Chemical and Morphological Characterization of *Crinis Carbonisatus*

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Research

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

Posted Date: August 26th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-60438/v1

Abstract

Background

Crinis Carbonisatus, prepared by pyrolysis of human hair, is traditional Chinese medicine used for increasing blood clotting and wound healing. Its use is explored in literature but no detailed structural study is reported.

Objective

This work is aimed at studying the chemical and morphological variation of *Crinis Carbonisatus* under given heating condition.

Materials and methods

Crinis Carbonisatus was obtained after pyrolysis of human hair at 300 °C in sealed ceramic pot. The obtained samples were characterized in terms of its physicochemical properties by scanning electron microscopy (SEM), Fourier Transform Infrared (FTIR) spectroscopy and X-ray Diffraction (XRD).

Results

Distinct morphology with nano-particulate structure was observed on SEM micrographs. FTIR spectroscopy of the samples revealed the presence of functional groups like –OH, $-COO^-$, -NH as well as methyl ($-CH_3$) and methylene ($-CH_2-$) groups. Graphite interlayer spacing peak appeared in XRD pattern only after 24 h of pyrolysis.

Conclusion

Pyrolysis converts the micron sized particles into the nanometric entities. Amorphous behavior of the materials decreases with the increase in pyrolysis time.

Background

Hair is a keratin filament having three major compartments; cortex medulla and cuticle. It is the characteristics feature of the mammals and usually grows to cover a body part or the whole. Cuticle is the outer covering of hair fiber composed of overlapping flattened cells [1, 2]. Cortex having cortical cell, comprises middle layer of the hair which contain macro fibrils of the keratin and matrix proteins. Medulla, the innermost layer has cells with air spaces and amorphous materials. This layer is responsible for hardness of hair filaments. Overall, hair fibers are keratinized dead cells [3, 4]. Biomolecules of the hair includes 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 [5]. Concentration of some of these metals may vary with age and sex as well as color [6]. Matrix protein contains 20 to 50 amino acids

which are structured in helical form. Serine, cystine, methanoine, theorine, glutamic acid, citrulline, cysteine, proline, tryptophan, glycine, alanine, valine etc. are the major amino acids. Functional groups like carboxylic acid (-COOH) and amino ($-NH_2$) 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 chain of protein. The matrix proteins having helical chains that are responsible for disulphide bonding are termed as keratin associated proteins. Although other binding structure like salt bond and hydrogen bonding are also present between two helices, but disulphide bonding has the most remarkable effect [7, 8].

Despite having same composition, thickness and morphology of hair can vary with geographical origin of the people. Human hair with Asian, African and Caucasian origin has diameter 100 μm, 80 μm, and 50 μm respectively. Also, the outer cuticle of hair from an Asian has more folded structure than of others [1]. Moreover, even a single strand of hair has different structure on its tip, middle and near scalp region. Diameter and cuticle deposition gradually decreases from root to tip. Cuticle may even be absent in the tip due to mechanical stress, friction and combing [9].

Human hair is not bio-degradable and is considered as a waste. Burning of hair in open environment generates harmful gases like ammonia, carbonyl sulphide, hydrogen sulphide, sulphur dioxide, phenols, nitriles, pyrrole and pyridines. This also imparts unpleasant and foul odor on locality [10, 11, 12]. However, controlled heat treatment of such municipal waste provides an alternative way to minimize the pollution. Heating rate, temperature and quenching phenomenon decides the resultant products. Pyrolysis and gasification are used to produce carbon product from waste such as fuels, chemicals, and solvents [13, 14]. 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 are 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 the pyrolysis. Char from the waste has large surface area and porosity which can be used for the manufacturing of active carbon [15, 16, 17].

Pyrolysis of human hair yields a black, shiny solid product at low and moderate temperature. Use of pyrolyzed hair was first reported by a Chinese herbalist Li Shi-Zhen in his book Ben Cao Gang in 16th century as a medicine. It was named as Xue Yu Tan and termed as *Crinis Carbonisatus* in English [6, 18]. This is still used in some parts of South Asian countries for fast relief as well as long term recovery of wound [19, 20, 21, 22].

Chemically modified pyrolyzed hair has been reported in literature. Guo, Y. *et.al.* (2016) prepared sample at 200 °C by hydrothermal treatment and reported the presence of carbon, nitrogen and oxygen by X-ray photoelectron spectroscopy. Fourier Transform Infrared (FTIR) result of the same work revealed the presence of carboxylic, alkyl, aryl, and amino functional group [23, 24, 25]. Qian, W. *et.al* (2014) and Chaudhari, K.N. *et.al* (2014) reported the crystalline nature of the pyrolyzed sample at 800 °C as confirmed by XRD. Crystalline behavior of sample increased with increase in carbonization temperature [19, 20, 26].

However, Altuntaş, D.B. *et.al* (2019) found that the crystalline nature decreases with increase in pyrolysis temperature when the hair sample is activated by $ZnCl_2$ [24]. Transmission Electron Microscopy (TEM) images of hydrothermally prepared carbonized sample showed the quantum dot of size 2–10 nm. However, TEM images of the pyrolyzed sample (800 °C) revealed the presence of the micro/meso porous channel like texture. While investigating the same sample by Scanning Electron Microscopy (SEM), images displayed graphite like carbon flakes structure with high level of disorder. This heteroatom doped carbon material had dimension in nanometer range with high surface area and better porosity [23]. Due to these properties, it has been found to be useful for intensifying oxygen reduction reaction in fuel cells and to increase the electrochemical performance in super capacitor [20, 21].

The works so far reported have been carried out on chemically modified pyrolyzed human hair. However, works related to structure and properties of the *Crinis Carbonisatus* have not been reported in 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 investigation of physicochemical properties of the substance.

Materials And Methods

Materials: Black colored human hair was collected from the donor (a seventeen year young girl). 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*: In order to study the vibrational nature of different bonding and functional groups of the sample, we carried out FTIR spectroscopic measurement. For this, we utilized IRTracer-100 spectrometer of the Shimandzu Company with the serial number A217053.

SEM: SEM experiments were carried out for both fresh neat hairs as well as pyrolyzed samples in order to study their morphology using a 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 morphology, structure and crystalline behavior of the samples. This was done by D_2 phaser Bruker X-ray Diffractometer with 0.154 nm X-ray.

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

The hair sample was then pyrolyzed using muffle furnace at controlled condition. At first narrow mouth ceramics pot with tight lid was collected from the local market. The pot was heated on burner to remove gaseous matter if any inside the pot. Then, dried sample was kept on the pot and the lid was sealed using

cement. At first sample was prepared by gradual increase of temperature up to 600 °C in 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 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 the room temperature, resultant material was white powder. Therefore, it was decided to prepare 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 frame. Thus, we used ceramic pot to prepare the samples, instead of closed iron vessel as used to prepare *Crinis Carbonisatus.* These samples were then characterized.

The yield of the substance was calculated using formula

$$\%\ Yield = \frac{Weight\ of\ product\ obtained}{Weight\ of\ hair\ sample\ used} \times 100\%$$

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 blister 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

SEM image in Fig. 1 shows the morphology of the hair filament (PH-0) at different magnifications. 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. G *et al* (2005). When the same filament was observed at higher magnification, well developed folded structure of the cuticle surrounding the whole filament is seen [9].

Further, SEM was carried on samples PH-1, PH-2 and PH-24 in order to study the structural variations on samples as the function of pyrolysis time. SEM image of these samples at low magnification reveal that all samples have similar morphological arrangements with irregular, wrinkled and uneven patterns consistent with previous reports [20]. SEM micrographs also indicate that human hair changes into crystal-like form after pyrolysis with some porous structure embedded on it. The morphological structures as well as arrangement are similar for PH-1 and PH-2 as shown in Fig. 2.

However, sample PH-24 at high resolution shows some crystal like behavior having lamellar flake- like structure. These flakes are similar to cuticle flake 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 increase in heating time interval from sample PH-1 to PH-24. Similar results were reported in earlier work [19].

FTIR spectroscopy is generally used to characterize the functional group of the 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 Fig. 4. Three FTIR spectra of PH-1, PH-2 and PH-24 have many common peaks with different transmittance level. 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 [27]. 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 aliphatic methylene -CH_2)-group [28]. The bands from 2800 cm^{-1} to 3000 cm^{-1} is due to the C-H stretching vibration and bands due to their deformation vibration generally appears from 1350 cm^{-1} to 1500 cm^{-1} [29, 30].

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 aromatic region as well as carbonyl group -C = O- can be noted by the absorption band from 1600 cm^{-1} to 1800 cm^{-1} [29, 30, 31]. Aromatic -C = C-C- and -C-H functional groups with a plane bends in a ring stretching are justified by 1454 cm^{-1} band. The OH stretching vibration of the phenol is reported between 1401 cm^{-1} to 1310 cm^{-1} [28]. 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 [24, 29]. Peak around 750 cm^{-1} confirmed the presence of the sulphides along with monocyclic and polycyclic aromatic groups in the carbonized samples [28] and Si-O-Si can be assigned to the 769 cm^{-1} peak [32]. 671 cm^{-1} marked the presence of bending peak of -COO^- group in all samples [33].

The presence of peaks for sulphur, nitrogen, oxygen and hydrogen shows that although pyrolysis has brought physical and chemical change in hair, the sample is not completely carbonized. The band of 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 secondary structure of keratin too 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 °C, energy is not sufficient to onset the decomposition and the process is not rate controlled.

XRD spectra of the sample PH-2 is given in Fig. 5. It shows two major peaks at 26.8º and 40.5°. Similar nature of spectra with peaks at 25.8º and 43° were obtained also in PH-24. The characteristic peak of graphite interlayer spacing (0.34 nm) peak at 26.8º shows that the sample contains predominately graphitic form. This graphitic form appeared as flake like morphology in SEM micrograph (Fig. 3b). Peak broadening when the heating time is increased shows that the particle size decreases when the sample is heated for longer time. Assuming that Scherrer relation holds, the particle size for PH-24 has decreased to 2.45 nm from 142.47 nm of PH-2. Moreover, increase in sharpness in spectrum indicates the formation of some crystal-like regular arrangement as supported by SEM image, with some fraction of amorphous structure. XRD results shows for increasing pyrolysis time shows that the amorphous nature does not change significantly which is far different than the results found by Altuntaş, D.B *et.al* (2019) [24, 34, 35].

Conclusions

In this study, we characterized the human hair as well as its pyrolyzed form (the *Crinis Carbonisatus*). The main findings are as follows:

1. Morphology of the Asian hair showed the laminar, folded structure of cuticle with diameter 100 μm which on pyrolysis form shows the laminar structural appearance with distinct fracture.
2. 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.
3. Moreover, pyrolysis was found to convert the micron sized particles into the nanometric entities as evidenced in SEM as well as XRD results.
4. Although, the *Crinis Carbonisatus* is mentioned as traditional Chinese medicine, here we cannot refer it to use as medicine. However, for pharmaceutical purposes, further elemental analysis as well different biological tests are needed.

Abbreviations

FTIR: Fourier transform infrared (spectroscopy)

PH-0: Non pyrolyzed neat human hair

PH-1: Pyrolyzed human hair at 300 ℃ for 1 hour

PH-2: Pyrolyzed human hair at 300 ℃ for 2 hour

PH-24: Pyrolyzed human hair at 300 ℃ for 24 hour

SEM: Scanning electron microscopy

TEM: Transmission electron microscopy

XRD: X-ray diffraction

Declarations

Ethical approval:

Not applicable.

Consent for publication:

Not applicable.

Availability of data and material:

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing Interest:

The authors declare that they have no competing interests.

Funding:

No funding was sought or obtained.

Author's contributions:

RA, NSP and TBK designed experiments and provided supervision of the work. TRB, BL, ML and PP performed experiments and drafted the first manuscript. All the authors read, gave feedbacks to the manuscript draft and approved the final version.

Acknowledgements:

We express our kind gratitude to Nepal Academy of Science and Technology (NAST), Khumaltar, Kathmandu, Nepal for FTIR and XRD studies.

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Figures

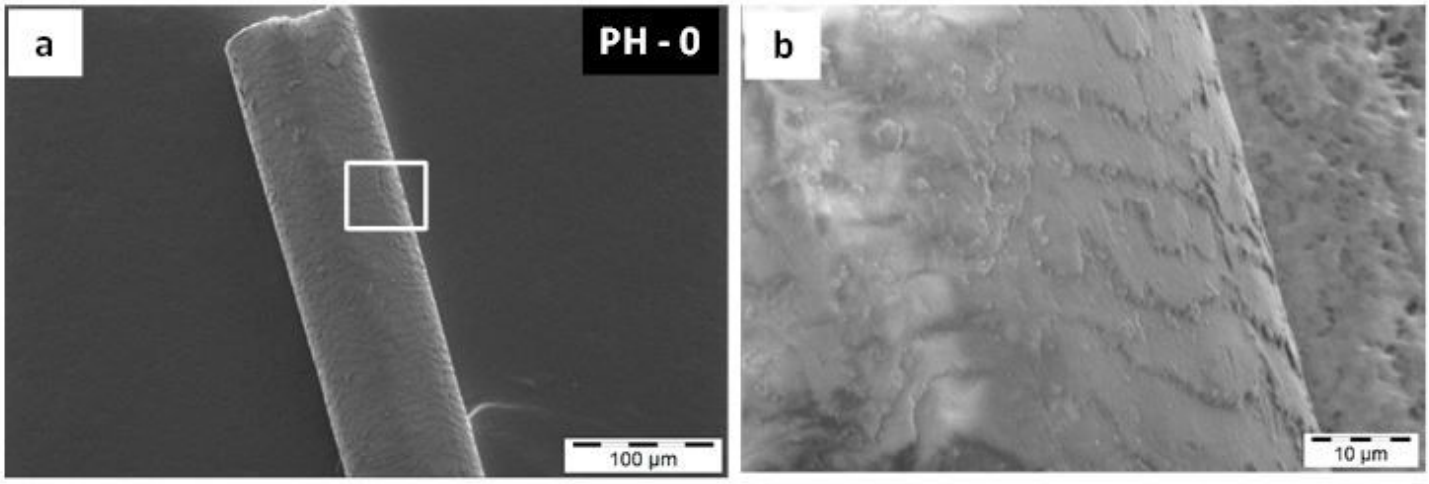

Figure 1

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

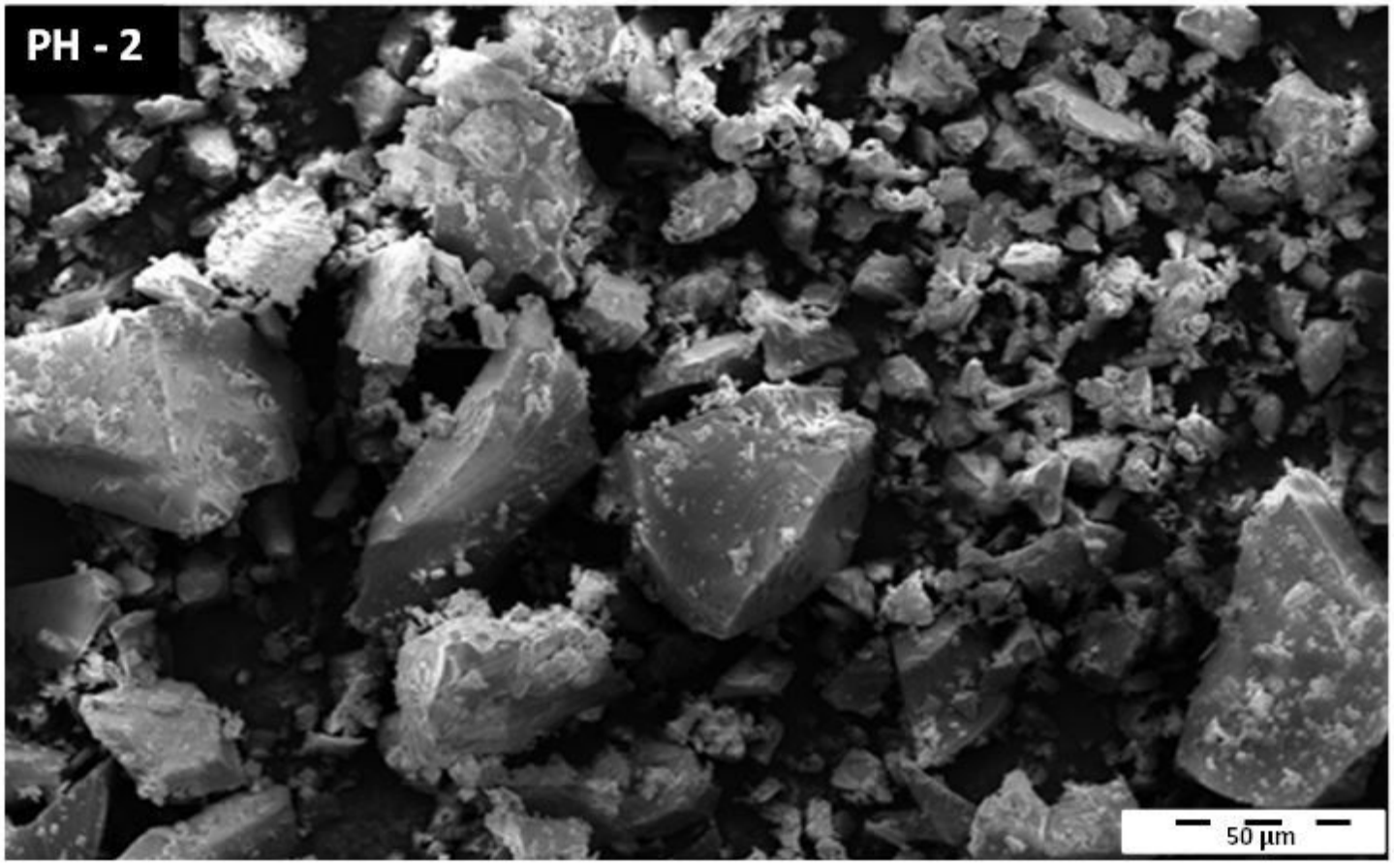

Figure 2

SEM image of PH-2 shows the crystalline like appearance with some irregular crystal-like structure

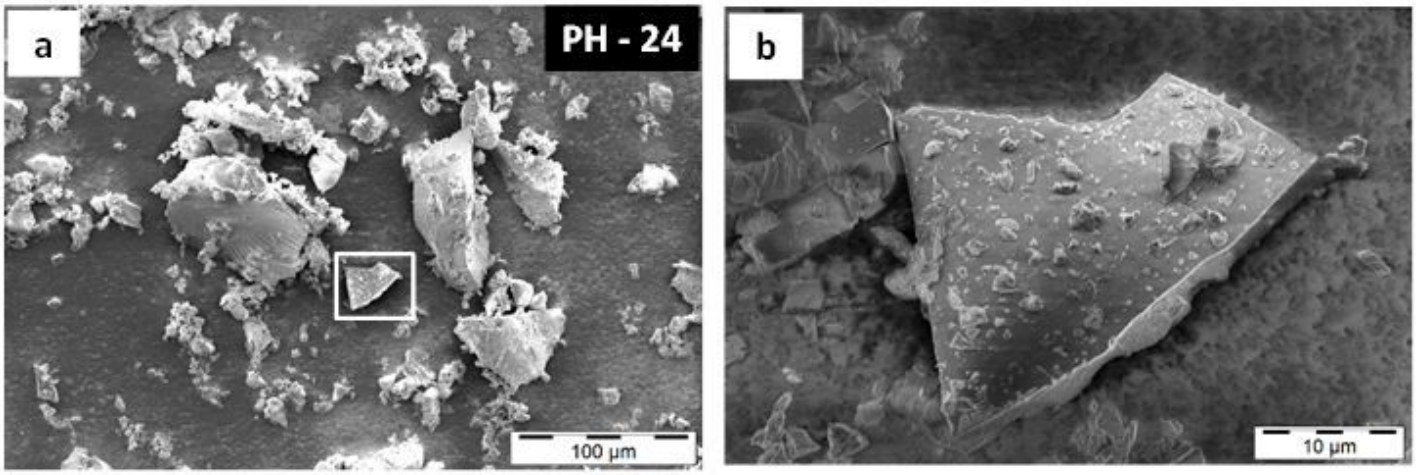

Figure 3

SEM images of PH-24 in 3a shows the lamellar flake-like morphology which on magnified form in 3b shows the graphite like flake structure

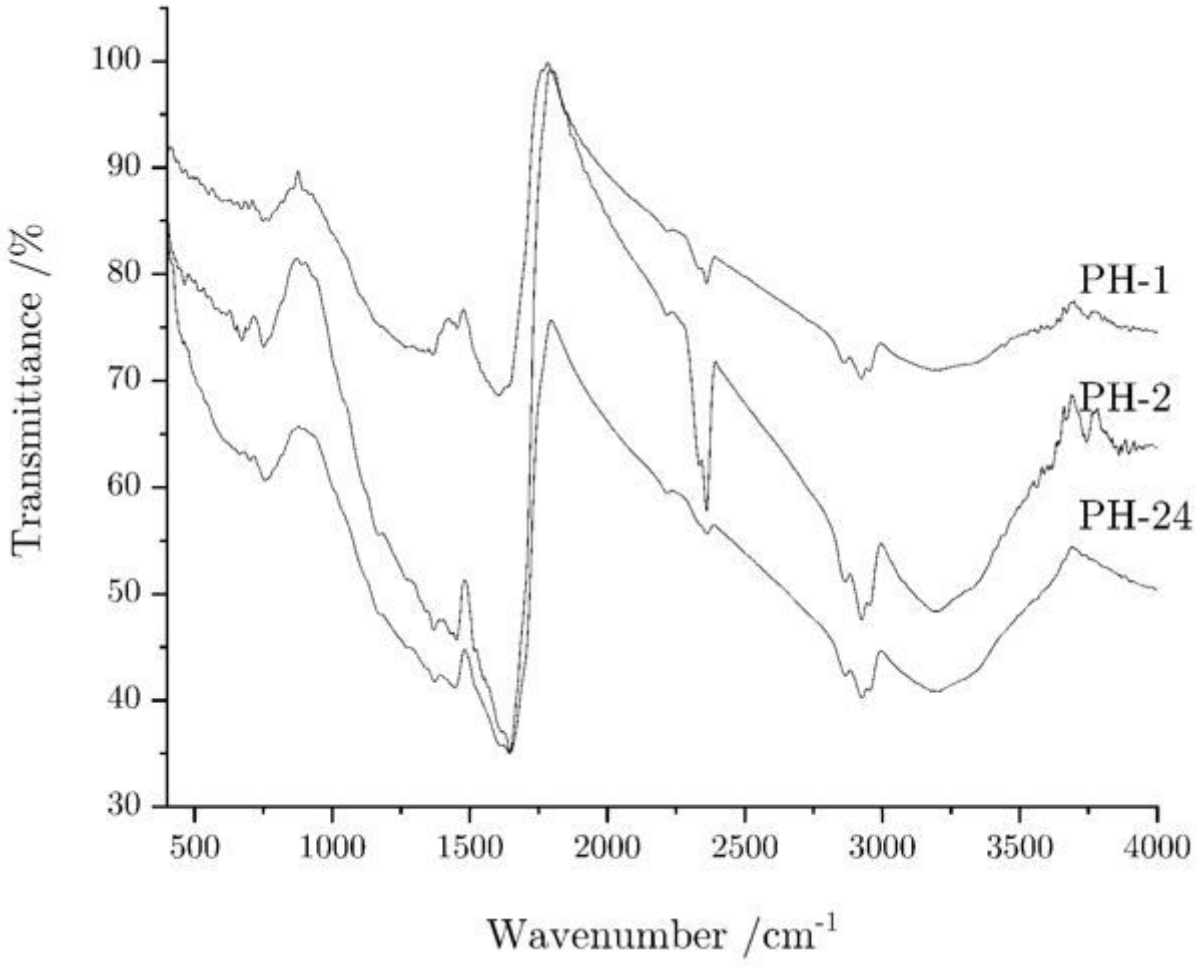

Figure 4

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

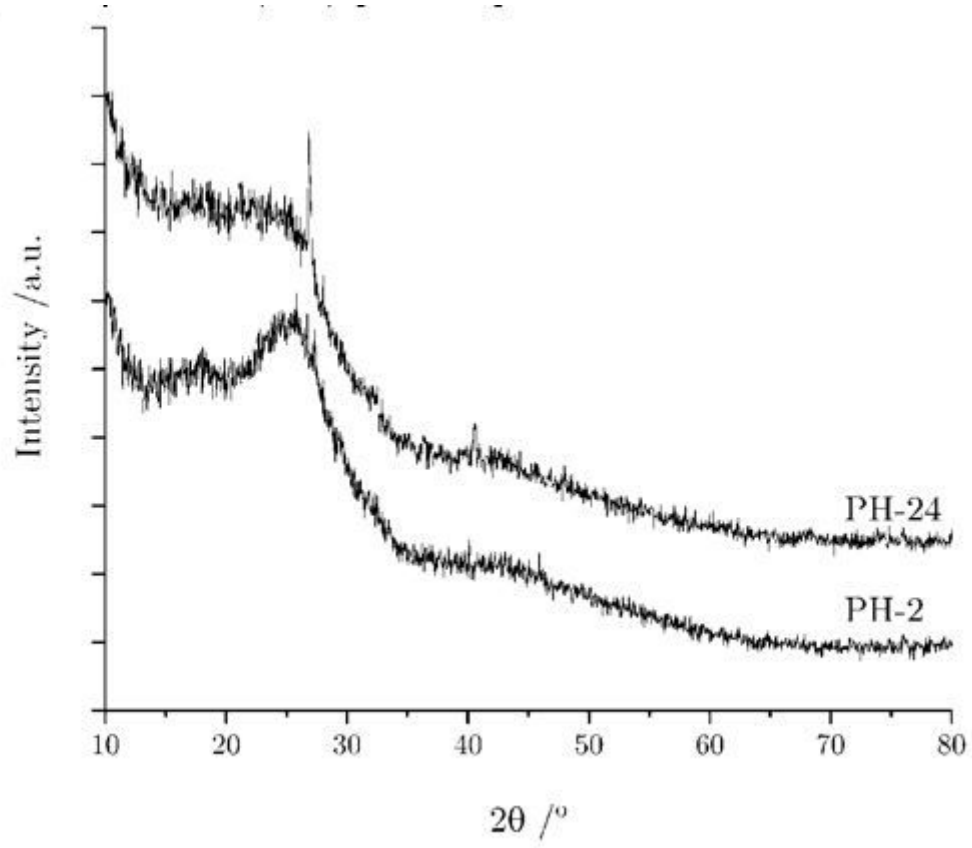

Figure 5

XRD spectra showing graphite interlayer spacing (0.34 nm) peak at 26.8 °C