



## Pharmaceutical Standardization

# Fourier transform infrared analysis of *Tamra Bhasma* at different levels: A preliminary study

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### Abstract

**Introduction:** *Tamra Bhasma*, one among the herbo-metallic preparations is extensively used in Ayurveda for different conditions. To make it safe to use, *Tamra* has to pass through a set of classical pharmaceutical procedures including a series of quenching in prescribed liquids, followed by incineration with black sulfide of mercury and herbal juice of *Citrus jambhiri* Lush. and corm of *Amorphophallus campanulatus* Linn. FTIR profiles of *Tamra Bhasma* at different levels is not available. **Aim:** To evaluate the chemical changes in *Tamra Bhasma* at different steps by following Fourier transform infrared (FTIR) spectroscopy. **Materials and Methods:** In current study, raw *Tamra*, intermediate samples obtained during purification, incineration and *Amritikarana* were analyzed using FTIR. **Results:** It was observed that *Shodhana* procedure leads in the formation of bonds between surface particles of *Tamra* and *Shodhana* media. These formed bonds on the surface of *Shodhita Tamra* samples gave various sharp peaks representing presence of many functional groups. **Conclusion:** The FTIR spectra revealed that both *Bhasma* samples contained organic compounds probably in the form of a complex with common functional groups like alkyl, methyl, etc., which need further studies for exact characterization of the complexes.

**Key words:** Copper, Fourier transform infrared, *Marana*, *Shodhana*, *Tamra Bhasma*

## Introduction

Ayurveda, the ancient system of medicine is being safely practiced in Indian subcontinent since ages. The drugs in this system of medicine are obtained from natural sources including plants, animals, marine and minerals that are converted into compound formulations by following specified guidelines mentioned in Ayurvedic classics. *Bhasmas* (calcined forms of metals and minerals) are one of such important formulations that are therapeutically useful in different disease conditions. Despite their utility, concerns are being raised regarding the safety and toxicity of traditional preparations and there is a need to address them appropriately.<sup>[1-4]</sup> *Tamra Bhasma* is one important *Bhasma* useful in many diseases.<sup>[5]</sup> If the metal is not processed properly, it may be hazardous to life.<sup>[6]</sup> It has to process through a set of classical pharmaceutical procedures known as *Shodhana*, *Marana* and *Amritikarana* etc., before its

internal administration. Properly processed *Tamra Bhasma* is useful in the treatment of *Udara* (ascitis), *Pandu* (anaemia), *Svasa* (bronchial asthma) and *Amlapitta* (hyperacidity) etc. disorders.<sup>[7]</sup> Though qualitative tests to judge proper formation of *Bhasma* are described in *Rasa* classics, they are not sufficient to provide quantitative information. Standardization of each involved step is become necessary to study possible reaction. Emphasis in the present paper is to see chemical changes in *Tamra Bhasma* at different steps by following Fourier transform infrared (FTIR) that may highlight the importance of Ayurvedic processing methods.

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## Materials and Methods

### Preparation of Tamra Bhasma

Tamra Bhasma was prepared by following classical guidelines as described in Ayurveda classics [Figure 1].

#### Samanya Shodhana

Copper scraps (0.5 mm thickness) of 99.89% purity copper were procured from Bharat Bridge Plate, Jamnagar. About 500 g of metallic copper was heated to red hot stage and quenched in *Tila Taila* (sesame oil), *Takra* (buttermilk), *Gomutra* (cow's urine), *Kanji* (sour gruel), and *Kulattha Kwatha* (decoction of seeds *Dolichos biflorus* Linn.) for 7 times in each media sequentially to obtain *Samanya Shodhita Tamra*.<sup>[8]</sup>

#### Vishesha Shodhana

*Samanya Shodhita Tamra* was subjected to *Swedana* (boiling) in *Gomutra* (cows urine) for 3 h.<sup>[9]</sup> It was shade-dried and used for *Marana* (incineration).

#### Marana

*Vishesha Shodhita Tamra* was mixed with equal quantity of *Kajjali* (black sulphide of mercury) and levigated with *Jambiri Nimbu Swarasa* (juice of *Citrus jambhiri* Lush.). *Chakrikas* (pellets) were prepared, shade-dried, placed in *Sharava* (earthen saucer) and covered by another *Sharava* of similar diameter. The junctions were sealed with double folded, mud smeared cloth. It was subjected to heat in electrical muffle furnace (EMF) at the temperature of 700°C for first *Putra* and 650°C for subsequent four *Putas* for 30 mins.<sup>[10]</sup> This process of incineration was repeated for five more times to obtain *Tamra Bhasma*.

#### Amritikarana

*Tamra Bhasma* was triturated with 1/2 part of *Shuddha Gandhaka* (processed sulphur) and levigated with *Jambiri Nimbu Swarasa*. After proper levigation, a round bolus was prepared and dried in shade. This bolus was kept in the pit of *Surana Kanda* (corm of *Amorphophallus campanulatus* Linn.) made by cutting it appropriately. Two halves of the corm were joined and sealed together. The corm was covered tightly with double layered, mud smeared cloth, allowed to become dry. Subjected to heat in EMF at 650°C for 30 min, and removed after *Swangasheeta* (self cooling). The bolus inside was taken out, triturated, weighed and stored in airtight glass container.<sup>[11]</sup>

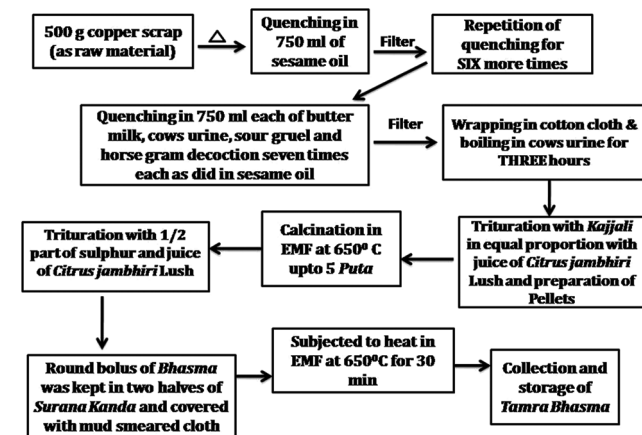


Figure 1: Flow sheet for preparation of Tamra Bhasma

### Analysis of samples

Samples of raw, *Samanya Shodhita*, *Vishesha Shodhana*, *Marita* and after *Amritikarana* were labeled and analyzed through FTIR [Table 1]. The 3000 Hyperion microscope with vertex 80 FTIR system instrument was used for the current study.

### Observations and Results

Fourier transform infrared spectroscopy was performed to detect the presence of functional groups or organic legends in different samples of *Tamra*. FTIR spectra are divided into four regions

Table 1: Sample codes for analysis

Sample	Code
Raw Tamra	RT
Taila Shodhita Tamra	TST
Takra Shodhita Tamra	TKST
Gomutra Shodhita Tamra	GST
Kanji Shodhita Tamra	KST
Kulattha Kwatha Shodhita Tamra	KKST
Vishesha Shodhita Tamra	VST
Tamra Bhasma without Amritikarana	TB
Tamra Bhasma with Amritikarana	TBA

Table 2: Number of peaks and their wavelength

Sample code	Number of peaks	Wavelength
RT	19	3941, 3887, 3828, 3766, 3725, 3594, 3479, 3320, 2924, 2860, 1591, 1459, 1039, 1008, 926, 824, 655, 603, 551
TST	20	3786, 3707, 3625, 3536, 3484, 3422, 3187, 3091, 3049, 3001, 2887, 1653, 1606, 1281, 1035, 975, 733, 690, 609, 519
TKST	19	3671, 3602, 3504, 3430, 3369, 3271, 3198, 3061, 2944, 2873, 1700, 1649, 1581, 1172, 1088, 1060, 791, 688, 582
GST	18	3525, 3489, 3423, 3325, 3245, 3184, 3109, 1668, 1630, 1592, 1439, 1406, 1376, 1070, 1058, 1028, 517, 615
KST	20	3890, 3462, 3327, 3211, 3015, 2932, 2875, 1613, 1415, 1357, 1151, 1094, 1054, 852, 764, 702, 645, 573, 537, 519
KKST	20	3806, 3579, 3480, 3431, 3358, 3319, 3259, 3168, 3112, 3050, 2916, 1649, 1598, 1402, 1090, 1059, 878, 804, 704, 635
VST	20	3585, 3544, 3504, 3435, 3368, 3264, 3208, 3173, 3132, 3107, 3029, 1665, 1574, 1473, 1419, 788, 750, 703, 615, 555
TB	18	3871, 3833, 3732, 3420, 2921, 2862, 1634, 1540, 1454, 1377, 1117, 990, 748, 694, 657, 602, 525, 437
TBA	15	3872, 3834, 3739, 3424, 2919, 2860, 1634, 1541, 1431, 1379, 1325, 1113, 1059, 668, 607

RT: Raw Tamra, TST: Taila Shodhita Tamra, TKST: Takra Shodhita Tamra, GST: Gomutra Shodhita Tamra, KST: Kanji Shodhita Tamra, KKST: Kulattha Kwatha Shodhita Tamra, VST: Vishesha Shodhita Tamra, TB: Tamra Bhasma without Amritikarana, TBA: Tamra Bhasma with Amritikarana

viz. Hydrogen stretching region ( $3700\text{--}2700\text{ cm}^{-1}$ ), triple bond region ( $2700\text{--}1950\text{ cm}^{-1}$ ), a double bond region ( $1950\text{--}1550\text{ cm}^{-1}$ ) and fingerprint region ( $1500\text{--}700\text{ cm}^{-1}$ ). FTIR spectra of all samples were taken in the region of  $551.70\text{--}3941\text{ cm}^{-1}$ . General overview of all the samples indicates the presence of large number of functional groups. Absorption peaks in Hydrogen stretching region ( $3700\text{--}2700\text{ cm}^{-1}$ ) are ordinary due to various O-H and N-H stretching vibrations, with the former tending to appear at higher wave numbers. Aliphatic C-H vibrations fall in between the region  $3000$  and  $2850\text{ cm}^{-1}$ . Most aliphatic compounds have a sufficient number of C-H bond to make this prominent peak [Table 2].

## Discussion

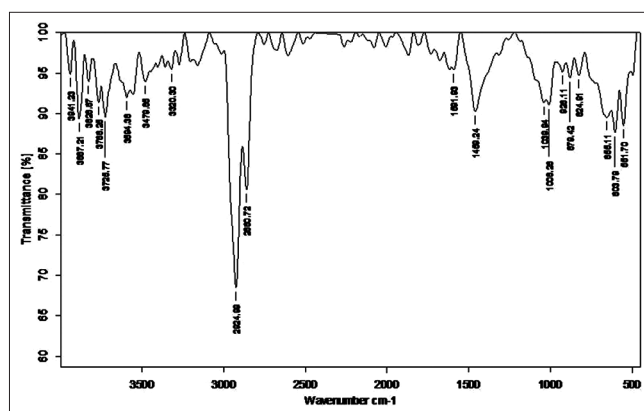
### FTIR of raw and Shodhita Tamra

Number of peaks obtained in hydrogen stretching region in sample raw *Tamra* (RT), *Taila Shodhita Tamra* (TST), *Takra Shodhita Tamra* (TKST), *Gomutra Shodhita Tamra* (GST), *Kanji Shodhita Tamra* (KST), *Kulattha Kwatha Shodhita Tamra* (KKST) and *Vishesh Shodhita Tamra* (VST) are 10, 11, 10, 7, 7, 11 and 11. No peaks were observed in any of the samples in Triple bond region ( $2700\text{--}1950\text{ cm}^{-1}$ ) which may indicate absence of highly complex structure. The carbonyl stretching vibration is characterized by absorption through double bond region ( $1950$  and  $1550\text{ cm}^{-1}$ ). Ketones, aldehydes, acids, amides and carbonates all have absorption peaks around  $1700\text{ cm}^{-1}$ . Esters, chlorides and acid aldehydes tend to absorb at slightly higher wavelengths; that is  $1770\text{--}1725\text{ cm}^{-1}$ . Conjugation tends to lower the absorption peaks by about  $20\text{ cm}^{-1}$ . Absorption peaks arising from C = C and C = N stretching vibrations are located in  $1690\text{--}1600\text{ cm}^{-1}$  range. The region between  $1650$  and  $1450\text{ cm}^{-1}$  provide important information about aromatic rings. Nearly all samples showed either one or more peak in this region. All samples of *Tamra* gave few peaks in the fingerprint region ( $1500$  and  $700\text{ cm}^{-1}$ ). Small differences in the structure and constitution of a molecule result in significant changes in the distribution peaks in the fingerprint region of the spectrum. Most single bonds give rise to absorption bands at these frequencies. The C-O-C stretching vibration in ethers and esters are found at about  $1200\text{ cm}^{-1}$  and the C-Cl stretching vibration at  $700\text{--}80\text{ cm}^{-1}$ . A number of inorganic groups such as sulfate, phosphate, nitrate and carbonate also absorb at wave numbers below  $1200\text{ cm}^{-1}$ .

C-H stretching vibrations near  $1380\text{ cm}^{-1}$  resulted in obtaining weak peak in GST, which is assigned to alkyl specifically methyl bond. Similar C-H stretching vibrations near  $2870\text{ cm}^{-1}$  wavelength gave a peak in all samples except in GST and KKST. These peaks are also representative of alkyl specifically methyl bond. There is considerable variation in obtained peaks due to stretching vibrations between C-H bonds. These C-H stretching vibrations are assigned to alkyl (methylene), vinyl (monosubstituted alkenes, cis-disubstituted alkenes, trisubstituted alkenes), aromatic benzene (monosubstituted benzene, ortho-disubstituted benzene, meta-disubstituted benzene and para-disubstituted benzene). All samples of *Shodhita Tamra* except KST showed peaks are raised due to C-C stretching vibrations of acyclic C-C bond. These peaks represent various forms alkene functional group such as mono-substituted,

1,1 di-substituted, cis-1,2-di-substituted, tri-substituted and tetra-substituted alkenes.

Stretching vibrations between C-C bond with C = O near  $1600\text{ cm}^{-1}$  gave peak in sample RT, TST, KKST and GST, which is assigned to dienes. There are few peaks detected in all samples in the range  $1640\text{--}1680\text{ cm}^{-1}$  except KST. All these peaks are due to C = C stretching vibrations. Multiple weak to strong peaks obtained near  $1450\text{ cm}^{-1}$  (in RT and *Tamra Bhasma* without *Amrittikarana*),  $1580\text{ cm}^{-1}$  (in TKST and VST) and  $1600\text{ cm}^{-1}$  (in RT, TST, GST and KKST) are representative of C = C stretching vibrations having aromatic structure. Stretching vibrations between C = O at  $1580\text{--}1690\text{ cm}^{-1}$  indicates presence of carboxylic acid and its derivatives such as unsaturated/aromatic carboxylic acids, amides, carboxylates salts and amino acid zwitterions is observed in samples of *Shodhita Tamra*. However, these peaks are not detected in both samples of *Tamra Bhasma*. In the classical text of Ayurveda, *Niramlatva* (absence of bitter or acidic test) is especially advised test for *Tamra Bhasma*. Hence, absence of carboxylic acid derivatives also supports proper formation of *Tamra Bhasma*. One peak obtained in sample TST, and two peaks in sample TKST are raised due to O-H stretching vibrations. These peaks are in the range  $3610\text{--}3670\text{ cm}^{-1}$  that is assigned to low concentrated phenol bonds. Similar O-H stretching vibrations are observed in all samples of *Shodhita Tamra* except in TST, which are in the range  $3200\text{--}3400\text{ cm}^{-1}$  and represent high concentrated alcohol or phenol bonds. Sample TST showed a sharp peak at  $3001.24\text{ cm}^{-1}$  is raised due to O-H stretching vibrations. Broad and weak to medium peak near  $3000\text{ cm}^{-1}$  is representative of either high concentrated carboxylic acid or secondary amines but peak obtained in sample TST is sharp, hence exact interpretation can't be withdrawn regarding assignment of this peak. All samples gave peaks in the range  $3400\text{--}3500\text{ cm}^{-1}$  that are obtained from N-H stretching vibrations and can be assigned to primary amines. All samples showed multiple peaks in the range  $2400\text{--}3200\text{ cm}^{-1}$  that are raised due to stretching vibrations between N-H bonds. These peaks indicate the presence of ammonium ions. Peak obtained below  $1380\text{ cm}^{-1}$  wavelength in samples of *Tamra* are due various stretching vibrations such as C-O, C-N, C-F, C-Cl, C-Br and N-O. These peaks represent various functional groups such as alcohol, esters, carboxylic acids, aliphatic amines, fluoroalkane, chloroalkane, bromoalkane and nitro compounds [Figures 2-8].



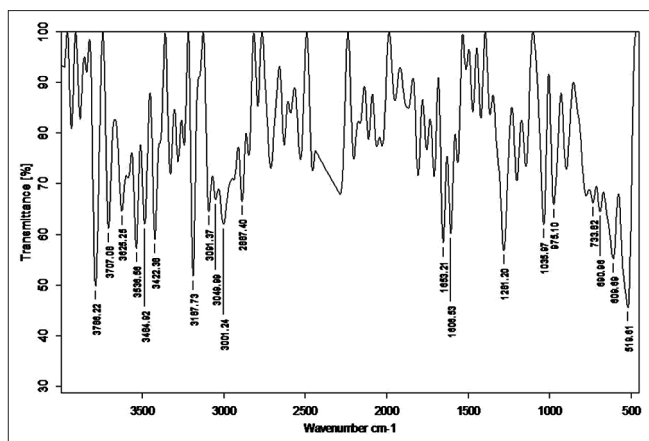


Figure 3: Fourier transform infrared analysis of Tila Taila Shodhita Tamra

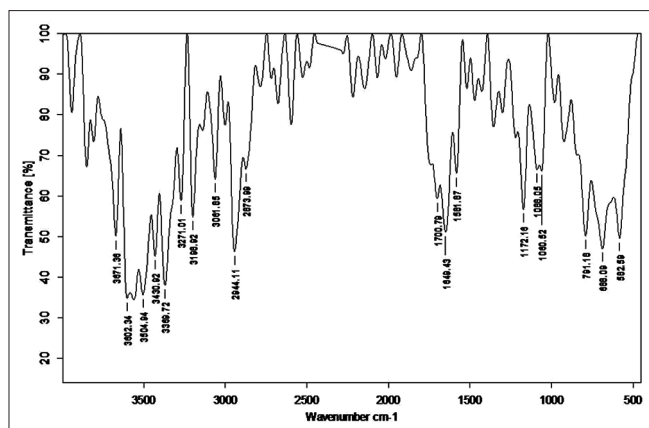


Figure 4: Fourier transform infrared analysis of Takra Shodhita Tamra

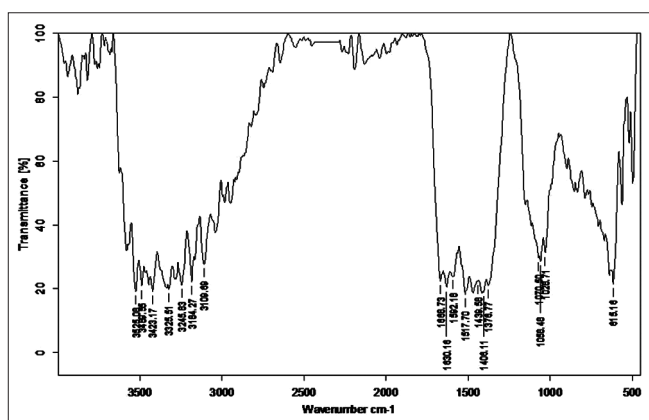


Figure 5: Fourier transform infrared analysis of Gomutra Shodhita Tamra

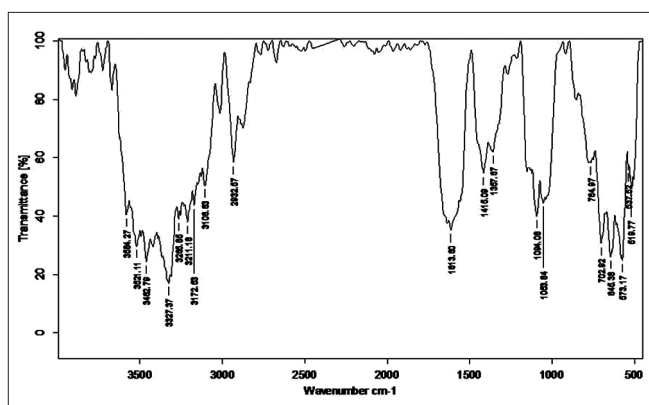


Figure 6: Fourier transform infrared analysis of Kanji Shodhita Tamra

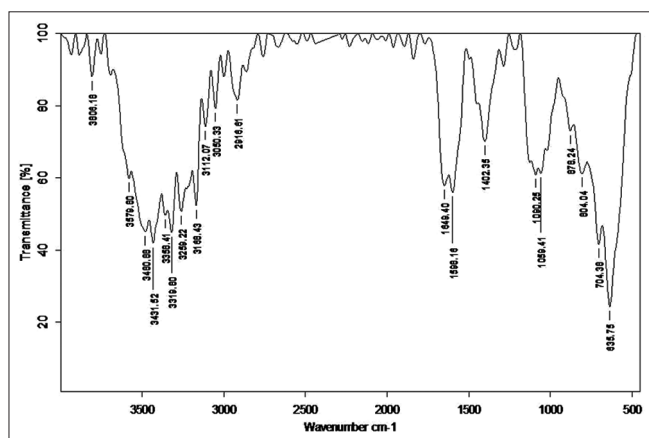


Figure 7: Fourier transform infrared analysis of Kulattha Kwatha Shodhita Tamra

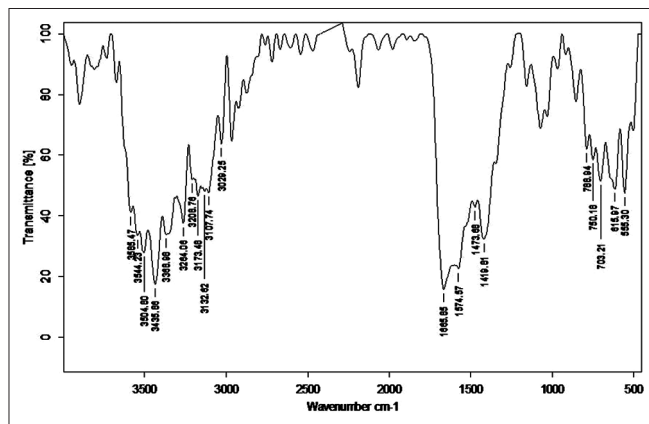
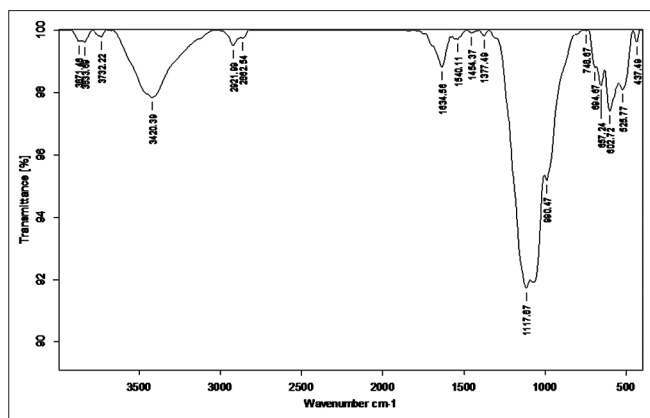


Figure 8: Fourier transform infrared analysis of Vishesh Shodhita Tamra

### FTIR of Tamra Bhasma without Amritikarana

Total 6, 0, 1 and 5 peaks were obtained in these regions respectively. Six peaks are obtained in a region below 650  $\text{cm}^{-1}$  that is not specifically classified. No peak was observed in triple bond region that indicates the absence of too much complex structure of Tamra Bhasma without

Amritikarana (TB). C-H stretching vibrations near 1380  $\text{cm}^{-1}$  resulted in obtaining weak peak that is assigned to alkyl specifically methyl bond. Medium to strong intensity of C-H stretching vibrations resulted in two peaks at 2862.54 and 22921.99  $\text{cm}^{-1}$  that are assigned to methyl and methylene ( $-\text{CH}_2$ ) type of bond. C-H stretching vibrations at 748.67, 694.67 and 748.67 resulted in obtaining three strong peaks out of these first two are assigned to aromatic

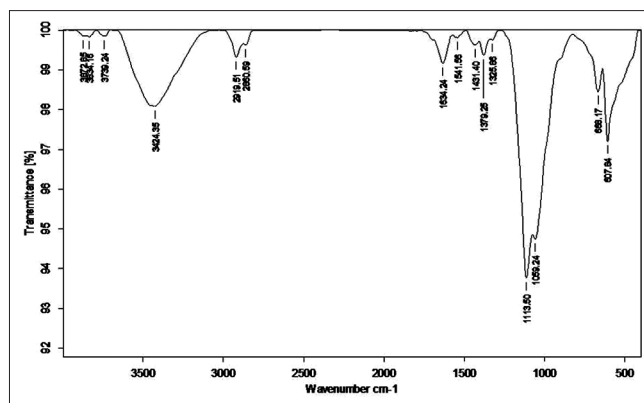


**Figure 9:** Fourier transform infrared of *Tamra Bhasma* without *Amritikarana*

specifically monosubstituted benzene bond and third peak is ortho-disub benzene bond. Three peaks (1634.56, 1634.56 and 1454.37) are due to C = C stretching vibrations. Two strong peaks (3420.39, 1634.56  $\text{cm}^{-1}$ ) are obtained which are assigned to the stretching vibrations between N-H bonds indicate presence of primary amines. C-X stretching vibrations represent bond between carbon and halogen atoms. Such six peaks are observed which are assigned to chloroalkanes (694.67, 657.24, 602.72  $\text{cm}^{-1}$ ), bromoalkanes (602.72, 525.77  $\text{cm}^{-1}$ ) and fluoroalkanes (1117.87  $\text{cm}^{-1}$ ). Weaker peak obtained at 1377.49  $\text{cm}^{-1}$  is raised due to N-O stretching vibrations and is assigned to aliphatic nitro compounds [Figure 9].

#### FTIR of *Tamra Bhasma* with *Amritikarana*

Total 6, 0, 1 and 5 peaks are obtained in above mentioned four FTIR regions respectively. Six peaks are obtained in an unknown region that is not specifically classified. Similar to sample TB, no peak is obtained in triple bond region. A given absorption band assigned to a functional group increases proportionately with the number of times that functional group occurs within the molecule. In this sample, Alkyl group is represented by four peaks raised due to C-H stretching vibrations at 1379.25, 2860.59  $\text{cm}^{-1}$  assigned to methyl and at 2860.59, 2919.51  $\text{cm}^{-1}$  to methylene. Two peaks (1634.24, 1634.24) are due to C = C stretching vibrations. Primary and secondary amines exhibit the most characteristic group frequencies, which are associated with the N-H bond. In this sample, primary amines are represented by the peak obtained at 3424.35 and 1634.24  $\text{cm}^{-1}$ . All these peaks are raised due to N-H stretching vibrations. However, similar N-H stretching vibrations resulted in multiple peaks at 2919.51, 2860.59  $\text{cm}^{-1}$  that are assigned to ammonium ions. Three peaks 1059.24, 1113.50 and 1113.50  $\text{cm}^{-1}$  were raised due to C-O stretching vibrations that are representative of alcohol, ether, ester group. Often, the frequency ranges for the different classes of carbonyl compound overlap, and the carbonyl frequency alone is not sufficient to characterize the functional group. In most cases, spectral information from the other component of the functional group is used for the characterization. Esters (C-O-C) and amides (C-N and N-H) are good examples. Three peaks at 1113.50, 1059.24 and 1634.24  $\text{cm}^{-1}$  were obtained due to C-N vibrations having similar conjugation effects to C = O. Tertiary amines are very comparable to ethers, and the main diagnostic information



**Figure 10:** Fourier transform infrared of *Tamra Bhasma* with *Amritikarana*

is gathered from the C = N vibrations only. An important exception is the methylamino (and dimethylamino) group. Five peaks were observed due to C-X stretching vibrations. One weaker peak at 1377.49  $\text{cm}^{-1}$  is raised due to N-O stretching vibrations similar in TB sample [Figure 10].

## Conclusion

It is evident from the present study that the treatment of raw *Tamra* with various treating liquids; some portion of *Tamra* gets converted into powder form and the surface consistency of copper changed from smooth to brittle rough. Hence, as per FTIR analysis, it can be interpreted that media used during *Shodhana* procedure and repeated strong heating followed by sudden cooling changes the surface consistency of *Tamra*. It also leads to the formation of bonds between surface particles of *Tamra* and atoms present in *Shodhana* media. These bonds on the surface of *Shodhita Tamra* samples gave various sharp peaks representing presence of many functional groups. FTIR analysis can be used as a tool to differentiate the *Bhasmas* prepared by different techniques. Present work can be considered as the first step towards identifying the followed methods through FTIR analysis. This is a preliminary analysis and exact nature of different peaks along with the characterization is to be carried-out.

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## Conflicts of interest

There are no conflicts of interest.

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## हिन्दी सारांश

### फौरीअर ट्रान्सफोर्म इन्फ्रारेड के विशेष संदर्भ से ताम्र भस्म का मानकिकरण

स्वप्निल वाय. चौधरी, धीरजसिंह एस. राजपूत, गालिब आर., प्रदीपकुमार प्रजापति

आयुर्वेद में ताम्र भस्म का विविध रोगों की चिकित्सा में अधिकतम उपयोग किया जाता है। उसके सुरक्षित उपयोग के लिए उसपर शोधन, मारण और अमृतीकरण प्रक्रिया की जाती है। अतः प्रस्तुत अध्ययन में कच्चे ताम्र का शोधन, मारण तथा अमृतीकरण के बाद में नमूनों का एफ.टी.आय.आर. विश्लेषण किया है। शोधन प्रक्रिया के बाद ताम्र के नमूनों में कार्यकारी गण के साथ अलग अलग पिक्स मिले हैं। एफ.टी.आय.आर. स्फेक्ट्रा में ताम्र भस्म के दोनो नमूनों में अल्काइल, मिथाइल, इत्यादि सेन्द्रिय योगों की श्रृंखला प्राप्त होती हैं।