatus*. These samples were then characterized.

The yield of the substance was calculated using the formula:

$$\text{"\% yield} = \text{wt. of product} / \text{wt. of hair sample} \times 100\text{"}$$

After pyrolysis, samples named PH-1, PH-2, and PH-24 were obtained. All samples were solid, black and glassy in texture. They were porous with some blisters beneath the surface. The average yield of the sample was found 35 %. It was found that the sample was insoluble in water but stable dispersion formed in glycerol.

Results and Discussion

The SEM image in Figure 1 shows the morphology of the hair filament (PH-0) at different magnifications. The low-magnification image shows that the hair has a diameter of 100 μm . This is similar to the diameter of other Asian hair used by Wei et al. (2005). When the same filament was observed at higher magnification, the well-developed folded structure of the cuticle surrounding the whole filament was seen.

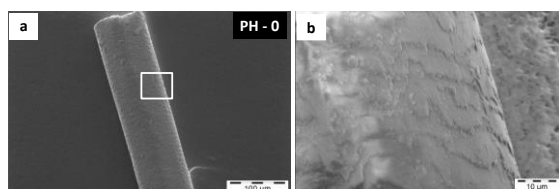


Figure 1: SEM images of the neat hair filament (PH-0) showing its morphology (a) at low magnification and (b) at high magnification.

Further, SEM was carried out on samples PH-1, PH-2 and PH-24 to study the structural variations on samples as the function of pyrolysis time. SEM images of these samples at low magnification reveal that all samples have similar morphological arrangements with irregular, wrinkled and uneven patterns consistent with previous reports (Qian et al., 2014). SEM micrographs also indicate that human hair changes into a crystal-like form after pyrolysis with some porous structure embedded in it. The morphological structures as well as arrangement are similar for PH-1 and PH-2 as shown in Figure 2.

However, the sample PH-24 at high resolution shows some crystal-like behaviour having a lamellar flake-like structure (Figure 3). These flakes are similar to cuticle flakes of untreated hair filaments whose structure may not have been lost during

pyrolysis or the sample might have converted to graphitic form with the increasing time duration of pyrolysis. This indicates the trend of irregular structure of the sample gradually becoming regular with an increase in the heating time interval from sample PH-1 to PH-24. Similar results were reported in earlier work (Saurez et al., 2002).

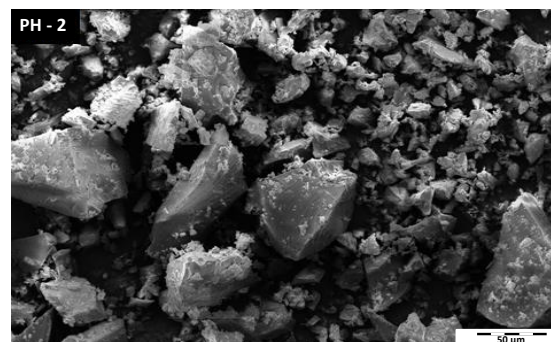


Figure 2: SEM image of PH-2 shows a crystalline-like appearance with some irregular crystal-like structure.

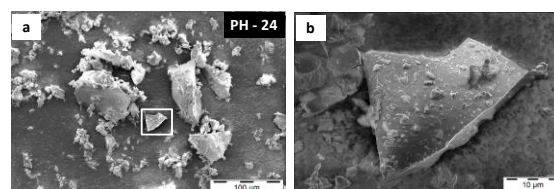


Figure 3: SEM images of PH-24 in Fig. 3a show the lamellar flake-like morphology which in magnified form in Fig. 3b shows the graphite-like flake structure.

FTIR spectroscopy is generally used to characterize the functional group of organic materials. Different bands are associated with the stretching and bending vibration of the different functional groups as can be seen in FTIR spectra in Figure 4. Three FTIR spectra of PH-1, PH-2 and PH-24 have many common peaks with different transmittance levels. The absorption band at 3750 cm^{-1} is associated with the N-H stretching which may be due to improper pyrolysis of the hair fiber. All the carbon samples have a wide band around 3250 cm^{-1} due to -OH stretching in hydroxyl functional groups (Liu et al., 2005).

Asymmetric and symmetric aliphatic methyl CH_3 can be reported by the band around 2924 cm^{-1} while the absorption peak at 2862 cm^{-1} is due to the aliphatic methylene $-\text{CH}_2-$ group (Sharma et al., (2002). The bands from 2800 cm^{-1} to 3000 cm^{-1} are due to the C-H stretching vibration and bands due to their deformation vibration generally appear from 1350 cm^{-1} to 1500 cm^{-1} (Grierson et al., 2011, Yorgum et al., 2001).

The absorption band at 2360 cm^{-1} is associated with the stretching of -C=C- bonding and 1604 cm^{-1} reveals the presence of -COO^- group, the aromatic and olefinic -C=C- vibrations in the aromatic region as well as carbonyl group -C=O- can be noted by the absorption band from 1600 cm^{-1} to 1800 cm^{-1} (Grierson et al., 2011; Jindo, 2014, Yorgum et al., 2001). Aromatic -C=C-C- and -C-H functional groups with plane bends in a ring stretching are justified by a 1454 cm^{-1} band. The OH stretching vibration of the phenol is reported between 1401 cm^{-1} to 1310 cm^{-1} (Sharma et al., 2002). The band due to functional groups -OH , -COO^- at 3749 cm^{-1} , 1605 cm^{-1} and 1365 cm^{-1} ascertains the presence of the carboxylic acid and its derivatives in the pyrolyzed sample (Altuntaş et al., 2019, Grierson et al., 2011). A peak around 750 cm^{-1} confirmed the presence of the sulphides along with monocyclic and polycyclic aromatic groups in the carbonized samples (Sharma et al., 2002) and Si-O-Si can be assigned to the 769 cm^{-1} (Apaydin & Putun, 2012) the peak 671 cm^{-1} marked the presence of bending peak of -COO^- group in all samples (Herrera & Voladez, 2005).

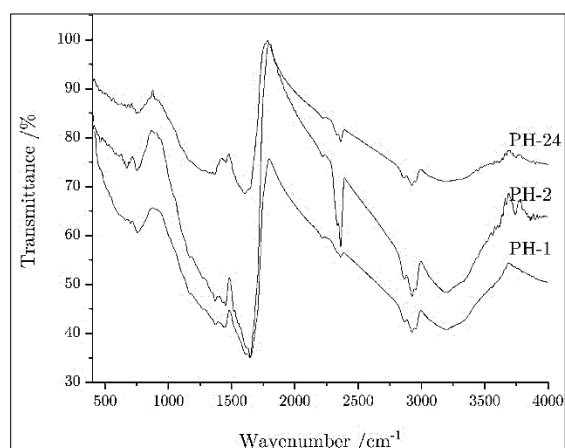


Figure 4: FTIR spectra of the Crinis Carbonisatus samples PH-1, PH-2 and PH-24 showing the presence of different functional groups.

The presence of peaks for sulphur, nitrogen, oxygen and hydrogen shows that although pyrolysis has brought a physical and chemical change in hair, the sample is not completely carbonized. The band of the N-H bond indicates that some parts of the protein moiety are still intact. In addition, the S-S bond between the cysteine residues shows that the footprint of the secondary structure of keratin is retained. In this regard, the presence of more stable groups such as hydroxyl, carbonyl and carboxyl in the pyrolyzed sample is expected. The peaks for these groups do not change significantly for all the

samples; suggesting that at $300\text{ }^{\circ}\text{C}$, energy is not sufficient to onset the decomposition and the process is not rate controlled.

XRD spectra of the samples are given in Figure 5. Both samples PH-2 and PH-24 show one distinct peak. The peak at 26.8° for PH-2 slightly shifts to 25.8° for PH-24; this corresponds to an increase in the d spacing from 0.332 nm to 0.344 nm . This is in the range of graphite interlayer spacing distance (Sgriccia et al., 2008). The increase in interlayer spacing is associated with the addition of defects in the graphite lattice which leads to increased amorphous nature by pushing the layers away from each other (Bacon, 1951). This is also evident from the change of the peak profile from sharp and narrow to weak and broad. Assuming that the Scherrer relation holds, the particle size for PH-24 has decreased to 2.45 nm from 142.47 nm of PH-2. XRD results for increasing pyrolysis time show that the amorphous nature does not change significantly, similar results were also found by Altuntaş et al. (2020) for different pyrolysis temperatures.

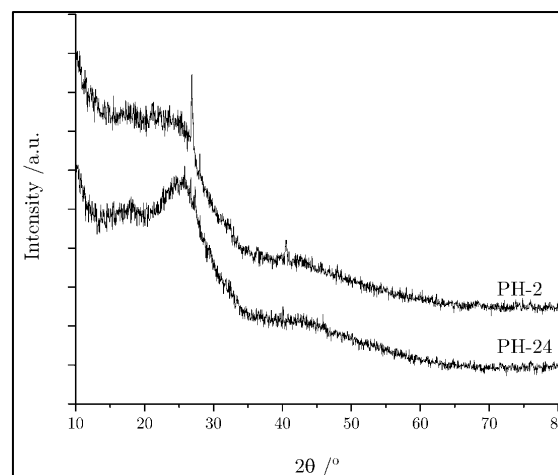


Figure 5: XRD spectra showing graphite interlayer spacing peaks at $26.8\text{ }^{\circ}\text{C}$ and 25.8° as indicated.

Conclusion

In this study, we characterized the human hair as well as its pyrolyzed form (that is, the Crinis Carbonisatus). Morphology of the Asian hair showed the laminar, folded structure of the cuticle with a diameter of $100\text{ }\mu\text{m}$ which in pyrolysis form shows the laminar structural appearance with distinct fracture. FTIR showed the presence of N-H, C=O, O-H, C-H, -CH_2 and other functional groups in the pyrolyzed form of hair. XRD peaks confirmed the presence of the graphitic form which can be seen

in SEM. Moreover, pyrolysis was found to convert the micron-sized particles into nanometric entities as evidenced in SEM as well as XRD results. Although Crinis Carbonisatus is mentioned as traditional Chinese medicine, here we cannot refer to its use as medicine. However, for pharmaceutical purposes, further elemental analysis as well as different biological tests are needed.

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