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Fourier Transform Infrared Spectroscopy for Sepia Melanin

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Abstract Melanin is of interest as a model system of understanding disorder in biological systems. The biological functionality of melanin depends on disorder which is considered as its essential part. This property distinguishes melanin from other much more intensively studied biomolecule systems such as nucleic acid, proteins and carbohydrates. Melanins have been reported to have a diverse number of functions in the biosystem, including photosensitization, metal ion chelation, photoprotection to absorb a broad range of electromagnetic radiation, antibiotic, thermoregulation. Melanins are found all over the body from the skin and blood to the nervous system but the role of melanin in all these system is unclear. FTIR spectroscopy technique is usually one of the most preferred techniques used to give a correct assignment of the observed spectral characteristic of functional groups corresponding to different absorption bands which are responsible of the absorption. FTIR is the characterization technique which is both rapid, non-destructive and requires small sized samples. In the material to be analysed, chemical bonds vibrate at a characteristic frequency representative of their structure, bond angle and length. FTIR spectrometer is important for the interpretation of the structure, binding capacity, affinity and sites of metal ions in melanin. These are important factors for better understanding the metals melanin complexity and its consequences**.** The analysis of sepia melanin by FTIR reveals that there is existence of functional groups that can be responsible for the binding cites of different metallic ions leading to many new applications of sepia melanin.

Keywords: *FTIR, melanin, sepia melanin, Sepia Officinalis*

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1. Introduction

Melanins are biopolymers widely distributed in many parts of living organisms. They constitute a major group of biological polymers responsible for much of diversity of coloration in animal world. Melanins are found in the skin, hair and eyes of many animal species, including humans, where they act as photoprotectants (absorbing harmful ultraviolet and visible radiation). Dark-skinned people have more melanin in their skin than light-skinned people have [1,2].

Other essential biopolymers such as proteins, nucleic acids and carbohydrates are well characterized. Their monomeric units and connectivity are well known and the methodologies for determination of the sequences of their connection are well-established. In contrary, structures of melanin are still unknown because till now; no available methods allowing to accurately determine their structures [3,4,5]. Here are some factors hindering accurate characterization of melanin:

- Melanins are insoluble in a broad range of solvents and pH.
- Their purification is difficult which leads to the heterogeneity in their structural features.
- Methods to accurately determine the ratio of the various units present in melanin are not yet found.
- The molecular structure and organization of melanin are complicated and not completely known [3,6].

To characterize completely the polymer, you must be able:

- To determine the molecular weight as well as the monomer(s). For monomers, it is necessary to know their sequences. When more than one type is present, it is necessary to determine their sequence and their special representation [7];
- To specify the location of all point : determine the location of all polyfunctional monomers [7];
- To specify the bonding between each pair of monomers [8].

Different studies have been done on conducting polymers for long time because of its interesting chemical complexity and this led to the identification of some of its chemical and physical properties. There still few analyzes that have been done on the identification of functional group in melanin by FTIR spectroscopy [3-9].

Melanin is of interest as a model system of understanding disorder in biological systems. The biological functionality of melanin depends on disorder which is considered as its essential part. This property distinguishes melanin from other much more intensively studied biomolecule systems such as nucleic acid, proteins and carbohydrates [7]. A great number of investigations on the role of melanin have been done, but till now it is still not well understood.

Fourier Transform Infrared Spectroscopy (FTIR) technique is usually one of the most preferred techniques used to give a correct assignment of the observed spectral characteristic of functional groups corresponding to different absorption bands which are responsible of the absorption [10]. The characterization of the material by using FTIR spectroscopy consists in making a spectrum of radiation energy absorbed by material molecules and interpretation of the obtained spectrum. FTIR is the characterization technique which is both rapid, non-destructive and requires small sized samples. In the material to be analysed, chemical bonds vibrate at a characteristic frequency representative of their structure, bond angle and length [11]. Their individual molecules have the ability to interact with incident radiation by absorbing the radiation at specific wavelengths. To individual absorption peaks, individual chemical bonds can be identified and assigned in order to identify qualitatively or quantitatively individual compounds in complex systems [12].

1.1. Sepia Melanin

Sepia melanin isolated from the ink sacs of *Sepia officinalis*, is commonly used as standard in many researches on melanin characteristics such as spectroscopy, photoreactivity, and morphology of this class of black pigments; because of its high purity as more than 98 % of melanosomes concentration in tissues is Eumelanin. Moreover, sepia melanin is relatively cheap since it is readily available and easily extracted; as it can be isolated simply by centrifugation and purified by washing with distilled water [13,14].

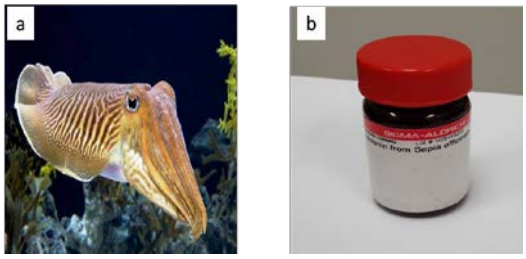

Figure 1. Sepia Officinalis (a) and the commercial sepia melanin from Sepia Officinalis (b) [13]

Sepia melanin (Figure 1b) is the melanin derived from the ink sack of various species of cephalopoda, more commonly from the cuttlefish Sepia officinalis (Figure 1a) [10]. This melanin is insoluble in organic solvents, acids, aqueous solutions, and only partially dissolves in alkaline solutions. Sepia ink from *Sepia officinalis* contains $CaCO_3$, $MgCO_3$, NaCl and Na_2SO_4, enzymes and other substances [14,15]. Purified sepia melanin is a black powder, hygroscopic that should be refrigerated at -20°C to avoid decomposition [13,14].

Melanins have applications in agriculture, medicine, cosmetic and pharmaceutical industries [16]. Many commercial products contain melanin as active ingredients; including creams that act as filters for single-response protection against UV radiation [5]. Melanin is used in cosmetics to fade defects of the skin diseases called "vitiligo" which is caused by a loss of melanin in the skin, due to destruction of melanin-forming cells known as melanocytes [4,14]. Melanin are used in medical field as a contrast in X-ray studies of the digestive system it has been shown that melanin can be used as a mean of contrast by being ingested by patients. The melanin is used in enjoying food from squid in its ink [9]. The production of sunglasses with a high ability to block UV radiations has been done by adding melanin to plastics. Melanin are also used to prevent damage to objects in museums or libraries because when coated to the internal surface of fluorescent lamp, they eliminate entirely the escape of UV light which usually occurs at a low level in these lamps [14]. Due to the presence of eumelanin polymer in sepia ink, sepia ink possesses antimicrobial activity [15]. Sepia (the name used for the ink from *Sepia officinalis*) was extensively used from Greco-Roman times through the 19th century as an ink and pigment used in writing, drawing and painting; because of its color and permanence. Sepia ink is also applied for inkjet printers as a nonpoisonous black ink [9,17]. Melanin coating is model coatings of coating for both biological investigations and smart surface science [3]. The hydroxyl and carboxylic functional groups present in melanin from *Sepia Officinallis* would be free binding site of different metallic ions that will increase its application including dye-sensitized solar cells [13].

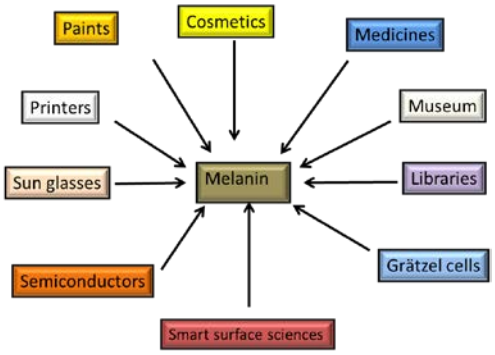

Figure 2. Some of applications of melanin

The summary of some applications of melanin is given (Figure 2). There are many researches being carried out on melanin. It is expected many new future applications and products that are based on melanins and this will lead to the increase in demand for melanin. Melanin is abundant because it can be extracted from animal tissues and plants in low cost [9]. One of the challenges for this method is that the melanin obtained generally has low purity and there is variation in composition in each batch. Luckily, this melanin can also be synthetized by chemical methods which guarantees its purity [9-18].

1.2. Fourier Transform Infrared Spectrometry

FTIR spectroscopy is measurement of the wavelength and intensity of the absorption of mid-infrared light by a sample. Mid-infrared light (4000 - 200 cm^{-1}) is energetic

enough to excite molecular vibrations to higher energy states [11].

The wavelength of many IR absorption bands is characteristic of specific types of chemical bonds, and IR spectroscopy finds its greatest utility for qualitative analysis of organic and organometallic molecules. IR spectroscopy is used to identify particular compound or a newly synthesized molecule [19].

For FTIR spectroscopy, IR radiation is passed through a sample. A part of the radiation is absorbed by the sample and the other part is transmitted. The FTIR spectrum represents the absorption and transmission of the molecular in creating a spectral fingerprint of the sample. To overcome the slow scanning speeds of older dispersion infrared instrument, the method that measures all of the infrared frequencies simultaneously, rather than individually is used.

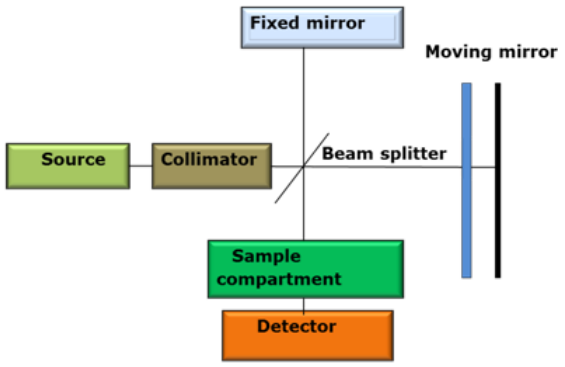

Figure 3. Block diagram of an FTIR spectrometer

A common FTIR spectrometer consists of a source, interferometer, sample compartment, detector, amplifier, A/D convertor, and a computer (Figure 3) [20]. The source generates radiation which passes the sample through the interferometer and reaches the detector. Then the signal is amplified and converted to digital signal by the amplifier and analog-to-digital converter, respectively. Eventually, the signal is transferred to a computer in which Fourier transform is carried out. FTIR spectrometer operates on principle called *Fourier transform*. The mathematical expression of Fourier transform $F(\omega)$ is given by:

$$F(\omega) = \frac{1}{2}\int_{-\infty}^{+\infty} f(r)e^{iwr}dr \qquad (1)$$

And the reverse Fourier transform $f(r)$ is given by

$$f(r) = \frac{1}{2\pi}\int_{-\infty}^{+\infty} F(\omega)e^{-i\omega r}d\omega \qquad (2)$$

where ω is angular frequency, r is the optical path difference, $F(\omega)$ is the spectrum, and $f(r)$ is called the interferogram.

The interferogram is determined experimentally in FTIR spectroscopy, and the corresponding spectrum (frequency against intensity plot), is computed using Fourier transform. This transformation is carried out automatically and the spectrum is displayed.

The core of FTIR spectrometers is the Michelson interferometer that is used to split one beam of light into two so that the paths of the two beams are different. The Michelson interferometer recombines the two beams and conducts them into the detector [20]. In the detector, the difference of the intensity of these two beams is measured as a function of the difference of the paths.

2.Materials and Experimental Methods

Sepia melanin powder (standard) from *Sepia officinalis* was obtained from Sigma-Aldrich (Chemie GmbH Kappelweg 1 D-91625 Schnelldorf, Germany) (Figure 4a). This purified sepia melanin is a black powder, hygroscopic that has been kept refrigerated at - 20° C to avoid any photo-chemical of photo-physical alterations. The Fourier Transform Infrared (FTIR) analysis was done on the pellet sample made from KBr (99.99%) (Figure 4b) from Fluka (Germany) was mixed with sepia melanin (Figure 4c).

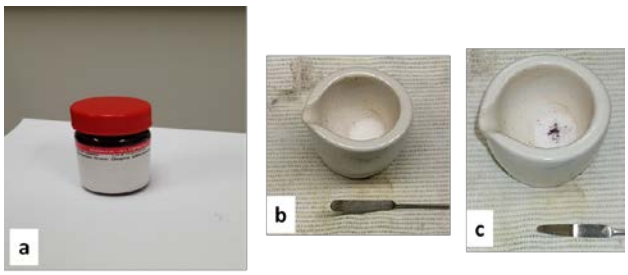

Figure 4. Sepia melanin powder (standard) from Sepia officinalis (a), KBr (b) KBr mixed with sepia melanin (c)

In preparing samples for FTIR investigation; the amount of sepia melanin (standard) was about 4 mg. This amount was mixed with about 1400 mg of KBr. In order to ensure that the produced pellets enable accurate spectra, the mixture was blended using a mortar and pestle (Figure 4).

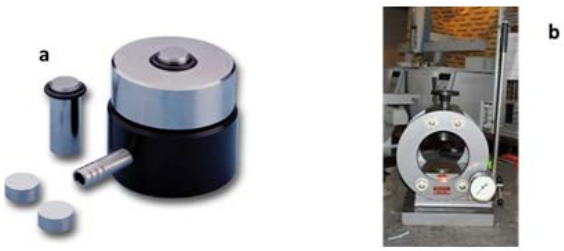

Figure 5. Aldrich® macro-micro KBr pellet die (a), BECKMAN 25 ton ring press 00-25(b)

The obtained powder was put in macro-micro KBr pellet die (Figure 5a) and compressed into pellets by using hydraulic press (Beckman 00-25 Glenrothes five Scotland) (Figure 5b). This pellet was ready for FTIR analysis.

Figure 6. Fourier Transform Infrared Spectroscopy (The Thermo Scientific Nicolet iS10)

3. Results and Discussion

The FTIR spectrum (Figure 7) was collected at resolution of 4 cm^{-1} in the transmission mode (4000–400 cm^{-1}) using Thermo Scientific Nicolet Is10 FTIR spectrophotometer (Figure 6). The broad absorption band centred about 3422 cm^{-1} was observed. This broad absorption band is the characteristic of O-H or N-H stretching vibration modes. The observation of this first band is in good agreement with the report of Centeno et al. saying that a broad absorption between 3600 - 3200 cm^{-1} spectral regions can be attributed to the O-H and N-H stretching vibrations of the carboxylic acid, and phenolic as well as aromatic amino functions presents in the indolic and pyrrolic systems [10,18]. Apte et al. observed this peak at 3438 cm^{-1} [21]. The other peaks have been observed at 2917, 2839, 1621, 1464, 1374, 1038, and 661.

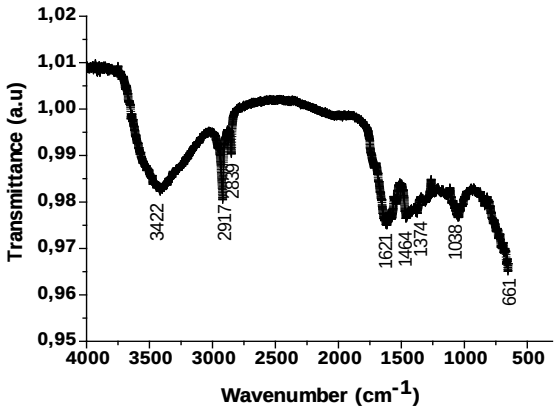

Figure 7. Room temperature IR vibrational spectra of sepia melanin spectra

Two absorption peaks were observed; the medium intensity band at 2917 cm^{-1} and the weak one at 2839 cm^{-1}, and these may be assigned to the stretching vibration of aliphatic C-H group. These values are in good agreement with stretching frequencies reported in literature [10,16]. The observation of two slightly different frequencies for the CH stretching may be the result of the fact that the hydrogen atoms in the components of sepia are located in different environments [16].

The characteristic strong band at 1621 cm^{-1} (between 1647 - 1531 cm^{-1}) is attributed to the bending vibrations modes of aromatic ring C=C and C=N bond of aromatic system in addition to C=O double bond (COOH) of carboxylic function. The mode between 1468 -1330 cm^{-1} can be due to aliphatic C-H groups and weak bands below 700 cm^{-1} ascribed to alkene C-H substitution in the melanin pigment [16-22].

The OH bending of the phenolic and carboxylic groups that was present in the 1400-1300 cm^{-1} area at the peak centered at 1374 cm^{-1} indicates the Indole ring vibration/CNC stretching [10]. The peak centered at 1038 cm^{-1} is the indication of CH in-plane/CH out-of plane deformation. Finally the weak bands below 700 cm^{-1} are assigned to the out-of-plane bending of the aromatic carbon-hydrogen bond in the sepia melanin [14].

The obtained FTIR spectrum is in agreement with the ones reported in the literature for closely related compounds, such as indole, pyrrole and substituted pyrrole. The compounds have been assigned by comparing them with other assignments for functional groups that have been published.

Table 1. Spectral Positions of FTIR Peaks, Respective Center Positions, and their Corresponding Assignments (Figure 7)

Peaks	Center (cm-1)	Assignments	Reference
1	3422	O-H or N-H stretching vibration modes	[10]
2	2917	Stretching vibration of aliphatic C-H group	[10,16]
3	2839	Stretching vibration of aliphatic C-H group	[16]
4	1621	bending vibrations modes of aromatic ring C=C and C=N bond of aromatic	[16,22]
5	1464	aliphatic C-H groups	[16,22]
6	1374	OH bending of the phenolic and carboxylic groups	[10]
7	1038	C-H in-plane/C-H out-of plane deformation	[14]
8	666	out-of-plane bending of the aromatic C-H bond	[14]

4. Conclusion

A great number of investigations on the role of melanin have been done, but till now it is still not well understood. Melanin is of interest as a model system of understanding disorder in biological systems. The biological functionality of melanin depends on disorder which is considered as its essential part. This property distinguishes melanin from other much more intensively studied biomolecule systems such as nucleic acid, proteins and carbohydrates. FTIR spectrometer is important for the interpretation of the structure, binding capacity, affinity and sites of metal ions in melanin. These are important factors for better understanding the metals melanin complexity and its consequences. The analysis of sepia melanin by FTIR reveals the existence of functional groups (C-H, COOH and N-H) that can be responsible for the binding cites of different metallic ions leading to many new applications of sepia melanin.

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