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SEM-EDAX Analysis of *Rasasindhura* prepared according to the reference of *Rasatarangini*

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ABSTRACT

Kupipakwa Rasayana's plays a major role in the field of therapeutics in *Rasashastra*. It is a procedure developed mainly for *Gandhaka Jarana* for which *Kupi* is used in the preparation. The procedure involves placing of *Kajjali* into the *Kupi* and subjecting it for paka for a specific period of time by supplying a uniform pattern of heat. Ancient Indian alchemist transformed lower metals to higher metals and in this way they used to potentiate and transform mercury to higher levels by the process of *Jarana*. In such experimentation of *Jarana*, *Kupipakwa* method was adopted. This article deals with the preparation of *Rasasindhura* followed by its analysis via SEM-EDX which proved that there was reduction in some of the elements present in the drug after subjecting it to heat. In elemental analysis of *Rasasindhura* there was presence of Sulphur and Mercury. Atomic % of Sulphur was 54.23 and Mercury atomic % was 45.77. There was no presence of heavy metals in the prepared sample of *Rasasindhura*. The particles present in the preparation were clearly distinctive and the particle size were clearly analysed which was in the range of 118 to 138.2 nm at a magnification of 20.00 K X.

Key words: *Rasasindhura*, *Sem-Edax*, *Kupipakwa Rasayana*.

INTRODUCTION

Kupipakwa Rasayanas is one among the most important medicinal preparations discovered in the field of *Rasashastra*. The term *Kupipakwa* mainly denotes pharmaceutical process that involves the use of glass bottle for the preparation. The product thus obtained is thereby used as a medicine for the

treatment of various diseases. *Rasasindhura*^[1] is one such preparation which includes ingredients such as Mercury and Sulphur. Both of these are first subjected to *Shodana*, after which, *Kajjali* is prepared by triturating *Parada* and *Gandhaka* till the product becomes lustreless. It is then placed in a *Kupi* which is completely layered by 7 layers of thick cloth and then it is subjected to heat. Heating process plays an important role in the preparation of *Kupipakwa Rasayanas*. A steady amount of heat is given and all the *Lakshanas* are observed precisely and required interventions are done. Once the *Samyak Lakshanas* of *Rasasindhura* is observed, it is later collected and used as a medicine.

Sem-Edax is one of the recent advancements used in the field of Nanotechnology which identifies the elements and particle size of the sample. *Rasasindhura* was analysed and showed the presence of Mercury and Sulphur, with its particle size ranging in nanometer scale.

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MATERIALS AND METHODS

Preparation of Rasasindhura

1. Shodana of Parada
2. Shodana of Gandhaka
3. Preparation of Kajjali
4. Preparation of Rasasindhura

1) Shodana of Parada^[2]

Apparatus used: Khalva Yantra (mortar and pestle), Spatula, Steel vessels, Cloth, Weighing balance.

Ingredients:

1. Ashuddha Parada: 800 gm
2. Suddha Churna: 800 gm
3. Lasuna (*Allium sativum*): 550gm
4. Saindhava: 275gm

Procedure (Fig. 1)

- 1kg of Ashuddha Parada was triturated with 1kg Suddha Churna in a Khalva Yantra for 72 hours.
- After 72 hours, Parada was separated from the Suddha Churna by filtering it through a two-folded clean cloth.
- 750 gm was the yield obtained after separating it from the lime powder.
- After which, the obtained Parada was again triturated in a Khalva Yantra with equal quantity of Lasuna (750gm) and half part of Saindhava Lavana (375 gm) till it forms a Kalka which is blackish in colour.
- Then this Kalka was washed with warm water carefully and Parada was collected carefully and filtered through a two folded clean cloth.
- 700gm of Shuddha Parada was obtained after the complete procedure (50gm loss while triturating it with Lasuna, Saindhava Lavana and after washing the kalka).

Observations

- Continuous trituration of Parada, made it to disintegrate into small globules and started mixing up with Suddha Churna.

- After 13 hours of trituration, Suddha Churna turned light greyish in color.
- After 27 hours of trituration, mixture turned dark greyish in colour and no free globules of Parada were seen in the mixture.
- While triturating Parada with Saindhava Lavana and Lasuna, after 3 hours of trituration it turned blackish in color.
- While doing Prakshalana of Kalka with water, Parada was seen to sediment at the base of the Khalva Yantra.

Precautions

- Trituration should be done carefully, to avoid spillage of Parada.
- Separation of Parada from Suddha Churna has to be done carefully and precisely in order to avoid wastage.
- Separation of Parada from the Kalka should be done very carefully due to its loss during washing.

Result

- Total time taken: 150 days
- Total time of trituration: 72 hours
- Weight of Parada obtained after trituration with Suddha Churna: 550gm
- Final yield of Parada: 500 gm
- Weight loss: 300

2) Shodana of Gandhaka^[3]

Name of Practical: Shodana of Gandhaka (Fig. 2)

Reference: R.R.S 3/21-23

Apparatus used: Khalva Yantra, Steel vessels, Gas stove, Spatula, Cloth, Weighing balance.

Ingredients:

1. Ashuddha Gandhaka: 600gm
2. Goghrita: 425gm
3. Godugdha: 3.5 litres

Procedure

- 600gm of *Ashuddha Gandhaka* was taken and powdered in a *Khalva Yantra*.
- The Powdered *Gandhaka* was taken in a steel vessel.
- Around 180gm of *Goghrita* was taken in an iron pan, placed over a stove and heated in *Mandagni*.
- 3.5 liters of *Godugdha* was taken in another steel vessel and a piece of cloth was tied over its mouth, which was then slightly smeared with *Gogritha*.
- *Gandhaka* was then carefully put in the Iron pan having *Ghritha* which was slightly hot.
- When *Gandhaka* totally melted in the *Ghritha*, the mixture was immediately poured into the vessel containing *Godugdha*.
- A solid mass of *Gandhaka* was formed at the bottom of the steel vessel containing Milk.
- This mass was taken out from the Milk and was washed thoroughly with hot water.
- Then again this *Gandhaka* was powdered and the same procedure was repeated 6 times.
- At the end of the procedure *Gandhaka* was washed with warm water and dried.
- Finally *Gandhaka* was powdered and placed in a container.

Observations

- When *Gandhaka* was melted in *Goghrita* it formed a homogenous mixture, which was yellowish red in color.
- The color of *Gandhaka* changed from yellow to brown after each step till the end of the procedure.
- Some physical impurities were observed over the cloth, after pouring the melted *Gandhaka* in Milk.
- After pouring melted *Gandhaka* into *Godugdha*, *Ghritha* was observed floating over the surface of *Godugdha* and colour of it was yellowish brown.

- *Shuddha Gandhaka* was obtained as granules at the bottom of the vessel containing *Dugdha* and its appearance was oily dull yellowish in color.

Precautions

- Heating has to be done in *Mandagni*.
- *Gandhaka* and *Go-ghritha* had to be constantly stirred while heating, to prevent it from getting burnt and becoming thick in consistency, which hinders the filtration process.
- When *Gandhaka* melts, it has to be immediately and cautiously poured into the Milk.
- *Gandhaka* has to be washed with hot water at the end of the process to remove the oil present over its surface and then should be dried.
- The completely dried *Gandhaka* has to be used.

Causes of weight loss

- Some particles of *Gandhaka* get adhered to the cloth during filtration.
- While washing some particles of *Gandhaka* gets wasted with water.

Result

- Time taken for practical: 1 day
- Quantity of *Shuddha Gandhaka* obtained: 500 gm
- Weight loss: 100 gms

3) Preparation of *Kajjali*^[4]

Name of Practical: *Samaguna Kajjali* preparation. (Fig. 3)

Reference: R.R.S

Apparatus used: *Khalva Yantra*, Weighing Balance etc.

Ingredients:

1. *Shuddha Parada*: 500gm
2. *Shuddha Gandhaka*: 500gm

Procedure

- *Shodita Parada* and *Shodita Gandhaka* were taken in equal quantity.
- It was then triturated in a *Khalva Yantra*.

- The color of *Parada* and *Gandhaka* disappeared and a black powder was formed.
- During the process of trituration, few drops of water was sprinkled over the powder to prevent it from spilling.
- Trituration was continued till the powder became jet black in colour and became fine and *Nischandra*.

Observations

- After 3 hours of trituration, the mixture of *Parada* and *Gandhaka* turned greyish in color.
- After 7 hours of trituration, the mixture turned blackish in color.
- After 9 hours of trituration, globules of *Parada* almost disappeared and the mixture turned dark black colour. But when rubbed between the fingers silvery particles was seen.
- After 46 hours of trituration, it appeared as though *Kajjali* was formed, but on examining under sunlight, free mercury particles was observed.
- After 72 hours of trituration, the mixture completely turned jet black in colour.
- On examination, *Kajjali* fulfilled the test of *Varitara*, *Rekhapurnatva* and *Nischandratva*.
- The entire powder became jet black in color, very fine, smooth and lustreless.

Precautions

- To prepare *Kajjali*, *Gandhaka* should always be taken in fine powder form.
- Trituration should be done slowly and carefully to prevent the loss of *Parada*.
- Few drops of water should be sprinkled over *Kajjali* to prevent spillage during trituration.
- *Khalva* should be kept covered when the process is not in progress.

Results

- No. of days taken: 135 days

- Total time taken for preparation of *Kajjali* : 72hrs
- Weight of *Kajjali* obtained: 850gm
- Total wt. loss: 150 gm.

Cause of weight loss

- Spillage of mixture during the process of trituration.
- *Kajjali* gets adhered to the *Khalva* which makes it difficult to collect after completion.
- Little quantity of *Kajjali* was lost while performing its confirmatory test.

Table 1: Showing organoleptic characteristics of *Kajjali*.

Organoleptic characteristics	Observation of <i>Kajjali</i>
Appearance	Black color
Touch	Smooth, Fine and <i>Rekhapurna</i>
Smell	Smell of <i>Gandhaka</i>
Form	Fine powder

4) Preparation of *Rasasindhura*^[5]

Name of Practical: *Samaguna Rasasindhura* preparation (4 batches). (Fig. 4)

Reference: R.T 6/162-167

Apparatus used: *Koshti Yantra*, *Kachakupi* (layered with 7 layers of *Multani Mitti*), *Iron Shalaka*, Cork, Copper sheet, electric muffle furnace etc.

Ingredients:

1. *Samaguna Kajjali* : 550gm
2. *Vatankura Swarasa* : Q.S

Procedure

The whole procedure was divided into 3 phases;

(1) *Purva Karma* (2) *Pradhana Karma* (3) *Paschat Karma*

1. *Purva Karma*

- Collection of all the essential equipment's and ingredients (*Kajjali*, *Kachakupi*, *Iron Shalaka*, *Copper sheet*, *Cork*, *Coal*, *Valuka yantra* etc) required for the preparation.

- Kachakupi had to be layered with mud smeared cloth (7 layers) and dried at each time.
- Preparation of *Kajjali*.
- *Kajjali* was given *Bhavana* with *Vatankura Swarasa* (3 times).
- After that *Kajjali* was carefully filled into the *Kachakupi* with the help of a funnel.

2. Pradhana Karma

- The *Kachakupi* containing *Kajjali* was placed in the *Valuka Yantra* and fixed in proper position.
- Heat was provided to the *Kachakupi* with the help of coal.
- Temperature was maintained throughout the procedure and temperature pattern was noted with the help of a Pyrometer.
- Temperature pattern given:
 - *Mrudu Agni* : Room temp. Upto 200°C (6hrs).
 - *Madhyam Agni* : 200-450°C (6 hrs).
 - *Teevra Agni* : 450-650°C (4hrs).
- Temperature started from room temperature and was gradually increased.
- The temperature was recorded one hourly.
- During the course of heating, red hot Iron shalaka was repeatedly inserted into the mouth of *Kachakupi* to burn the accumulated sulphur present at the neck of the bottle to prevent blocking.
- With rise of temperature, flames increased and finally it was replaced by a blue flame, which gradually decreased in size.
- After the disappearance of blue flame, the bottom of *Kachakupi* becomes red hot. (*Suryodaya Lakshana*)
- Immediately copper sheet was placed over the mouth of the *Kachakupi*.
- After the appearance of white particles over the Copper sheet, the mouth of *Kachakupi* was then immediately corked and sealed.

- Then *Teevra Agni* was given for 4 hours after which it was allowed to self-cool.

3. Paschata Karma

- After 24 hours of self cooling, *Kupi* was removed from the *Valuka Yantra*.
- The layer of *Kapadamitti* which was blackened was removed by scrapping out with the help of knife and the external surface of the *Kupi* was cleaned with a wet cloth carefully.
- A string soaked in petrol was tied 1 inch below the level of compound on the external surface of *Kupi* and set to fire carefully.
- When the string was burnt, *Kupi* was wrapped by a wet cloth near the neck region, where the string was tied.
- The *Kupi* was broken exactly at the level of string.
- The bottle was broken and the sublimate deposited at the neck of *Kachakupi* was collected and weighed.

Observations

- When heating was started the room temperature was 29°C.
- After 2 hours 26 mins of heating, slight yellowish white fumes started to emerge out from the mouth of *Kachakupi*, the temperature at this stage was 230°C.
- After 5 hours 13 minutes of heating, yellow fumes started to come out profusely with a strong smell of *Gandhaka* and the temperature recorded at that time was 250°C.
- After 6 hours 16 mins dark yellow fumes increased and the temperature recorded at that time was 427°C (temperature maintained at 430°C for 2 hours).
- After 6 hours 29 mins the fumes disappeared completely.
- After 7 hours 36 mins blue flame started to come out from the mouth of the *Kachakupi* and the temperature recorded at that time was 493°C.

- The height of the flame was nearly 5 inches.
- After 7 hours 43 mins at 530°C, the size of blue flame increased to 6 inches.
- Blue flame was present for 4 hours after which it gradually decreased in size and the temperature recorded at that time was 578°C.
- After 12 hours, the flame disappeared and the temperature recorded at that time was 585°C.
- The bottom of the *Kachakupi* also became red hot (*Suryodaya Lakshana*).
- Copper sheet test showed presence of white mercury particles over it when placed over the mouth of the *Kupi*.
- At this point corking of *Kachakupi* was done carefully and sealed.
- The temperature recorded at that time was 603°C.
- Temperature was increased to 650°C after corking and heating was continued for further 4 hours and stopped.

Table 2: Showing the observations during the preparation of Rasasindhura.

Time	Temperature	Observation
10.00 am	30°C	Kupisthapana
11.00 am	102°C	<i>Kajjali</i> was dried
12.00am	154°C	<i>Kajjali</i> was dried
1.00pm	203°C	<i>Kajjali</i> was melted
2.00pm	246°C	Appearance of yellow fumes
3.00pm	301°C	Appearance of yellow fumes
4.00pm	343°C	Appearance of yellow fumes
5.00pm	378°C	Reduction in fumes
6.00pm	457°C	Appearance of blue flame
7.00pm	457°C	Presence of blue flame

8.00pm	476°C	Presence of blue flame
9.00pm	489°C	Presence of blue flame
10.00pm	490°C	Presence of blue flame
11.00pm	508°C	Presence of blue flame
12.00am	510°C	Reduction in size of blue flame
1.00am	523°C	Absence of flame
1.30am	550°C	Suryodaya lakshana

Result

Total time taken : 16hrs

Quantity of Rasasindhura obtained :

- With firewood: 40gm (150gms of *Kajjali*)
- With coal: 92gm (150gm of *Kajjali*)
- With muffle furnace: 94 gm (150gm of *Kajjali*)

Analytical Data

Table 3: Showing results of Rasasindhura by SEM-EDX.

Element	Weight %	Atomic %
S	15.92	54.23
As	0.00	0.00
Mo	0.00	0.00
Cd	0.00	0.00
Hg	84.08	45.77
Pb	0.00	0.00

- In elemental analysis of *Rasasindhura* there was presence of Sulphur and Mercury.
- Sulphur weight % is 15.92 and atomic % is 54.23.
- Mercury weight % is 84.08 and atomic % is 45.77.
- There is no presence of heavy metals in the prepared sample of *Rasasindhura*.

- The particles present in the preparation were clearly distinctive.
- The particle size were clearly analysed and were in the range of 118 nm to 138.2 nm at a magnification of 20.00 K X.
- The particle size were clearly analysed and were in the range of 56.02 nm to 60.95 nm at a magnification of 80.00 K X.

Table 4: Showing Bhasma Pareeksha done for Rasasindhura.

Bhasmas	Colour	Nischandratvam	Variatara	Rekha purna	Unama	Slakshnatvam
Rasasindhura	Bright Reddish orange	Shiny particles observed under sunlight	+	+	+	+

Figure 1.1: Shodana of Parada

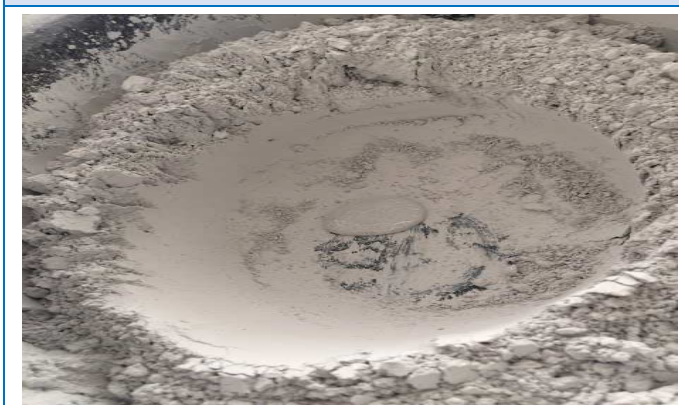


Figure 1.2: Shodana of Parada



Figure 1.3: Shodana of Parada



Figure 1.4: Shodana of Parada



Figure 2.1: Shodana of Gandhaka



Figure 2.2: Shodana of Gandhaka



Figure 2.3: Shodhita Gandhaka



Figure 3.1: Kajjali



Figure 3.2: Vatankura



Figure 3.3: Bhavana of Kajjali with Vatankura Swarasa



Figure 3.4: Suryodaya Lakshana



Figure 3.5: Copper sheet test

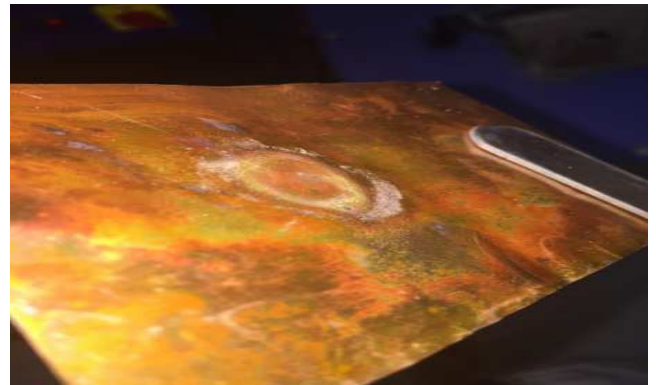


Figure 4.1: Rasasindhura

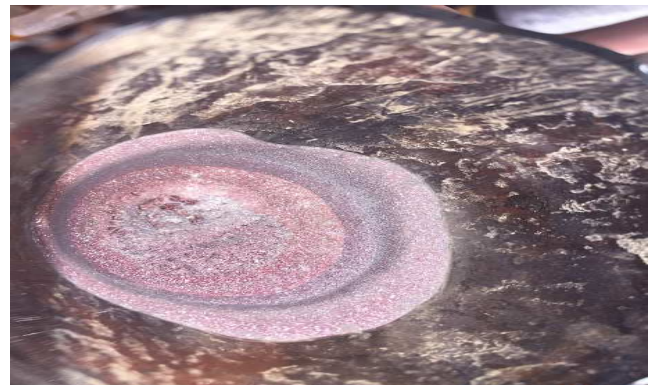


Figure 4.2: Rasasindhura



DISCUSSION

Discussion on Pharmaceutical Process

A) Shodana Procedures

1. Shodana of Parada

- According to the reference taken, it required lot of man power.
- More duration of time : 6 months
- Lot of care has to be taken to prevent wastage of mercury.
- Lot of loss happens during the procedure.
- Difficult method for large quantity *Shodana*. (preferred for small quantities)
- Proves to be efficacious as it purifies mercury to the fullest which was proved by analysis of the product.

2. Shodana of Gandhaka

- According to the reference taken, it required lot of man power.
- After complete *Shodana*, *Gandhaka* gets slightly charred and brown in colour (In case of large quantity taken for *Shodana*).
- Probable suggestion is to use this method for lesser quantity as it doesn't get charred due to earlier melting phase.
- *Bhudhara* method can be adopted for large quantity *Shodana*.

B) Preparation of Kajjali

- A smooth *Khalva* has to be preferred in order to avoid wastage of *Kajjali* i.e. *Kajjali* tends to get stuck at the broken surfaces of the *Khalva* there by leading to loss if the *Khalva* isn't smooth.
- For large quantity preparation, its better to take a wide mouthed and increased depth *Khalva Yantra* as it will avoid the spillage of the product during trituration.
- During trituration maximum care has to be taken as *Kajjali* tends to fall off the *Khalva* if done

vigorously and hence has to be done slowly and cautiously.

- Pressure has to be applied during trituration.
- Slight water can be sprinkled over *Kajjali* to prevent it from spilling.
- While removing the *Kajjali* out of the *Khalva*, a lot of it will be present at the base after complete removal, here a brush and a spoon could be used to remove it out completely to avoid wastage.
- *Kajjali* has to be examined under sunlight to see the presence of shiny particles.

C) Preparation of Rasasindhura

Preparation was done in 2 methods:

- a) Classical method
- b) Muffle furnace

a) Classical Method

Classical method proved to be tough with requirement of lot of manpower and preparation was done in 2 ways:

- Using firewood
- Using coal

Using firewood

- Lot of wastage of time and power.
- Excess amount of firewood was required (200 kg).
- Total time spent: 20 hrs
- Yeild: Not a success, *Rasasindhura* seemed to be charred.
- Temperature couldn't be maintained well, as a result of which the product wasn't fruitful.
- Also the *Bhatti* used was not properly made i.e. it was big in size.
- *Rasasindhura* obtained was at the *Talastha Bhaaga*.

Using coal

- Easier when compared to the use of Firewood.
- Less amount of coal was required (11 kg).

- Temperature could be easily maintained.
- Time period was lesser compared to the use of firewood: 8 hours
- *Rasasindhura* was collected at the kanta pradesha (Shreeshta lakshana)
- Yeild- Success, but the actual Sindhura Varna was not very prominent.

b) Muffle Furnace

- Bottle has to be arranged in the right manner.
- Temperature could be easily maintained as per the classics.
- Easy procedure.
- No manpower required.
- Duration of 7½ hours in total
- While doing *Sandhibandana* of *Kupi*, precise *Bandhana* had to be done as due the heat of the muffle it chars the coating.
- Yeild: Perfect formation of *Rasasindhura* and more Yeild when compared to classical method. (Temperature has to be maintained properly for proper yield)
- Probably, *Rasa Sindhura* can be prepared by conventional methods for large scale production, as it is faster, easier, gives much better yield and doesn't affect the quality.

Discussion on Analytical Data

SEM-EDAX

- *Rasasindhura* proved to have no presence of heavy metals after the process and the percentage of Hg weight % was 84.08 and atomic % of 45.77 and that of Sulphur weight % was 15.92 and atomic % of 54.23.
- Particle size of *Rasasindhura* was in the range of 56.02 to 60 nm at a magnification of 80.00 K X and 118 to 60 nm at a magnification of 20.00 K X.
- EDX shows the chemical composition consist of Mercury and Sulphur and the particle size was in

nanometer, so absorption of drug will be more with quicker action.

- The atomic % of Hg is less and weight % is more because Hg is a heavy metal.
- During the preparation of *Rasasindhura*, extra Sulphur will be burnt off and hence the Hg % is more.

Discussion on *Bhasma Pareeksha*

Bhasmas are unique preparations in *Rasashastra*, for its preparation *Marana* has to be done. Before the process of *Marana*, *Shodana* of the metal has to be done. For the obtainment of a pure *Bhasma*, *Bhasma Pariksha* plays an important role.

1) *Rekhapurna*

This *Pareeksha* mainly deals with the particle size of the *Bhasmas* and also deals with its softness. The *Bhasma* can only pass this *Pareeksha* when the diameter of the particles is less than the breadth of grooves on the finger surface. Also, deals with the consistency of the particles. The particles of the *Bhasma* only get entangled over the fingers if they are smooth and soft. If they are hard in consistency they will not get adhered to the finger surface though they are sufficiently small.

2) *Varitara*

The probable cause behind floating of *Bhasma* over water can be described as the atoms of water are bounded with each other due to an attractive force in between them due to which they remain in contact with each other forming a flat surface. When a fine powder is spread on its surface tension of the water doesn't allow the particle to enter / sink thus, keeping them floating. Hence, can be considered as *laghu* (particles having light weight). *Bhasma* particles which are *laghu* will float on water and if it contains any unconverted heavy particles of metal it tends to sink. *Acharya Vagbhatta* states that *Bhasma* becomes ready for consumption only if its *Varitara*.

3) *Unama*

Additional test to confirm the *Varitara Pareeksha*. It is similar to that of *Varitara* test but further on stating

the *Laghutva* of the particle which will not allow the grain to sink.

4) *Nischandratva*

Test is carried out to check the presence of free metal, if its present there will be presence of lustre.

CONCLUSION

Rasasindhura prepared by using muffle furnace proved to be easier compared to other methods. SEM- EDAX revealed to have no presence of heavy metals and the percentage of Hg weight % was 84.08 and atomic % of 45.77 and that of Sulphur weight % was 15.92 and atomic % of 54.23. Particle size of *Rasasindhura* was in the range of 56.02 to 60 nm at a magnification of 80.00 K X and 118 to 60 nm at a magnification of 20.00 K X. EDX shows the chemical composition consist of Mercury and Sulphur and the particle size was in nanometre, so absorption of drug will be more with quicker action.

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