Chapter 6

X-ray and Optical Spectroscopic Studies on Phytocomplex Asphaltum (Shilajeet)

6.1. Introduction

About 200 million years ago, India was a large island off the Australian coast separated from the Eurasian continent, by Tethys Sea. During this Mesozoic period, the salty water of Tethys sea separated both supercontinents Gondwana (in the South) and Laurasia (in the North). Today’s North America was in the Laurasia super continent at that time, and South America, Australia, Asia, Antarctica, Africa were in Gondwana supercontinent. These supercontinents drifted towards each other very slowly and finally collided. The Himalayan region of Alpine mountain ranges resulted from collision of these two supercontinents. These young mountain ranges extended from Spain to Indonesia, through NW Africa, Alps and Himalayas of the Asia. The Indian continent uniformly drifted towards North at the rate of ~9 m per century. This movement or drift ultimately lead to the disappearance of Tethys Sea as shown in Fig. 6.1. Even today remnants of extinct Tethys Sea are observed in Aral, Black, Caspian and Mediterranean seas. Nearly 40-50 million year ago, Indian continent collided with the Asian continent, which caused sea bed of the Tethys sea to be pushed up and kept moving up to the eventual formation of the Himalaya mountains, which is still rising at ~1cm per year. The rise of Himalaya and creation of Shivalik range of mountains is shown in Fig. 6.2. In the aqueous environ of the Tethys Sea, life evolved as a function of solar energy and geothermal energy, and contained very small number of chemical elements. This process of evolution led to mineral rich fertile soil of the sea bed to give birth to the lush green dense forest. As the ground continued to be pushed up to form mountains, many plants were trapped beneath the rocks and soil, and were preserved for thousands of years in the pristine environment and ultimately formed humus [1].
The genesis of Phytocomplex asphaltum

The Phytocomplex originated from the geochemical transformation of organic carbon of aquatic and terrestrial ecosystems between mineral elements and living organisms found in the vicinity of parent rock. The lithophillic vegetation and marine fossil fauna underwent several thermal, geo chemical and biological changes.
with the passage of time. Due to microbial action and high pressure from the weight of top rocks, this entrapped humus transformed into dense, viscous, mineral rich substance. These plants were never exposed to any pesticides or fertilizers or chemicals of any form. The biological and chemical breakdown of residues of plants and animals synthesized a composite ensemble of plant, animal and microbial substances. This compound had faced many climatic conditions - hot summers, freezing winters, monsoon rainfalls, etc., and hence produced by the gradual decomposition of embedded substances by the action of microorganisms. These vegetation fossils were compressed beneath the layers of rocks for hundreds of years. The existing conditions of high temperature and pressure metamorphosed these substances into a phytocomplex compound.

This carbon footprint of plants and biological origins is found in the terrestrial and aquatic environment of the ecological system of the ancient pristine world. The botanical name of this natural phytocomplex is Asphaltum. The popular name of this compound is Shilajeet, its few other names are mumie, mummimyo, silajitu, hazar-ul-musa, momiai faqurual yahud, etc. It is a solid or elastic greasy resin of variable consistency with a smooth or non-uniform porous surface having blackish brown to dark black color. Fig. 6.3 shows the rudimentary form of Shilajeet having shining surface. It has a distinctive coniferous smell (odor of cow’s urine, i.e., go-mutra), is astringent and slightly bitter in taste. This semi-hard substance is exuded from the crevices of sedimentary steep rocks having an altitude between 1000 to 5000 m. It is occasionally obtained from the debris of rocks after landslides, excavations, fracking, and tunnels creations in hilly areas also. Its sites of occurrence includes regions from Himalayan range in East to West from Arunachal Pradesh to Kashmir, also many high altitude places of Bhutan, China, Nepal, Pakistan, Tibet (Himalayan belt), Afghanistan (Hindukush area), Australia, Kazakhstan (Tien-Shan, Ural and Caucasus) and Norway.

According to the texts in traditional Indian medical compendium Charak Samihta Sutras, Shilajeet is a Rasayana which rejuvenates (or synergies or strengthens) the human beings and nurtures the health of all tissues of the body. The medicine systems of Ayurveda, Siddha and Unani regard it as an elixir or panacea for curing for all the diseases.
Its dictionary meaning in Sanskrit is ‘conqueror of rocks’, *i.e.*, ‘destroyer of weakness’. For thousands of years, in many countries, who were following traditional system of medicine, many miraculous healing properties have been attributed to the intake of Shilajeet. In 1870’s, during the era of British rule, a royal explorer Sir Martin Edward Stanley observed that the monkeys in plains of Northern India gets bald and sluggish by the age of 10 years. But, he failed to notice any aged monkey in higher altitude of Himalayas during months of his expedition and exploration. He found that monkeys regularly ate black resin exudate coming out from the cracks from the rocks. Generally they do not eat anything oozing out from the soil of the Earth. This odd behavior of monkeys compelled him to investigate this edible resin. The local people of the area told him about the rejuvenating aspect of this resin, called as *Silajitu* which was used by Indian Yogis, Saints and Tibetan monks selectively since long time. These facts about Shilajeet have been mentioned in the travelogue written by him.

Many naturally occurring microbial metabolites of the rock rhizospheres are found in it. This exudate substance is an admixture of an inorganic material, fossils of entrapped plants and animals [2]. During hot summers, this material becomes less viscous and finally oozes out of the cracks of rock thus named as Shilajeet [3]. Many studies have identified benzoic acid, tannic acid, fatty acid, sterols, albuminoids, glycosides, calcium, potassium, nitrogen, silica, resin, vegetable matter, zinc, iron, phosphorous, magnesium, iodine, sulfur, chloride, moisture, gums and a number of vitamins in it. Since oligoelements like zinc, iron, copper, lead, silver were found in
the bryophytes tissues also, this confirmed its animal origin. These heterogeneous compounds are ubiquitous in nature. The observed variance in composition of Shilajeet is attributed to species of plants involved, geological nature of entrapping rock, altitude, humidity, moisture, prevailing temperature and pressure etc. [4].

The physical appearance of Shilajeet is similar to organic compound bitumen coal tar which is viscous black liquid extracted from the destructive distillation of coal. This millenary product of nature mainly consists of humus. It is made up of humic acid, fulvic acid and ulmic acid (~60-80 %), mineral matter (~20-40 %) and minor trace elements (~5 %). The classification of these main components is done according to their differential solubility in water at different pH levels. Humic acid is an organic acid which is a dark sub fraction, soluble only in solutions with pH of 7 and above i.e., in alkaline or basic medium, but precipitates at pH<3. It is insoluble in short chained alcohol. It has molecular weight in the range of 10-35000 Da. The average chemical formula of humic acid is C_{187}H_{186}O_{89}N_9S. Fulvic acid is also an organic acid which is soluble in water solution regardless of its pH (both acidic and basic). It is the major component of Shilajeet (~70 %). Generally these are multivalent organic acids, having low molecular weight (50-2000 Da) [5]. The average chemical formula of fulvic acid is given by C_{135}H_{182}O_{95}N_5S_2. Ulmic acid is found in very small quantities (<2 %) and is soluble in only petroleum based solvents/products. Fulvic acid has lower molecular weight than humic acid but has greater functional group density. Humic acid contains more C, H, N and but less S and O than fulvic acid. There exists high content of phenolic OH, ketonic C=O and carboxylic COOH groups in fulvic acid, where as humic acid have more aromatic, ether and heterocyclic groups than fulvic acid. In humic acid, there is a variation in number and position of the functional group, aromatic and aliphatic chains due to difference in their conditions of its conception, pathway of genesis and prevailing physical conditions. Humins are also present in Shilajeet as insoluble fractions of mineralized form of condensed molecules. The ratio C:H for humic acid is 1:1 indicating the significant degree of aromatic character where as its low value of ratio O:C signifies lower acidic functional groups. In case of fulvic acid, ratio C:H<1:1 implies decreased aromatic character and
Fig. 6.4: Humic acid fragments (a) the ball and stick model (b) space filling model [6].

Fig. 6.5: Structural model of humic acid [7].
ratio O:C >1:2 signify more acidic character. It had been reported by one group [3] that Shilajeet is a remnant of rock humus containing minerals of rocks and other organic substances of rock rhizospheres (microorganism, bacteria, fungi, algae, mosses, lichens, actinomycetes). It contains both organic and inorganic poly condensates compounds. The marine fossil, fauna and lithophilic vegetation forms complexes with salts, minerals and metal oxides under geo chemical and bio chemical processes. The density of these strongly polar supramolecules ranges from 1.1 to 1.8 g cm$^{-3}$. The exact structure of humic acid and fulvic acid is not known, but few researchers [6,7] proposed few models containing both aromatic and aliphatic structures for humic acid, substituted with oxygen containing functional group which are shown in Figs. 6.4-6.6. For fulvic acid, free and bound phenolic OH group, quinone structure, nitrogen and oxygen acts as bridge units and COOH groups placed variously on aromatic ring.

**Structure of Shilajeet, fulvic acid and humic acid**

One of the popular aphrodisiac drugs sold for enhancing body energy is based on natural phytocomplex asphaltum, *i.e.*, Shilajeet which is claimed to synergize the human body, and cure many bodily deficiencies including erectile dysfunction. The huge market potential, exorbitant cost, embarrassments associated with sexual impotency, along with Shilajeet, the phosphodiasterase type-5 (PDE-5) inhibitors are among the popular drugs freely sold over the internet too. The Food and Drug Administration (FDA), US had approved only three PDE-5 drugs namely, Viagra (sildenafil citrate) from pfizer, Calis (tadalafil) from el Lilly and Lentre (vardenafil) from Bayer. These chemicals and their analogues are expected to be adulterated into the dietary supplements to enhance efficacy and reduce cost. The neutraceuticals are opted in lieu of prescription drugs, assuming no latent side effects. Many users of PDE-5 have reported of flushing, dyspepsia, muscle aches, rare occurrence of visual side effects, headache, flushing, and running nose. PDE-5 is primarily distributed within the arterial wall smooth muscle of male organs, and it acts selectively in these areas without inducing vasodilatations in other areas of the body [8]. PDE-5 analogues are prevalent which provide similar physiological effects but have the structural modifications to the parent compound [9-11]. Sildenafil is an inhibitor of
Fig. 6.6: Structure of Shilajeet, fulvic acid and humic acid [7].

PDE-5 enzyme, popularly prescribed to the patients of pulmonary artery hypertension, anti-depressant, diabetes and erectile dysfunction in man. The part of structure of Sildenafil is shown in Fig. 6.7. It is also used for the treatment of pulmonary edema caused by high altitude illness. Many groups have reported an increase in life span of
flowers kept in vase and there is slowing down of plant ripening with the help of PDE-5. Some studies have also shown that sildenafil is very effective in jet-lag recovery. Many adverse effects have been reported by taking PDE-5 inhibitors along with nitrate medications unknowingly by the patients of diabetes, hyperlipidemia or ischemic heart as blood pressure drops suddenly. The major side-effect of its overdose is color blindness in the patients so this drug should be taken under strict medical subscription. The methods of chromatography and voltammetry have been reported for analysis of falsification of these drugs which are time consuming techniques and require an extensive sample preparation. XRF studies of sildenafil citrate have also been reported in literature [12].

![Chemical structure of sildenafil](image)

Fig. 6.7: Chemical structure of sildenafil [12].

Some people prefer to consume natural supplements like Shilajeet to avoid potential side effects of PDE-5 inhibitors. Mostly these healthcare products claim to be safer as they contain natural extracts and deliver miraculous results. To achieve improvement in efficacy for obtaining the desired pharmacological effect in drugs in short time and easy profit making prompts few manufacturers to make deliberate addition of sildenafil citrate, tadalafil and similar compounds in large quantity in their products. The adulterations of Shilajeet with tadalafil and sildenafil citrate had forced many developed countries to ban its sale. The counterfeits of these products pose a potential risk for health of consumer, so there is an urgent need to identify such adulterations. Table 6.1 shows the details of samples of Shilajeet which were bought from local market for ascertaining the adulterations.
Spectroscopic analysis have proved to be an efficient technique in finding counterfeits since they allow characterizing the samples by directly measuring substances in solid state with minimal sample preparation. Its straight forward operational steps provide very reliable and fast results. The Fourier transform infra-red spectroscopy (FTIR) and Raman spectroscopy are comparatively inexpensive techniques. In the present study, to distinguish between authentic and counterfeit Shilajeet, Raman spectroscopic technique was used. It is a less time consuming and reliable method, without any need of sample preparation. Raman spectroscopy characterizes the solid state samples directly, so this efficient technique relying on straightforward operational steps provides trustworthy results quickly. The Raman spectra’s were obtained in the midIR region between 1150 and 700 cm\(^{-1}\).

Table 6.1: List of the various drugs/products containing Shilajeet under study.

<table>
<thead>
<tr>
<th>Sample label</th>
<th>Drug (Brand)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA1</td>
<td>Shilajeet Nepal (Unbranded)</td>
</tr>
<tr>
<td>SA2</td>
<td>Shilajeet capsules (Dabur brand)</td>
</tr>
<tr>
<td>SA3</td>
<td>Nice Musli capsules (Nice Pharma)</td>
</tr>
<tr>
<td>SA4</td>
<td>Shilajeet Gold capsules (Dabur brand)</td>
</tr>
<tr>
<td>SA5</td>
<td>Shilajeet capsules (Patanjali brand)</td>
</tr>
<tr>
<td>SA6</td>
<td>Shilajeetwati Badi capsules (Baidya nath asli)</td>
</tr>
<tr>
<td>SA7</td>
<td>Shilajeet capsules (Fidalgo brand)</td>
</tr>
<tr>
<td>SA8</td>
<td>Shilajeet Shudh capsules (Divyajyoti brand)</td>
</tr>
<tr>
<td>SA9</td>
<td>Shilajeet Sat (R.T) (Divyajyoti brand)</td>
</tr>
<tr>
<td>SA10</td>
<td>Shilajeet pure (Nidalco brand)</td>
</tr>
<tr>
<td>SA11</td>
<td>Shilajeet Rasayan (Divyajyoti pharma brand)</td>
</tr>
<tr>
<td>SA12</td>
<td>Shilajeet Upper Himalyan (Unbranded)</td>
</tr>
</tbody>
</table>
6.2. Material and methods

6.2.1 Objective of research

The XRF technique has been preferred for elemental quantification due to its multi-element analytical capability, lower detection limit, high resolution, greater reliability, capability of analyzing metals and non-metals alike and less time consumed for sample preparation. The spectroscopy techniques are widely used to study the characteristic functional groups of phytocomplexes. In the present study, raw and purified of Shilajeet samples were purchased from local market and humic acid (99.99 % pure) was procured from Sigma Aldrich. The detailed analysis was performed by using techniques of SEM, XRF, FTIR, and UV-VIS spectroscopy for its elemental composition, nature, origin and formation of organo-metallic complexes. The fingerprints of molecular vibrational (FTIR) spectra, \( \text{viz.} \), the position, width and intensity of absorbed peaks gave us information about functional groups and their binding sites. The metal tagging capability of Shilajeet was evaluated with the help of WDXRF setup. The observed metal tagging of Al and Hg by Shilajeet can be further improved so as to develop a potent remediation technique for water pollution.

6.2.2 WDXRF spectroscopy

In the present work, WDXRF spectrometer (S8 TIGER, Bruker AXS, Germany) was used for elemental analysis of samples and analyzing metal tagging capability. This spectrometer is based on end-window \( _{45}\text{Rh} \) anode tube equipped with 4 kW intensity X-ray generator having a maximum voltage of 60 kV and a maximum tube current of 170 mA. The \text{spectraplus} analysis software has been used in standard less, best detection mode. The irregularly shaped sticky black crystals of raw Shilajeet were embedded with non-crystalline impurities. Prior to analysis, these samples were mixed thoroughly in a smooth agate mortar using pestle. The moisture was removed by oven drying the samples at 60° C. These dried samples were filled in micro cup assembly for hand pressing by applying the pressure of about 2 tonne using pallet making machine to form a glossy transparent pellet. The hydraulic press (INSMART, India)
capable of producing maximum pressure up to 40 ton was used for the pallet formation. Nearly 10 g of the dry sample was finely grounded and pressed with a pressure of 20 tonne to form a stable pellet. A vibratory cup milling machine (INSMART, India) using 2 HP motor grinded these samples up to size of 5 micron. This method of sample preparation is simple, fast and cost effective. The physical examination of glossy pallets for any structural anomaly was important step during this analysis of elemental characterization. This procedure was repeated for analyzing the metal tagging capability using supernatant solution too. WDXRF technique fails to provide an absolute quantification due to unknown matrix of samples.

6.2.3 FTIR spectroscopy

Fourier Transform Infra Red (FTIR) spectra were recorded on the dried material dispersed in KBr pellets performed in the range 4000-225 cm\(^{-1}\) in the 32 scan mode using Thermo Fisher spectrometer (Nicholet 6700, USA). The KBr pellets were made immediately before analysis by pressing a mixture of 3 mg sample and 800 mg KBr (99.99 % pure, Sigma Aldrich). The spectrometer was initially blanked with KBr. All spectra's were recorded at room temperature, then data obtained was signal averaged into a single spectra using dedicated computer software Omnic in the spectrometer and default baseline corrections were applied to minimize unavoidable shifts. Each spectrum was normalized by applying the normalization factor. Special attention was given to the mid infra-red region (the prime region of interest 4000-400 cm\(^{-1}\)) of absorbance spectra's. First three samples of Shilajeet were prepared by dissolving it in deionized water of different pH of values 4.0, 7.0 and 9.2 and then fixed quantity of mercury was added in this aqueous media. These were continuously stirred so that organo-metallic interaction could take place. After some time, the supernatant of this mixture was removed and added to an adequate amount of KBr and dried.

6.2.4 UV-Vis spectroscopy

UV-Vis spectra of various samples in liquid solutions were recorded by using 1cm quartz cuvette in the wave length range 200-800 nm by using spectrometer (Evolution 300, Thermo Scientific USA). The analyte solution included pure Shilajeet, raw Shilajeet, humic acid, saturated solution of Shilajeet at pH 4.0, 7.0, and 9.2 with
tagged Hg. Since the uncharacteristic spectra were yielded at UV-Vis wavelengths. The absorbance ratio at 465 and 665 nm, i.e., the ratio \( E_4/E_6 \) was also determined. The spectral comparisons were made to reach the conclusion.

6.2.5 Scanning electron microscopy

Scanning electron micrographs of the powdered samples of raw and pure Shilajeet at various magnifications was obtained using a Scanning transmission electron microscope (JSM6100, JEOL, JAPAN) with a 5 kV accelerating voltage with image analyzer. Its electron source consisted of lanthanum hexaboride (LaB\(_6\)). The setup is linked to ion and turbo suction pumps to achieve the desired vacuum. The samples were coated with very thin gold film to make conductive and mounted on pegs made up of Al. The source provided a very intense, stable and focused beam of electrons. The surface of samples was made electrically conductive in a sputtering apparatus during taking SEM pictographs. The microscope consisting of the secondary electrons detector and backscatter electrons was used for imaging of the samples. The microscope had viewing angle of 360° on the rotating platform. The pressure inside the chamber lies between 0.1 to 1 mb. The digital image of specimen was taken from phosphor screen with quality upto 4000 x 4000 pixels.

6.2.6 Raman spectroscopy

Raman spectrograph (iHR-550 JY HORIBA, Japan) equipped with thermoelectrically Peltier cooled CCD detector was used for recording the Raman spectra of samples. This spectrograph is installed at the Department of Physics, Panjab University, Chandigarh. Before taking measurements, this spectrograph was calibrated with known Raman lines of CCl\(_4\). Edge filters have been used for elimination of elastically scattered light of higher wavelengths. Laser excitation lines at 488.0 nm have been provided by the air cooled Ar\(^+\) ion source (Newport, USA). Plasma lines of the lasers have been filtered out with the help of narrow band pass filter. Raman spectra's in range of 60-2250 cm\(^{-1}\) were dispersed with gratings of 2400 g/mm. The specifications of Raman spectrograph used in the present study has focal length 550 mm, resolution 0.025 nm (using 1200 g/mm grating) and Synapse CCD Detection System using
SynerJY Software. It has three interchangeable gratings 1200 g/mm, 1800 g/mm and 2400 g/mm. Measurements were carried out in 90° geometry with the linearly polarized incident light and the scattered light was detected unpolarized in CCD. A silicon (Si) based semiconductor CCD having an array of photosensitive elements, each one of which generates photoelectrons and stores them as small charge in the potential well, to record spectra. The major advantages of the CCD are the low readout noise and sensitivity in a significantly wide range of wavelength (120-1000 nm) in comparison to other multichannel detectors.

The essential requirement for a Raman active vibrational mode is the change in polarizability depending on tightness of the electrons bound to the nuclei of the substance. In the present investigations, the silver coated mirror-like glass slides have been used to surface-enhanced the Raman spectra (SERS) of humic acid, Shilajeet (raw and purified), dietary supplements and sildenafil citrate. All the chemicals used in the present investigations were reagent grade, and doubly distilled water was used for preparation of solutions. Electro-less deposition of Ag thin films was achieved by following the standard procedure. Prior to use, all glassware was washed by rinsing in 2 M HNO₃ with deionised water and dried. In 300 mL of distilled water, 2 g of AgNO₃ was added and then 0.7 M NH₄OH was added drop-wise upto the time the white precipitate disappears. The glass substrate is an ordinary microscope slide (of size: 75 mm x 25 mm x 1.45 mm) which was fully immersed in this solution. The solution was magnetically stirred for about 5 hrs to get chemical deposition of Ag on glass substrate. The mirror-like Ag film exhibited highly intense SER spectra of samples exposed to the laser. The Raman spectra of humic acid, Shilajeet (raw and purified), dietary supplements and sildenafil citrate were recorded. Twenty spectra were recorded for each sample and cumulative spectra was generated by adding them so as to get rid of statistical fluctuation peaks and using the software SynerJY the background peaks and fluorescence peaks were suitably deduced.
6.3. Results and discussion

6.3.1 Physical characteristics

The samples of Shilajeet from different region possessed identical physico-chemical characteristics, having similar to bitumen like semi hard and glossy features, except localized surface impurities. The blackish brown color, astringent taste with typical odor of cow’s urine was observed in all the unpurified raw and purified samples. These raw samples were found to be completely soluble in water irrespective of their origin.

6.3.2 Elemental analysis of Shilajeet using WDXRF setup

The major and trace elemental concentrations of Shilajeet recorded by WDXRF setup have been determined for. Many elements like K, Ca, Cl, Si, Fe, S, P, Mn, Mg, Sr, Mo, Rb, Ru, Br, Zn, Ti, Cu, Ni and Na were observed in the samples. The variation in the content of C, H, N and S of the samples is due to change in place of origin, isolation method and inherent error of sampling and analysis. The quantitative analysis of all the samples showed nearly same composition indicating the similar physico-chemical characteristics. The fluorescence analysis have established presence of many elements in Shilajeet like K, Ca, Cl, Si, Fe, S, P, Mn, Mg, Sr, Mo, Rb, Ru, Br, Zn, Ti, Cu, Ni and Na. Table 6.2 gives the mean concentration of observed elements. It was observed that there is variance in the concentration of different sample of Shilajeet due to different locations of its procurement and physical conditions involved in its genesis. The mean composition of different observed elements in Shilajeet by WDXRF technique was compared with the composition of metal ions (and metalloid elements) in mineral constituents of Shilajeet reported by S. Ghosal [13]. The advantage of WDXRF analysis is that few elements Cl, Sr, Rb, Ru, Br, Ti and Ni were recorded upto trace level in the samples. Further, it may be added that the elements $_{23}$V and $_{74}$W were not detected in the present analysis, but Ghosal had reported their concentration as 0.002-0.01 % and 0.001-0.08 % respectively.
<table>
<thead>
<tr>
<th>Element</th>
<th>Mean Composition (% by wt)</th>
<th>Ghosal [13] (% by wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium (\text{\textit{19}}K)</td>
<td>6.97%</td>
<td>0.1-0.8 %</td>
</tr>
<tr>
<td>Calcium (\text{\textit{20}}Ca)</td>
<td>1.77%</td>
<td>2.0-16.0 %</td>
</tr>
<tr>
<td>Chlorine element (\text{\textit{17}}Cl)</td>
<td>1.11%</td>
<td>ND</td>
</tr>
<tr>
<td>Silicon (\text{\textit{14}}Si)</td>
<td>0.34%</td>
<td>0.8-1.2 %</td>
</tr>
<tr>
<td>Iron (\text{\textit{26}}Fe)</td>
<td>0.34%</td>
<td>0.5-1.8 %</td>
</tr>
<tr>
<td>Sulfur (\text{\textit{16}}S)</td>
<td>0.33%</td>
<td>0.2-1.1 %</td>
</tr>
<tr>
<td>Phosphorus (\text{\textit{15}}P)</td>
<td>0.20%</td>
<td>0.2-2.5 %</td>
</tr>
<tr>
<td>Manganese (\text{\textit{25}}Mn)</td>
<td>645 ppm</td>
<td>0.008-0.01 %</td>
</tr>
<tr>
<td>Magnesium (\text{\textit{12}}Mg)</td>
<td>547 ppm</td>
<td>5.0-8.0 %</td>
</tr>
<tr>
<td>Strontium (\text{\textit{38}}Sr)</td>
<td>429 ppm</td>
<td>ND</td>
</tr>
<tr>
<td>Molybdenum (\text{\textit{42}}Mo)</td>
<td>416 ppm</td>
<td>0.002-0.003 %</td>
</tr>
<tr>
<td>Rubidium (\text{\textit{37}}Rb)</td>
<td>403 ppm</td>
<td>ND</td>
</tr>
<tr>
<td>Ruthenium (\text{\textit{44}}Ru)</td>
<td>242 ppm</td>
<td>ND</td>
</tr>
<tr>
<td>Bromine (\text{\textit{35}}Br)</td>
<td>197 ppm</td>
<td>ND</td>
</tr>
<tr>
<td>Zinc (\text{\textit{30}}Zn)</td>
<td>186 ppm</td>
<td>0.002-0.003 %</td>
</tr>
<tr>
<td>Titanium (\text{\textit{22}}Ti)</td>
<td>134 ppm</td>
<td>ND</td>
</tr>
<tr>
<td>Copper (\text{\textit{29}}Cu)</td>
<td>126 ppm</td>
<td>0.005-0.01 %</td>
</tr>
<tr>
<td>Aluminum (\text{\textit{13}}Al)</td>
<td>114 ppm</td>
<td>0.08-1.0 %</td>
</tr>
<tr>
<td>Nickel (\text{\textit{28}}Ni)</td>
<td>75 ppm</td>
<td>ND</td>
</tr>
<tr>
<td>Sodium (\text{\textit{11}}Na)</td>
<td>47 ppm</td>
<td>0.1-0.5 %</td>
</tr>
<tr>
<td>Vanadium (\text{\textit{23}}V)</td>
<td>ND</td>
<td>0.002-0.01 %</td>
</tr>
<tr>
<td>Tungsten (\text{\textit{74}}W)</td>
<td>ND</td>
<td>0.001-0.08 %</td>
</tr>
</tbody>
</table>

Here “ND” stands for ‘not detected’
6.3.3 Scanning electron pictographs

The scanning electron microscopy was performed for raw and purified Shilajeet at different magnifications. Fig. 6.8 shows SEM picture of natural raw Shilajeet at magnifications of 1000X showed particles existing at loose greasy, spongy, glossy and sticky surface which tended to be amassed. Fig. 6.9 shows SEM picture of purified Shilajeet at magnification of 230X having ensemble of very fine micro pores which were relatively open faced, having different sizes, and adsorption occurs at these sites of voids. The core structure slackly held the humus compound in its intrinsic voids either by adsorption/interaction or by weak bonds [14]. The secondary metabolites of plants trapped in these voids were not decomposed chemically or biologically. The carboxylic and hydroxyl groups attached to its aromatic rings makes an organo-metallic complexes of Mg, Fe, Cu, Al, Mn, Hg, which got immobilized by filling the voids of the perforation of the lattice-like structure of Shilajeet [15].

Fig. 6.8: Natural Shilajeet SEM picture at magnification 1000 X with 10 μm bar.
6.3.4 Fourier Transform Infra Red (FTIR) spectra

In this work, the pH mediated metal tagging effect of parent supra molecule Shilajeet was studied using FTIR spectroscopy to analyze the newly formed organo-metallic complexes. The chemical breakdown of animal and plant residues under particular geo-thermal conditions leads to the genesis of humic substances containing lignins, leaf polyphenols, proteins and lipids along with humic, fulvic and ulmic acid. Humic acids (HA) are black colored organic polymers having high molecular weight and exhibit the hydrophilic character, attributed to functional groups attached to aromatic rings such as hydroxyl and carboxylic acids. The conspicuous changes in infrared spectral bands of functional groups established the high metal binding capacities. In the region of interest, i.e., midIR spectra, the absorption bands of various samples were nearly identical due to the similar chemical constituents. The adsorption of metals on binding sites of Shilajeet is governed by pH of the aqueous solution. With increasing pH, significant changes were observed in spectra. Three different FTIR
spectra of Hg in Shilajeet saturated solution at pH value of 4.0, 7.0 and 9.2 are shown in Fig.6.10. The FTIR spectra exhibited few broad band's along with characteristic peaks, in raw samples of Shilajeet the regions of 3500-2000 cm\(^{-1}\), 1830-1520 cm\(^{-1}\), and 1260-990 cm\(^{-1}\) are considered to be an organic matter bands. There was a distinct additional peak belonging to carboxyl region in the acidic medium, partly due to strong adsorption exhibited by hydrogen ions because the speciation of metal ions and the ionization of surface functional groups which depends upon the pH of liquid. The interpretations of spectra are as follow

(a) In the region 3450-3350 cm\(^{-1}\) of all the spectra's, a strong absorption band at 3400 cm\(^{-1}\) is seen due to vibration of hydrogen bonded –OH (hydroxyl) of the COOH (phenolic) functional groups. The narrowness of peaks suggests the presence of lower molecular weight compounds in the sample. A broad band around 3400 cm\(^{-1}\) may be prominently due to absorption by hydrogen bond in the humic acids. It may be assigned to O–H (or N–H) stretching in plant-derived materials. The fulvic acids absorb moderately in this region. The broadness of band may be due to binding of metal ions or by increased hydrogen bonding.

(b) The sharp peak observed in the region 2950-2850 cm\(^{-1}\) marked the presence of methyl and methylene groups. In this region, absorption peaks at about 2925 cm\(^{-1}\) may be due the asymmetric aliphatic C–H stretching band. In this region, main absorbance is due to humic acids but the fulvic acids exhibited intermediate one. The 2850 cm\(^{-1}\) peak showed the symmetric stretching vibrations of aliphatic C–H bonds. The shoulder on humic acid spectra is formed by the carboxylic acid O–H stretching band at 2600 cm\(^{-1}\).

(c) The marked spectral signature of humic and fulvic acids is the C=O bond stretching band of carboxylic acid, carbonyl groups and esters at the deep shoulder at 1700 cm\(^{-1}\). A sharp and small peak at about 1710 cm\(^{-1}\) is attributed to C–O or C=O band stretch of carboxyl and carbonyl functional group. The deep shoulder at 1700 cm\(^{-1}\) is the marked spectral signature of humic and fulvic acids showing the C=O bond stretching band of carboxylic acid, carbonyl groups and esters.
(d) The region 1640-1600 cm\(^{-1}\) belonged to aromatic C=C double bonds conjugated with C=O. This band is regarded as characteristic to humic compounds. The 1650-1600 cm\(^{-1}\) showed the aromatic C=C double bonds conjugated with C=O. At 1610-1620 cm\(^{-1}\) aromatic bands presence was confirmed.

(e) The bands at 1640 and 1560 cm\(^{-1}\) are attributed to the presence of amides having peptide linkage containing N due to free radical coupling of nitrogenous substances in humic acids. The sharpening of peak at 1630-1624 cm\(^{-1}\) implied that routes of vibrational relaxation to lower energy level decreased by binding of metal ions. The peak at 1517 cm\(^{-1}\), is due to presence both aromatic bands. The observed peaks at 1510 cm\(^{-1}\) is due to aliphatic stretching.

(f) The peak at 1430 cm\(^{-1}\) represented aliphatic C–H stretching. The humic acid absorbed strongly in the region between 1400 and 1350 cm\(^{-1}\) which is dominated by phenolic, or COO\(^{-}\) stretching vibrations of oxidized carbon and CH\(_3\) bending. The band at 1385 cm\(^{-1}\) is due to O–H deformation, C–O\(^{-}\) stretch of phenols, COO\(^{-}\) vibrations and C–H deformations of CH\(_3\). The possible mineral influence is shown by the high absorbance peak at 1383 cm\(^{-1}\). The information about an additional peak at 1283 cm\(^{-1}\) in the spectra can be obtained by the comparison of control and metal tagged Shilajeet. It may be probably be due the fact that carboxyl group (COOH), hydroxyl, carbonyl, amino and phosphoryl functionalities helped in tagging of the metallic ions. The characteristic O-H stretching in metal treated samples is indicative of non-bonded hydroxyl groups on metal-oxygen interaction.

(g) The region between 1250-1200 cm\(^{-1}\), where 1220 cm\(^{-1}\) peak existed, gave an information about phosphodiester functional group indicating asymmetric stretching of –P=O band. Phosphate moieties (phosphate metabolites, sugar phosphate esters) chelate or tag the metallic ions. In the sample, the fulvic and humic acids absorbed in the region around 1230 cm\(^{-1}\) for aromatic –CH and at 1160-1030 cm\(^{-1}\), due to –COH stretching. The absorption band near 1220 cm\(^{-1}\) is regarded as due to OH bending deformations and C–O stretching vibration primarily due to carboxyl group. The absorption region around 1200 cm\(^{-1}\) has shown C–O stretching, O–H bending deformations primarily due to carboxyl group.
A very strong peak around 1035 cm\(^{-1}\) is attributed to alcoholic and polysaccharide C–O stretch and O–H deformation.

The interpretation of the region between 1030 and 400 cm\(^{-1}\) is difficult as absorbance varied widely amongst samples containing the humic and fulvic acids. This may be due to different organic origin and uncertain mineral content. Any other band outside this region may be predominantly due to absorption of mineral or formation of mixture of organic-mineral absorption. In the present study all the samples exhibited characteristics peaks of hydroxyl, methyl, methylene, aromatic bond, carbonyl, carboxyl, phenol, alcohol and polysaccharide while relative intensities of specific bands vary owing to different genesis [16].

The sharpening and broadening of particular bands implied metals binding to the humic acid at carboxylic acid sites and –OH groups respectively. Studies of \(pH\) effects on solid samples of humic or fulvic acids or Shilajeet are scarce. In the present study, an insight into the \(pH\)-mediated effect on the protonation of carboxyl and other functional groups was attempted using FTIR spectroscopy. As \(pH\) of mother solution is increased, some significant spectral changes, related to the formation of carboxylate anions, \(i.e.,\) the increase of the bands at 1614-1627 cm\(^{-1}\) (asymmetric stretching), and 1145 cm\(^{-1}\) (symmetric vibration) and additional peaks at 1393 cm\(^{-1}\) and 1283 cm\(^{-1}\) were observed as shown in Fig. 6.11. The formation of radicals is favored by OH\(^-\) ions providing few spatial arrangements, voids or “cavities” within humic matter, where less partial pressure of oxygen existed and the radicals were shielded from external oxygen, there by formation of long-lived radicals. Experimentally it was observed that within humic matter such cavities are the protected sites showing hydrophobicity, as shown in SEM pictures in Figs. 6.8 and 6.9, and they may be disappearing by raising \(pH\) because of conformational changes [17,18]. As \(pH\) is modified from acidic to alkaline, the orbicular loop-like configurations changes to uniformly existing little aggregates in the solution [19].
Fig. 6.10: FTIR spectra showing Metal tagging capability of Shilajeet at different $pH$ 9.2 (top), 4.0 (middle) and 7.0 (bottom). The additional peaks at 1393 cm$^{-1}$ and 1283 cm$^{-1}$ corresponds to metal tagging to carboxyl functional group.
Fig. 6.11: FTIR spectra of Shilajeet purified (top), humic acid purified (middle) and Shilajeet raw (bottom).
6.3.5 Scavenging by Shilajeet: Removal of toxic metals from water

It was observed that presence of Shilajeet in HPLC water at pH 7.0 facilitated the deposition of mercury on the surface of copper stripe. After the time interval of 8 hours, the copper piece was fully coated with sheath of mercury as shown in Fig. 6.12. This deposition occurred in the absence of any electric potential.

![Electro-less deposition of mercury on copper.](image)

This finding of Hg metal deposition on surface can be further improved to scavenge toxic metals from water just by adding small amount of Shilajeet in the medium. The thin sheath of mercury was formed at all the surface area of fully immersed copper stripe. The WDXRF analysis of the surface sheath so formed revealed that the 96.76% composition of mercury using the best detection mode. Further intensive investigations are required to exploit this toxic metal scavenging capability of Shilajeet onto the surface of copper, i.e., *pseudo-electroplating* or *electro-less deposition.*
6.3.6 Occurrence of horizontal plateau in the middle portion of metallic tagging curve of mercury and aluminum

Another pilot experiment about metal tagging by Shilajeet was performed using WDXRF technique. This study was made without using the copper stripe. All the chemicals used in this investigation were regent grade. The mother solution was

![Mercury tagging curve](image1)

![Aluminum tagging curve](image2)

Fig. 6.13: Al and Hg tagging curves via pathway of Shilajeet.
prepared by adding known quantity of aluminum (0.8 g) of Shilajeet (2.0 g) in 100 ml of HPLC water at 7 pH. Similarly another such solution was prepared by mercury (10.7 g) in Shilajeet (2.0 g) in 100 ml of HPLC water at 7 pH. In this investigation there was no copper stripe to provide binding sites. These solutions were stirred and after regular interval of time the supernatant solution was taken for WDXRF analysis. The graphs were plotted between amount of metal (in ppm) and time elapsed for both metals Al and Hg. The amount of metal content in water increased as elapsed time increased. The presence of Shilajeet in water facilitated the dissolution of metals. It was interesting to observe a horizontal plateau in both graphs, i.e., metals were not picked by Shilajeet linearly for some time. The plausible reason for this nearly flat middle region in the metal binding curves of mercury and aluminum, may be the falling down of tagged metals under effect of gravity, from the binding sites as their size increased due to clinging together of metals, i.e., tending to amass. The bare binding sites again became available for more adsorption to take place after some time, further tagging of metal occurs which is shown by the rising curve again in Fig. 6.13.

6.3.7 UV-Vis spectrographs of metal tagged solutions

The UV-Vis spectra of various metal tagged samples of Shilajeet at different pH values of 4.0, 7.0 and 9.2 in deionized water with Hg, raw and purified Shilajeet, and humic acid were studied. The UV-Vis spectra were obtained using a 1 cm quartz cuvette in the range from 200 nm to 800 nm for humic acid and Shilajeet (raw and purified) as shown in Fig. 6.14. The humus exhibits uncharacteristic spectra in the UV and visible region, so no maxima was observed in the visible region but end absorption existed in the UV region. The UV-Vis spectra had uncharacteristic peak for humic acid (Sigma Aldrich, Switzerland) with a slight hump in the vicinity of 260 - 280 nm, without any of sharp maxima peak. Further Hg tagged water solution at different pH (4.0, 7.0 and 9.2) was analyzed using its UV-Vis spectra as shown in Fig.6.15. The absorption of radiation by double bonds C=C, C=O and N=N of the unsaturated or aromatic components is assumed to be responsible for it. The variation in humification process created different density of aromatic compounds which is shown by difference in humps of UV-Vis spectra. It is fact that the range of parameter
Fig. 6.14: UV-Vis spectra of Shilajeet purified, humic acid, Shilajeet raw.

In humification process created different density of aromatic compounds which is shown by difference in humps of UV-Vis spectra. It is fact that the range of parameter E4/E6 (ratio of the absorbance of the solution at 465 and 665 nm) exists between in the range 3.5-10.8 for Shilajeet of various origins[20], and in the present study its mean value was found to be 5.37. The low value of E4/E6 ratio implied more complexity in its core.
structure associated with lower molecular weights. The absorbance ratio E4/E6 depends inversely to the degree of condensation of aromatic functional groups, its high value implied low degree of aromatic condensation and existence of relatively large proportions of aliphatic structures.
Further, it is observed that UV-Vis spectra of Shilajeet in water at different pH in the presence of Hg were shifting towards higher wavelength, \( i.e., \) red shift was recorded in the complexes formed by tagging of mercury. It seems that the lowest unoccupied molecular orbital (LUMO) is where Hg is expected to bind. Further evidence in this respect may be drawn by performing more intensive IR studies on humus substances, \( \text{viz.} \), fulvic acid, humic acid and Shilajeet [19] shifting down in energy and LUMO may be located at the carboxylic group.

### 6.3.8 Raman spectrographs

The technique of Raman spectroscopy was used to ascertain falsification of Shilajeet drug by sildenafil citrate. This technique is preferred due easy sample preparation, less time consuming and fast results. Table 6.1 shows the list of various samples investigated for falsification by matching of Raman peaks. The characteristic peaks of expected adulterants, \( i.e., \) sildenafil citrate are shown in Fig. 6.16. Raman spectra of only six samples are shown in Figs.6.17-6.18 where as twelve popular brands of Shilajeet drugs were investigated. The surface-enhanced Raman spectra’s of these drugs were matched for characteristic peaks of expected adulterants. There is no matching of peaks for the remaining samples thus it may be concluded that they are not adulterated. It is better to buy branded drugs of Shilajeet manufactured by established pharmaceutical companies only, not from roadside vendors, quacks etc. who sell neutraceuticals in loose or powdered form, without stating their contents. Various samples of Shilajeet were also characterized and their physico-chemical and spectral properties were compared. Such results are presented for the first time herein as per our knowledge. The folk medicines (neutraceuticals) can establish themselves in the mainstream medicines only if their claims are scientifically proven. The efficacy of natural health care products is believed to be due to presence of minerals and metals in it. In the present study, the Shilajeet exhibited high mineral content and metal tagging activity. Now a day’s Shilajeet is popularly used as aphrodisiac, a synergizer, both as raw and pharmaceutical drugs, the present study was undertaken to know the elemental composition of various samples of Shilajeet. The concentrations (in ppm) of its constituent elements K, Ca, Cl, Si, Fe, S, P, Mn, Mg, Sr, Mo, Rb, Ru,
Fig. 6.16: Typical Raman spectra of sildenafil citrate showing main peaks in the region (a) 1800-950 cm\(^{-1}\) (b) 3800-2700 cm\(^{-1}\).
Fig. 6.17: Typical Raman spectra of samples SA3, SA5 and SA7 used for matching of peaks with sildenafil citrate.
Fig. 6.18: Raman spectra of samples SA8, SA9, and SA13 used for matching of peaks with sildenafil citrate.
Br, Zn, Ti, Cu, Ni, and Na are observed as 6.97%, 1.77%, 1.11%, 0.34%, 0.34%, 0.33%, 0.20%, 645 ppm, 547 ppm, 429 ppm, 416 ppm, 403 ppm, 242 ppm, 197 ppm, 187 ppm, 134 ppm, 126 ppm, 114 ppm, 75 ppm and 47 ppm, respectively. Trace elements are very essential in the biological systems but if their quantity exceeds maximum permissible limit they become toxic too. This native raw product, Shilajeet is sold freely in rudimentary form. Before it is ingested by the people, it should be purified properly and it’s elemental composition be considered.

In an effort to ascertain counterfeiting of products of Shilajeet by sildenafil citrate, the technique of surface enhanced Raman spectroscopy was used. The results of Raman analysis indicated that in the region around broad band at 1584 cm\(^{-1}\), there is matching of 1583 cm\(^{-1}\) band of sildenafil citrate with sample SA3. However due to the absence of matching other signature peaks, nothing conclusive can be said about the adulteration of product. In the remaining samples, no matching of peaks was observed. Thus Raman spectroscopy provided a vital probing technique to establish the counterfeit drugs. More over availability of Shilajeet in rudimentary form is very localized which makes it scarce whereas its main components humic acid fulvic acid are low-priced and abundant, and primarily responsible for observed metal tagging property, so more intensive studies are required on them. Thus efforts need to be made for Shilajeet’s synthesis in the laboratory under optimum conditions of temperature and pressure on humus.
References


