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RESEARCH ARTICLE

Formulation, Standardization and Comparative Evaluation of Ancient Nanomedicine *Varatika Bhasma*

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Abstract:
Bhasmas are unique *Ayurvedic* metallic/mineral preparations with herbal juices, widely recommended to treat variety of chronic ailments. *Varatika bhasma* is a traditional *Ayurvedic* medicinal preparation, used for protection against ulcers. *Varatika* is external shell of sea animal Cypreamonetalinn. Chemically varatika is identified as carbonate of calcium. In the present research an attempt has been made to carry out a comparative standardization of formulated varatika *bhasma* (VB1) prepared as per standard classical methods and marketed *Varatika bhasma*(VB2). These formulations where evaluated for physical properties, chemical characterization using sophisticated instrumentation techniques such as particle size, AAS, SEM, XRD. Physico-chemical standards where determined for both the samples.The X-ray Diffraction analysis of both formulations exhibited crystalline nature and nano-sized particles. In SEM study of VB2 shows well-defined fibre like structures while VB1 shows relatively compact microcrystalline aggregates with loss of grain boundaries.

Keywords: *Varatika, bhasma*, Standardization, *Ayurveda*

Introduction

Ayurveda is an ancient traditional medicine system, originated in India and evolved and practiced over thousands of years. *Bhasma* is the well-known potent preparation of the traditional *Ayurveda*. *Bhasma* literally means 'ash' which is obtained after incineration.[1]*Bhasmas* are unique *Ayurvedic* metallic preparations with herbal juices/fruits or decoction, widely recommended for treatment of a variety of chronic ailments. *Bhasmas* are biologically produced metallic nanoparticles obtained by calcination into ash and are taken along with milk, butter, honey, ghee, etc.[2]This group of medicines can work even in smaller doses and may even control incurable diseases effectively. In present era *Ayurvedic* physicians uses medicines which are made from minerals, metals, animals as well as from vegetable products. Among these preparations products obtained from mineral-metals, are supposed to be harmful to human body.

But it is very surprising to know that in *rasa-shashtra* text the side effects are already mentioned if we use these medicines if they are not prepared properly. To avoid such side effects different *Ayurvedic* pharmaceutical processing techniques such as *shodhana, marana,*etc to convert metallic preparation into nontoxic form of medicines are mentioned and also standardization of that processes according to *Ayurveda* as well as modern analytical methods for standardization is also given in the *Ayurveda* texts. Standard is the numerical value which qualifies the parameters and thus determines the quality and purity of the material.[3]

Varatika is identified as the external shell of sea animal *Cypreamoneta*linn. It occurs in the coastal areas of sea. *Cypreamoneta* is commonly known as money cowry, because the shells where historically widely used in many Pacific and Indian ocean countries as a form of exchange. Chemically, *Varatika* is identified as Carbonate of Calcium. Since ancient days *Varatika* is used for playing as well as for medicinal purposes. *Dharana*(amulet) of *Varatika* is practiced for the treatment of *Balagraha*(viral infections of children) and also used in ulcer protection.[4]

Materials and methods

Varatika (*Cypreamonetalinn*) procured from local market of Ahmednagar (Maharashtra), India. Procurement of lemon from market time to time according to need is done. *Nimbu Swaras* is the fresh lemon juice obtained from the fruits of *Citrus lemon* (Family: Rutaceae). The lemon juice was filtered using muslin cloth.

1. Pharmaceutical processing[5]

a. *Shodhana*(Purification) of raw *varatika*
b. *Marana*(Calcination) of purified *varatika*
c. *Bhavana*(Trituration)

Preparation of *varatikabhasma*

***Shodhana*- method of purification.**

About 100gm of raw *varatika* was subjected to *sodhana* process. Raw *varatika* was heated with lemon juice and water(1:6) for about 3 hours. After heating process these purified *varatika* were removed, cooled and dried.

***Marana*- Incineration and *bhavana*- trituration[6]**

The lemon juice treated *varatika* were directly placed in silica crucibles which were subjected to heating in the muffle furnace for about 550°C for 3hrs. For this heating process i.e. *Gajaputa*, temperature was gradually increased to 550°C in about 180mins. When the temperature reaches 550°C, the temperature was maintained constant for about 3hrs and after 3hrs the temperature was decreased gradually in about 180mins to cool. After this process the *varatika* becomes brittle. These brittle *varatika* where collected and powdered with the help of mortar and pestle. The powdered *varatika* is given bhavana(trituration) with 40ml of *nimbuswarasa* (lemon juice) for 3hrs.Then *Cakrikas* (pellets) were prepared and dried. After drying these pellets were subjected to second *puta*for 800°C. The process was repeated for third time, 30 ml of *nimbu swarasa*was used and it was heated for 3 hours. After cooling white coloured *Varatika Bhasma* was obtained[6]. Here *bhavana* refers to the process of grinding the mineral drugs in the liquids like juices or decoction of herbs, cow's milk, urines or any such specified liquids. The quantity of liquid should be sufficient to immerse the mineral powder. The grinding was continued, until liquid added dried up and semisolid consistency was achieved. This makes on *bhavana* and the same process was repeated.

Instrumental analysis

Particle size analysis

Particle size of VB1 and VB2 were measured by using Malvern Zetasizer(Ver. 6.20). This technique determines the mean particle diameter and range of particle size distribution (PDI)

X-ray Diffraction (XRD)

X-ray powder diffraction pattern VB1 and VB2 were recorded on Rigaku Ultima(IV) XRD (serial no JD37BD62-3026) make japan diffractometer having Cu-K and #945. The source was operated at 40kV and 40mA.

Scanning Electron Microscopy(SEM)

The surface morphology of *bhasma* was examined by using Scanning Electron Microscope (FEI-Novananosem-450)

Particle Size

Figure 3 shows particle size distribution of VB1 and VB2. By using Malvern Zetasizer, mean particle size was found to be about 141 nm and 219.6 nm resp.The particle size distribution graph was found to be bell shaped with even distribution range. Polydispersibility index found to be 0.405 and 0.518 resp. which indicates the particles were in monodisperse form. The size range indicates that theparticles arenano sized.

B.Chemical characteristics.

Apurnabhavta

Apurnabhavata means incapability to retain its original metallic form. For this test varatika *bhasma* is mixed with equal quantity of *mitra panchaka* (seeds of *Abrusprecatorius,* ghee, jaggery, borax and honey) and it is sealed in *sarava samputa* and is heated at 800°C and after self-cooling particular product is observed. Lustrous particles in it show presence of free metal which is inactive after incineration.[7]

Niruttha

Niruttha is to test inability to regain metallic form of metallic *bhasma*. In this test varatika *bhasma* is mixed with fixed weight of silver leaf and kept in*saravasamputa*, and is heated at 800°C and after self-cooling, the weight of silver is taken. Increase in weight of silver leaf indicates improperly prepared *bhasma*. [8]

Amlapariksha

A pinch of prepared *bhasma* was mixed with a little amount of *dahi* (curd) in a clean and dry petri dish and is involves *bhavana* (wet trituration) and *puta*system of

observed for any colour change. No colour change of *dahi* should be observed. The same colour of lemon juice taken in a test tube and the same result should be observed. [9]

Standardization by Analytical methods

Standardization by analytical methods of both formulations was carried out by sophisticated analytical techniques such as AAS, SEM and XRD.

Atomic absorption spectroscopy.

The AAS study was conducted to determine the concentration of elements present in both formulations. The results showed that the elements calcium, phosphorous, zinc, sodium, nitrogen, sulphur and potassium were seen in major concentrations of 1.99%, 0.63%, 1.44%, 2.10%, 0.77%, 0.99% and 3.50% respectively for VB1 and 1.20%, 0.92%, 2.48%, 1.40%, 1.03%, 1.07% and 3.42% for VB2 respectively. And the elements such as copper, iron, manganese, boron and molybdenum where seen in minor concentrations of 0.42ppm, 119.9ppm, 20.01ppm, 0.06ppm and 0.03ppm respectively for VB1 and 0.47ppm, 114.01ppm, 23.99ppm, 0.04ppm and 0.02ppm respectively for VB2.

X-Ray Diffraction.

The XRD pattern of both VB1 and VB2 are shown in fig. 3. The diffraction peaks of VB1 and VB2 are at 2Θ=36˚, 32.06, 37.16' 37.36, 53.62, 53.72, 67.28, 67.38 and 29.16, 29.38, 31.42, 47.38, 48.42, 65.38, 65.54, 65.66 respectively.Comparative analysis of XRD results between VB1 and VB2 shows the major reflection peaks of both samples are at identical positions. The high intensity of lines in the XRD pattern suggests that the drug is present in crystalline form.

Scanning Electron Microscopy

SEM images of VB1 and VB2 showed difference in size and agglomeration of the particles (fig. 4).The results showed well-defined fibre like structures while the formulated '*Varatika bhasma*' showed slightly agglomerated fibers, relatively compact microcrystalline aggregates with loss of grain boundaries. The average particle size of sample was found to be about 1µm while that of sample. Agglomeration of the particles is due to repeated cycles of calcinations involved in preparation. The influence of method of preparation on morphology, particularly the calcination temperature and duration, has also been reported.

Discussion

Varatika, a mineral drug of animal origin is used in Ayurvedic therapeutics in many diseases. It is the key ingredient in drugs prescribed in gastrointestinal symptoms. The various stages of formulation techniques like *shodhana* (which involves roasting, with addition of herbal juices and continuous stirring) and *marana* [which heating], the particle size reduces significantly, which may facilitate absorption and assimilation of the drug into the body system. The micro to nano size of the formulations was confirmed by the analytical techniques, which could be specified as the criterion for the final product conforming to all the traditional parameters under *bhasma pariksha*(examination of properly prepared *bhasma*). The results of the present study revealed that *varatika bhasma* is an efficient herbo-metallic preparation. Attempts have been made to comparatively evaluate metallic *ayurvedic* formulations using sophisticated analytical tools such as AAS, XRD and SEM.

Conclusion

The present study includes that, purifying plant agents helps in the formation of absorbable bhasma. Nature of this purified and formulated bhasma can be determined only after carrying out some chemical analysis such as particle size analysis which indicates reduction in particle size, which is due to purification processes. There is notable difference found in the particle size. SEM data also further confirmed this.XRD pattern confirmed the formation of more crystalline compound which is due to the impact of various purification and incineration processes. The ayurvedic parameters such as *Apurnabhava, Niruttha, Nishchandratvam, Varitara, Unama, Rekhapurnatvam, Slakshnatvam, Sukshmatva and AnjanaSannibha* confirmed the softness and fineness of the formulations.The nano sized particles (*Bhasma*) of metals and minerals are being used as effective medicines and also in various aspects such as detection and diagnosis of diseases.Thus, modern techniques can assist in proper characterization of Ayurvedic dosage forms and standardization of Ayurvedic medicines.

(a) (b)

Fig 1.*Varatika* before purification (a) and after purification (b).

Table 1: Observation made during *Shodhana* process with *nimbuswarasa*

Sample	Colour	Weight
Raw drug	Yellowish white	100gm
Purified drug	Greyish white	99.5gm

Table 2: Observations made during *marana* (incineration) process.

Puta	Qty. of *Swarasa*used	Grinding hours	Weight
First	-	-	99.5gm
Second	40	3hrs	96.2gm
Third	30	3hrs	74.51gm

Standardization of *Bhasma*[7]

Ancient *Ayurvedic* Methods of Analysis

Physical Characteristics
- *1.Verna*
- *2.Nisvadutam*
- *3.Nishchandratvam*
- *4.Varitara*
- *5.Unama*
- *6.Rekhapurmatvam*
- *7.Slakshnatvam*
- *8.Sukshmatva*
- *9.AnjanaSannibha*
- *10.Avani*
- *11.*Particle size

Chemical Characteristics
- *1.Apurnabhavta*
- *2.Niruttha*
- *3.Amla Pariksha*

Advanced Methods of Analysis

Analytical Methods
- 1.AAS
- 2. XRD
- 3.SEM

Physiochemical Method
- 1.Ash Values
  - a.Total Ash
  - b.Acid Insoluble Ash
  - c.Water Soluble Ash
- 2.pH
- 3.LOD

Fig 2: *Varatikabhasma*(final product)

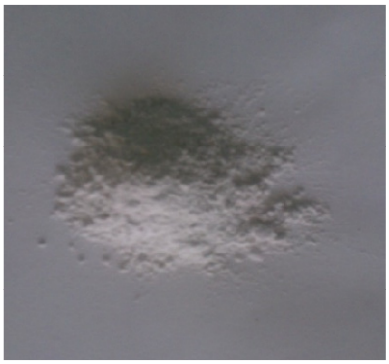

Table no. 3. Physical characteristics of VB1 and VB2.[8,9,10,11,12]

Sr.no.	Physical characteristics	VB1	VB2
1	*Verna* (colour dull white)	✓	✓
2	*Nisvadutam*(tasteless)	✓	✓
3	*Nishchandratvam* (lusterless)	✓	✓
4	*Varitara*(float)	✓	✓
5	*Unama*	✓	✓
6	*Rekhapurmatvam* (furrow filling)	✓	✓
7	*Slakshnatvam* (tactile sensation)	✓	✓
8	*Sukshmatva* (fineness)	✓	✓
9	*AnjanaSannibha* (softness)	✓	✓
10	*Avani*	✓	✓

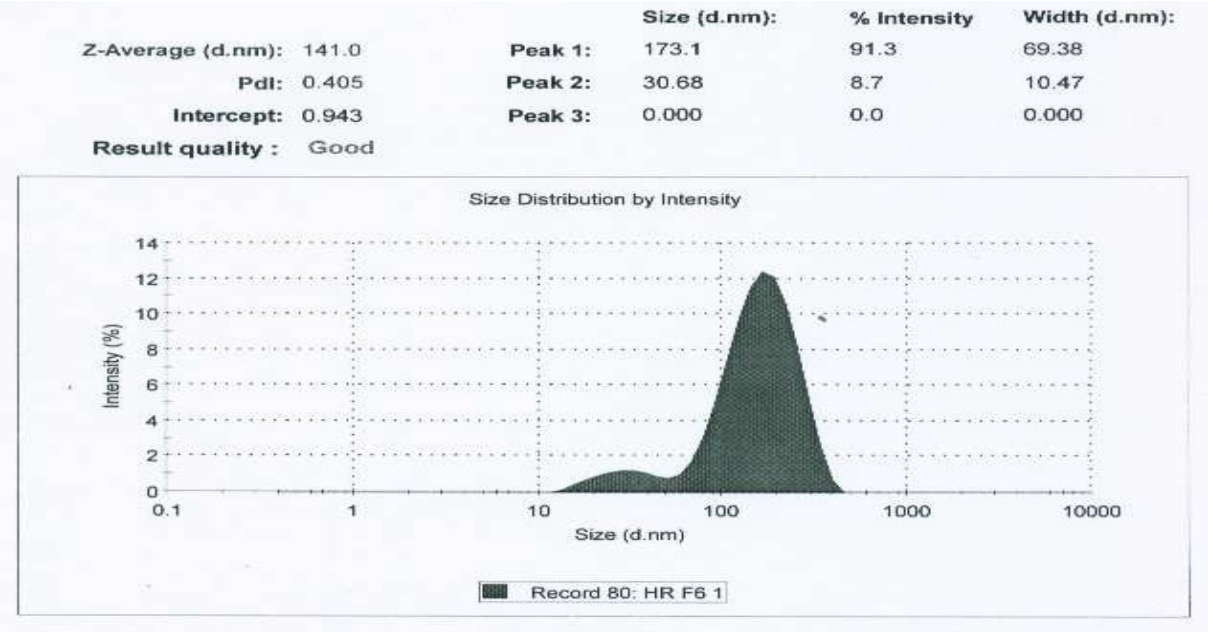

Fig. 3(i) Particle Size Analysis of VB1

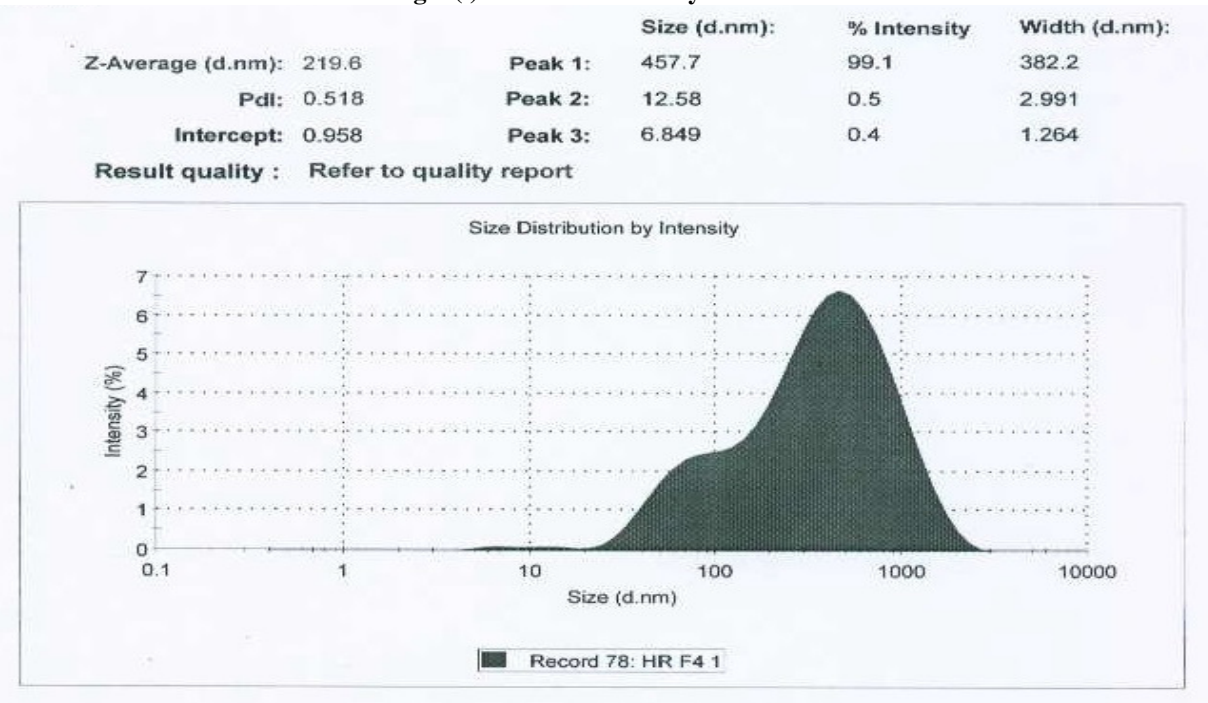

Fig. 3(ii) Particle Size Analysis of VB2

Table no. 4. Chemical characteristics of VB1 and VB2.

Sr.no.	Characteristics	VB1	VB2
1	*Apurnabhavta*	✓	✓
2	*Niruttha*	✓	✓
3	*AmlaPariksha*	✓	✓

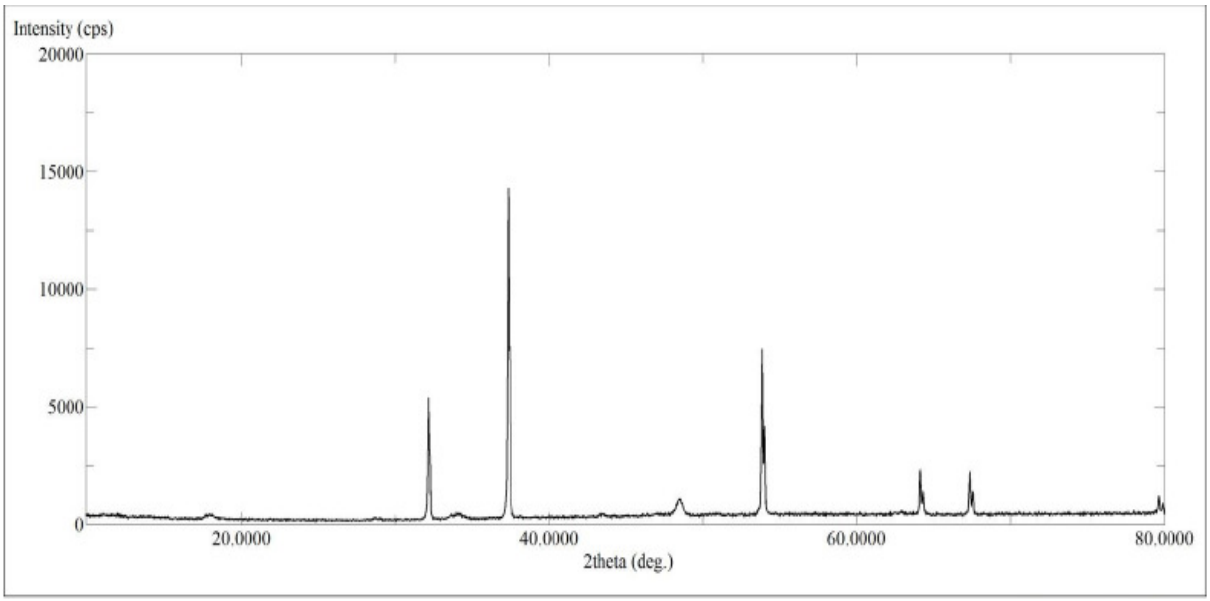

Fig. 4(i) XRD for VB1

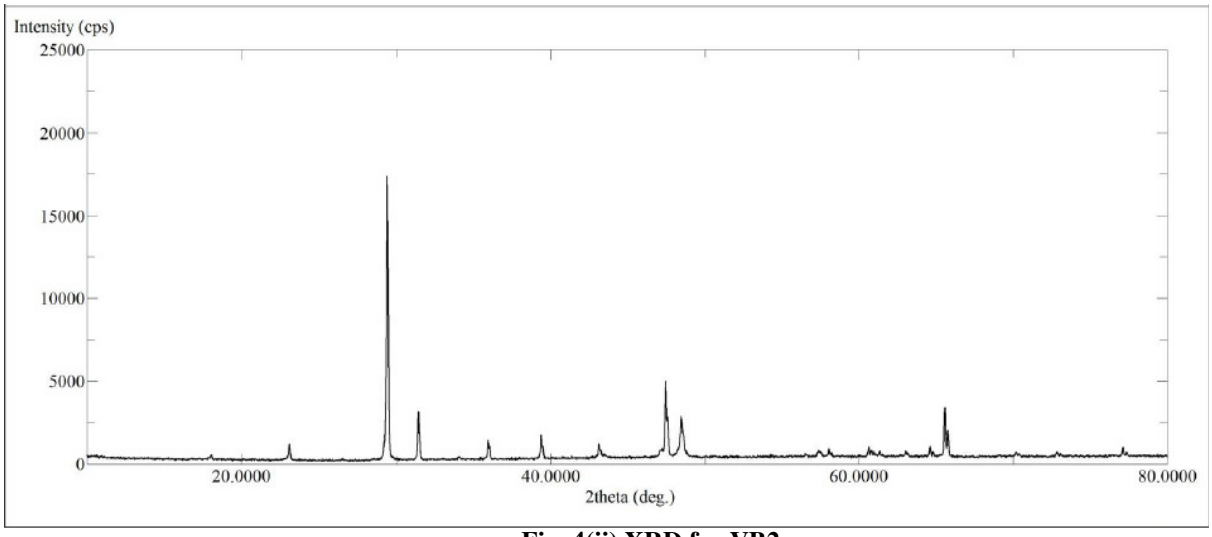

Fig. 4(ii) XRD for VB2

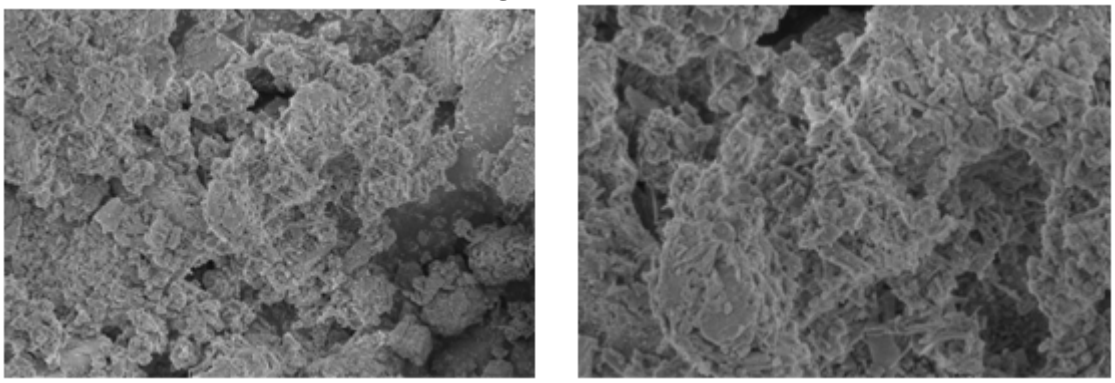

Fig.5(a) SEM image(x50000)VB1.(b) SEM image(x50000) VB2

Table no. 5. Physiochemical properties

Sr. No.	Test	VB1	VB2

1	Odour	Odourless	Odourless
2	Taste	Tasteless	Tasteless
3	Solubility	Soluble in dil. HCL	Soluble in dil. HCL
4	Total ash	94%	91%
5	Acid insoluble ash	66.66%	76.66%
6	Water soluble ash	73.33	80
7	pH	11.30	10.00
8	LOD	0.6500	0.6466

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