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Comparative pharmaceutical evaluation of different samples of Yellow orpiment (*Haratala*) and Ayurvedic mineral formulation (*Rasamanikya*)

Research Article

Nilima Narayanrao Wadnerwar[1*]

1. Professor, Department of Agadtantra, Mahatma Gandhi Ayurved College, Hospital & Research Centre, Salod (H), Datta Meghe Institute of Higher Education and Research (Deemed to be University), Wardha, Maharashtra, India.

Abstract

Background: The present work aimed to study the Physical and Physico-chemical Properties of raw *Haratala* (Yellow Orpiment), processed *Haratala,* and its self-prepared formulation *Rasamanikya* with different market preparations of *Rasamanikya* by using advance methods such as ICP-AES (qualitative and quantitative analysis), XRD and FEGSEM so that the particle size and elemental content can be estimated and compared in all the samples. This is an attempt to study the classical method of preparation as well as effect of purification process on the constituents of the drug and their size. Methods: Raw *Patra Haratala* (Orpiment) and three different samples of *Rasamanikya* were procured from authentic sources. *Shodhana* (purification) of *Haratala* was conducted by two different methods. *Rasamanikya* was prepared from the *Haratala* processed by two different methods. All the eight samples were subjected to physicochemical analysis, ICPAES, XRD and FEGSEM. Results: Arsenic content is decreased in all the samples except CSH in comparison with ASH. In ICPAES, number of elements is reduced in CSH, KSH and RMCSH in comparison with ASH whereas it is increased in RMKSH, RMMSA, RMMSB, and RMMSC. XRD analysis reveals that Average Crystal Size is minimum in RMMSA and maximum in RMMSC. Average Lattice Strain is minimum in ASH and maximum in RMMSC. Orpiment was detected in all the samples except RMKSH, RMMSB and RMMSC. FEG SEM indicates that the gross particle size of all the samples varies from 1um to 100nm at resolution ranging from 200 to 100000 magnifications. The nanoparticles are visualized at 50000 to 100000 magnifications. Conclusion: Advanced techniques like ICPAES, XRD and FEGSEM are very helpful in estimating the heavy metal content and particle size in Ayurvedic medicine. It is necessary from safety point of view. Small size particles increase the absorption of the drug in the body which causes increase in the bioavailability and potency of the drug. Hence the dose of the formulation may be reduced. Thereby the untoward effects of the high doses can be avoided.

Keywords: *Haratala,* Yellow Orpiment*, Rasamanikya,* ICPAES, XRD, FEGSEM.

Introduction

Use of classical mineral formulation in the treatment is one of the specialties in Ayurveda. As the minerals cannot be used in their natural or raw form, some procedures of their purification or detoxification and incineration are described. It is expected that the converted form should be more potent, easily digestible, absorbable, and free from untoward effects. (1) But after these procedures what exact changes occur in the physical and chemical structure of the drug is a mystery. Certain attempts are being carried out to understand this mystery with the help of some advanced techniques.

Haratala (Yellow orpiment) is a widely used mineral in various formulations of Ayurveda as a single or an auxiliary drug. It has various method of purification. *Rasamanikya* is a formulation in which only purified *Haratala* is used. *Rasamanikya* is light, micro fine powder, prepared by processing *Haratala* in Lime water. Processed/ purified *Haratala* is sandwiched between two Mica sheets and is heated till it turns to Ruby colour. (2) It is widely used in all the corners of India for Fever, Cough, Dyspnoea, Piles, Fistula, Chronic wounds, and blood and skin diseases. (3)

The present study is conducted to study the Physical and Physico-chemical Properties of raw *Haratala,* processed *Haratala,* and its self-prepared formulation *Rasamanikya* with different market preparations of *Rasamanikya* by using advance methods such as ICP-AES (qualitative and quantitative analysis), XRD and FEGSEM so that the Arsenic content can be estimated and compared in all the samples. This is an attempt to study the classical method of preparation as well as effect of purification process on the constituents of the drug and their size.

Material and Methods

Pharmaceutical-analytical study was conducted after receiving IEC Approval**.**

*** Corresponding Author:**

Nilima Wadnerwar

Professor, Department of Agadtantra, Mahatma Gandhi Ayurved College, Hospital & Research Centre, Salod (H), Datta Meghe Institute of Higher Education and Research (Deemed to be University), Wardha, Maharashtra, India.

Email Id: dr.nilima_wadnerwar@rediffmail.com

Material

Table 1: Materials required for the study

SN.	Name of Drugs	Quantity
1	Raw *Haratala* (Yellow orpiment)	500 gm
2	*Churna* ($CaCO_3$ / Lime)	50 gm
3	*Churnodaka* (Lime water)	10 litres
4	*Kushamanda Swarasa* (*Benincasa hispida* Thunb. Cogn. juice)	q.s.
5	*Kanji* (Sour gruel)	q.s.
6	*Dadhiamla* (Supernatant of curd)	q.s.
7	*Shweta Abhraka patra* (Mica sheets)	q.s
8	Market samples of *Rasamanikya* (RMMSA)	100 gm
9	Market samples of *Rasamanikya* (RMMSB)	100 gm
10	Market samples of *Rasamanikya* (RMMSC)	100 gm

Methods

Raw *Patra Haratala* and three different samples of *Rasamanikya* were purchased from authentic sources. *Shodhana* (purification) of *Haratala* was conducted by two different methods. *Rasamanikya* was prepared from the *Haratala* processed by two different methods. All the eight samples were subjected to physicochemical analysis, ICPAES, XRD and FEGSEM.

Processing *(Shodhana)* of *Haratala*

Method I:

Raw *Patra Haratala* in coarse powder form was boiled in Lime water for three hours. Purified *Haratala* was dried and made into powder. (3)

Method II:

Raw *Patra Haratala* in coarse powder form was boiled in fresh juice of *Benicasa hispida* for three hours. Next day it was boiled in supernatant of curd for three hours. On the third day, it was boiled in sour gruel for three hours. The same procedures were repeated three times. Purified *Haratala* was dried and made into powder. (4, 5, 6)

Preparation of *Rasamanikya*:

Method I:

Processed *Haratala* in lime water was sandwiched between two Mica sheets and heated till it turns to Ruby colour. After cooling, mica sheets were separated and *Rasamanikya* was made into fine powder. (2)

Method II:

Processed *Haratala* by method II was sandwiched between two Mica sheets and placed in an earthen dish covered by another dish. It was heated over cow dung cakes. After cooling, mica sheets were separated and *Rasmanikya* was made into fine powder. (4, 5, 6)

Analytical Study of all the samples:

All the eight samples were coded as follows.

- ASH: *Ashuddha* (Raw) *Haratala*
- CSH: *Haratala* processed by method I
- KSH: *Haratala* processed by method II
- RMCSH: *Rasamanikya* prepared by method I
- RMKSH: *Rasamanikya* prepared by method II
- RMMSA: Market sample of *Rasamanikya*
- RMMSB: Market sample of *Rasamanikya*
- RMMSC: Market sample of *Rasamanikya*

Analytical Parameters

Physicochemical analysis of raw drug and finished products included Total Ash Value, Water soluble Ash value, Acid insoluble Ash value, Water extractive value, Alcohol extractive value, pH, Conductivity and Particle Size by sieve analysis. Physicochemical analysis was performed according to the methods and standards as mentioned in Ayurvedic Pharmacopoea of India (API).

ICP-AES (Qualitative and quantitative), XRD (X-Ray Diffraction) and FEGSEM were conducted at Sophisticated Analytical Instrument Facility (SAIF) Indian Institute of Technology, Bombay.

ICP AES

Instrument details

Make: SPECTRO Analytical Instruments GmbH, Germany, Model: ARCOS, Simultaneous ICP Spectrometer

Specification

- R.F. Generator: Maximum of 1.6 KW, 27.12 MHz
- Plasma: Radial plasma, having capability to analyse aqueous solutions with high dissolved solid content even up to 30 wt %. Aqueous solutions can be acidic, basic or neutral.
- Spectrometer: Wavelength Range: 130 nm to 770 nm, Resolution: approx. 9 pico meter, having capability to scan full spectrum to have qualitative information about the content of the sample.
- Detector: Charge Coupled Devices (CCD)
- Vertical Torch assembly having fully demountable quartz torch with individual tubes as well as a Ceramic Fully demountable torch for HF based solutions.
- Nebulizers: Concentric, cross flow, organic nebulizer (hydrocarbons, solvents) as well as cross flow nebulizer for HF containing solutions.
- Spray Chambers: HF Resistant Cyclonic Chamber and hydrocarbon solution spray chamber. Spray chambers suitable for cross flow Nebulizers are available

XRD

Instrument details

- Diffractometer system=EMPYREAN
- Measurement program=C:\PANalytical\Data Collector\Programs\clay_praagya_4 to 70
- Goniometer=Theta/Theta; Minimum step size 2 Theta:0.0001; Minimum step size Omega:0.0001
- Sample stage=Reflection-transmission spinner; Minimum step size Phi:0.1

FEG SEM

Instrument details

- Model: JSM-7600F
- SEI Resolution: 1.0nm at 15 kv
- Magnification: Low: 25X to 10,000X; High: 100X to 1,000,000X at 4x5 photo size
- Accerating Voltage: 0.1 to 30 kv
- Probe Current Range: 1 pA to ≥ 200 nA

Specifications

The JEOL JSM-7600F FEG-SEM combines two proven technologies an electron column with semi-in-lens detectors and an in the lens Schottky field emission gun to deliver ultrahigh resolution combined with wide range of probe currents for all applications (1pA to more than 200 nA).

The JSM-7600F successfully integrates a full set of detectors that make it ideal for: anotechnology, material science, biology, compositional and micro-structural analysis.

Results and Discussion

Analytical study is the core part in the drug research. This is necessary to ensure about the standards of preparation and quality of final product. In the present study, advanced techniques are used to serve the purpose.

Moisture content is less in all the samples. Total ash value and water soluble and acid insoluble ash value is more in ASH, CSH, KSH, RMCSH, and RMKSH. Increased total ash value indicates the presence of inorganic content in the samples. Water soluble extractive values are high in market samples of RM. Alcohol soluble extractive value are high in ASH, CSH, KSH, RMCSH, RMKSH.

Water soluble ash value indicates that ASH, CSH, KSH, RMCSH and RMKSH are more soluble in water in comparison with the market samples. Acid insoluble value reflects that the market samples RMMSA, RMMSB and RMMSC are more soluble in gastric juice indicating their enhanced bioavailability in comparison with the other samples (table 2).

Table 2: Observations of physicochemical analysis

SN.	Analytical Parameter		ASH %	CSH %	KSH %	RMCSH	RMKSH	RMMSA	RMMSB	RMMSC
1	Moisture content at		0.72	0.33	0.24	0.36	0.43	0.16%	1.66%	0.4%
2	Total Ash Value		6.38	6.31	7.38	8.34	6.38	1.1%	1.76%	1.1%
3	Water soluble Ash		3.56	2.66	4.21	4.11	4.22	0.61	0.69	0.76
4	Acid insoluble Ash		1.75	2.5	2.64	2.56	2.34	0.53%	1.06%	0.56%
5	Water soluble extractive value		5.53	5.67	7.83	7.25	6.83	37%	30%	39%
6	Alcohol soluble extractive value		9.42	10.43	9.32	6.46	10.47	6%	4%	7%
7	pH		5.1	5.1	5.2	5.3	5.4	6.03	6.00	6.09
8	Particle Size (By Sieve Analysis)		80 mesh	80 mesh	80 mesh	80 mesh	80 mesh	100 mesh	100 mesh	100 mesh
9	ICP-AES for Arsenic		47.23	49.69	46.09	41.77	42.13	45.11	43.77	45.68
10	XRD	Average Crystal Size	141.59 nm	123.05 nm	122.99 nm	146.82 nm	175.29 nm	110.31 nm	167.88 nm	296.51 nm
		Average Lattice Strain	0.0014	0.0017	0.0017	0.0018	0.0021	0.0028	0.0046	0.0391

Haratala (Yellow orpiment) is a combination of Arsenic and Sulphur. Arsenic content is decreased in all the samples in comparison with ASH. In ICPAES, number of elements is reduced in CSH, KSH and RMCSH in comparison with ASH whereas it is increased in RMKSH, RMMSA, RMMSB, and RMMSC. In qualitative analysis, it was found that the number of element is decreased in CSH, KSH and RMCSH whereas they are increased in other samples (table 3). All the other elements or heavy metals are within the permissible limit (table 4).

After processing, the Arsenic content in all samples is reduced in comparison with raw *Haratala*. It may be due to the production of Arsine gas due to leaking of Arsenic in liquid media used for processing. (7) The element which are not present in ASH but are present in rest of the samples may be due to the media used for processing (*shodhana*).

XRD Crystallite size and lattice strain was calculated by using Scherer equation with tiny tool software available online. XRD analysis reveals that Average Crystal Size is minimum in RMMSA (110.308 nm) and maximum in RMMSC (110.308 nm). Average Lattice Strain is minimum in ASH (0.0014) and maximum in RMMSC (0.0391) (table 2). Lattice strain denotes the bonding or strength between the crystals. Orpiment was detected in all the samples except RMKSH, RMMSB and RMMSC. No pattern list was visible in RMKSH and RMMSC. In RMMSA and RMMSB quartz (O_2 Si_1) and Arsenolite (AS_2O_3) were detected respectively. The number of peaks reduced in processed samples of Orpiment CSH (50) and KSH (56) in comparison with standard unprocessed orpiment ASH (57). The peaks are again reduced in samples of *Rasamanikya* prepared from CSH and KSH (table 5). The highest peak in CSH, KSH, RMCSH and RMMSA corresponds to that of ASH at position 18°2θ at 100% intensity (Fig.1, 2, 3, 4, 6).

Table 3: Qualitative and Quantitative Analysis Report of Inductively Coupled Plasma Atomic Emission Spectrophotometry (ICP-AES)

Sample	Qualitative Analysis Report (presence of elements)	Number of elements detected	Quantitative Analysis Report / Arsenic (As) estimation:- %	Quantitative Analysis Report / Sulphur (S) estimation:- %
ASH	Ag, Al, As, Ba, Ca, Fe, Hg, Mg, S, Si, Sr, Zn	12	47.235	24.671
CSH	As, Ca, Mg, S, Sr, Zn	6	49.692	26.653
KSH	As, Ca, Mg, S, Sr, Zn	6	46.094	25.523
RMCSH	As, Ca, Fe, Mg, S, Si, Sr, Zn	8	41.777	22.390
RMKSH	Al, As, Ba, Ca, Fe, Hg, K, Mg, Mn, S, Si, Sr, Ti, Zn	14	42.131	22.788
RMMSA	Ag, Al, As, Ba, Ca, Cr, Cu, Fe, Hg, K, Mg, Mn, S, Si, Sr, Ti, Zn	17	45.113	24.488
RMMSB	Ag, Al, As, B, Ba, Ca, Cr, Cu, Fe, Hg, K, Mg, Mn, S, Si, Sr, Ti, Zn	18	43.773	24.633
RMMSC	Ag, Al, As, B, Ba, Ca, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, S, Si, Sr, Ti, Zn	19	45.688	24.239

Table 4: Quantitative estimation of elements by ICPAES

Sample	Hg	Cu	Si	Sr	Zn	Ba	Ca	Cr	Fe	Mg
	%	%	%	%	%	%	%	%	%	%
ASH	ND	-	0.07725	0.00047	0.00029	0.00002	0.2349	-	0.00253	0.00200
CSH	ND	-	ND	0.00019	0.00028	-	0.0518	-	-	0.00228
KSH	ND	-	ND	0.00007	0.00077	-	0.0815	-	-	0.00037
RMCSH	ND	-	ND	0.00012	0.00126	-	0.0865	-	0.00966	0.00407
RMKSH	0.00468	-	ND	0.00013	0.00100	0.000096	0.1170	-	0.01588	0.00584
RMMSA	0.01597	0.00183	0.11413	0.00040	0.00517	0.000429	0.1460	0.00066	0.06825	0.00095
RMMSB	0.0387	0.00067	0.32515	0.00150	0.00475	0.002375	0.3160	0.00180	0.04906	0.00361
RMMSC	0.00847	0.00177	0.14902	0.00027	0.00043	0.000567	0.1837	0.00190	0.03170	0.00420

Note: ND means less than 0.01ppm

Fig. 1: XRD Pattern of Sample ASH

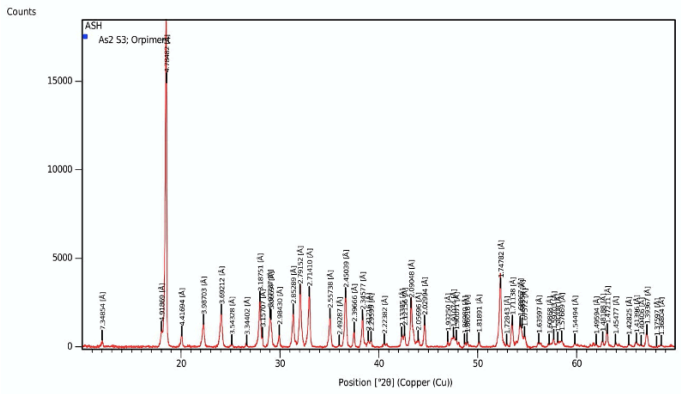

Fig. 2: XRD Pattern of Sample CSH

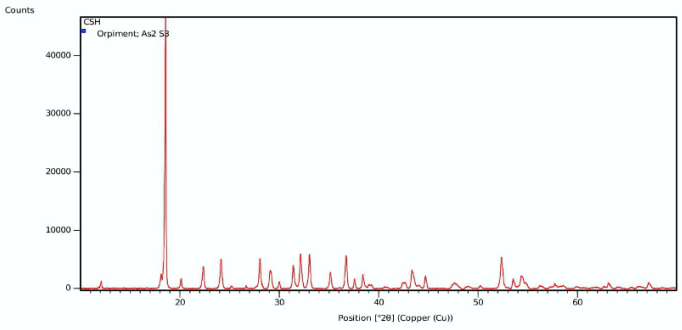

Fig. 3: XRD Pattern of Sample KSH

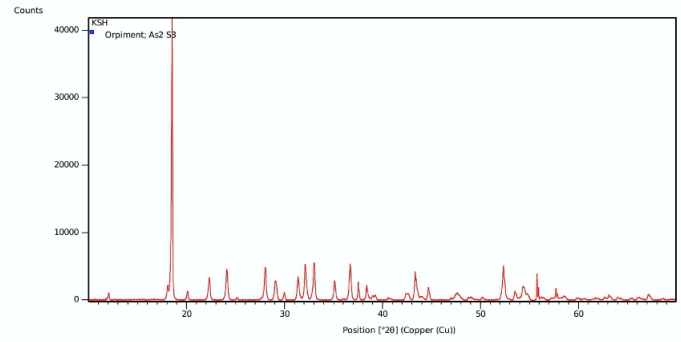

Fig.4: XRD Pattern of Sample RMCSH

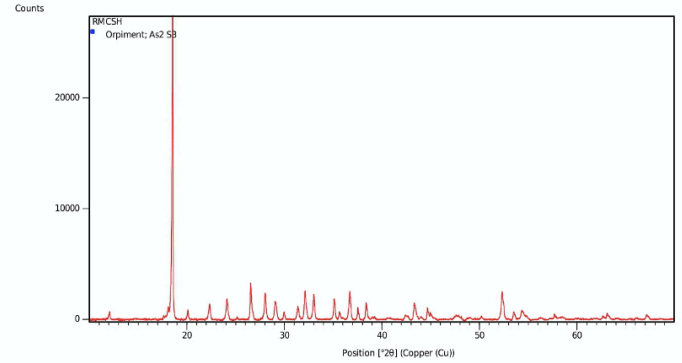

Fig. 5: XRD Pattern of Sample RMKSH

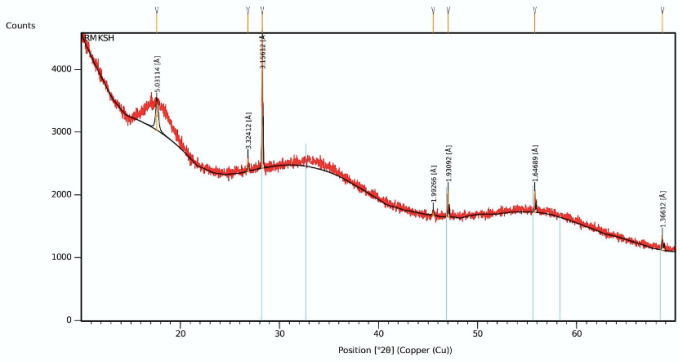

Fig. 6: XRD Pattern of RMMSA

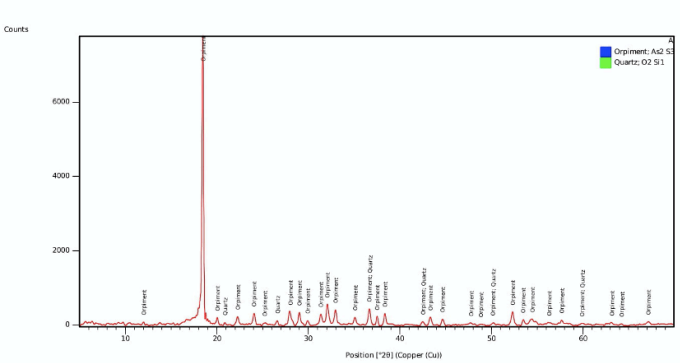

Fig.7: XRD Pattern of RMMSB

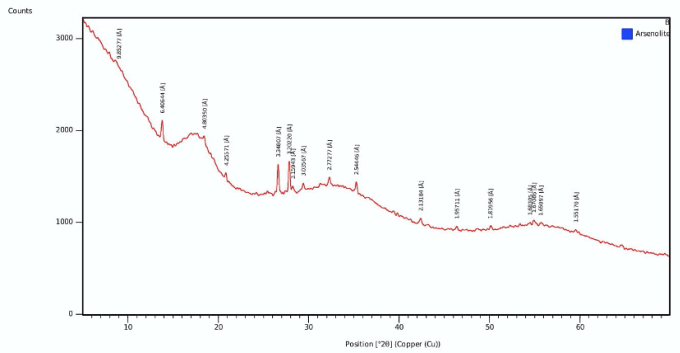

Fig. 8: XRD Pattern of RMMSC

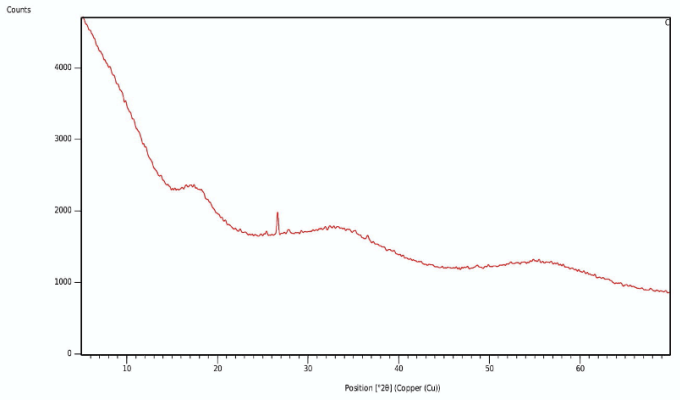

Table 5: XRD analysis Pattern List

Samples	Ref. Code	Score	Compound Name	Displ. [°2θ]	Scale Fac.	Chemical Formula	Number of peaks
ASH	98-001-5239	53	Orpiment	0.000	0.543	As_2S_3	57
CSH	98-001-5239	48	Orpiment	0.000	0.426	As_2S_3	50
KSH	98-001-5239	49	Orpiment	0.000	0.499	As_2S_3	56
RMCSH	98-001-5239	38	Orpiment	0.000	0.444	As_2S_3	45
RMKSH	No Pattern list						7
RMMSA	98-001-5239	44	Orpiment	0.000	0.429	As_2S_3	33
	98-015-6198	32	Quartz	0.000	0.019	O_2Si_1	
RMMSB	98-001-6850	34	Arsenolite	0.000	0.881	As_2O_3	17
RMMSC	No Pattern list						2

SEM images were taken at magnification of 200, 250, 400, 5000, 10000, 25000, 50000 and 100000 with 10.0kV voltage and sample width 4.2-15.0 mm. FEG SEM indicates that the gross particle size of all the samples varies from 1µm to 100nm at resolution ranging from 200 to 100000 magnifications. The nano particles are visualised at 50000 to 100000 magnifications. In CSH, RMMSA, RMMSB and RMMSC, it is also observed at 250 magnifications. Fine particles denote the amorphous nature of the final product which is water soluble and increases the absorption of the drug. In other words, the bioavailability of the formulation enhances due to its amorphous nature.

The particle size is one of the factors that are related to drug absorption and chemical reaction. Finer the particle size more will be the chemical reaction and absorption. Absorption may require consideration of additional criteria, but overall it can be concluded that the formulations have considerable level of absorption and initiating faster chemical reaction upon administration. The results of the present study are different from that of previous researches (8, 9, 10) as the method of processing and preparation of formulation are different.

Conclusion

The pharmaceutical process conducted in the form of purification and heating the drug has reduced the quantity and size of main content that is Arsenic. Small size particles increase the absorption of the drug in the body which causes increase in the bioavailability and potency of the drug. Hence the dose of the formulation may be reduced. Thereby the untoward effects of the high doses can be avoided.

Conflict of Interest: Nil

Fig. 9: SEM Images of all samples

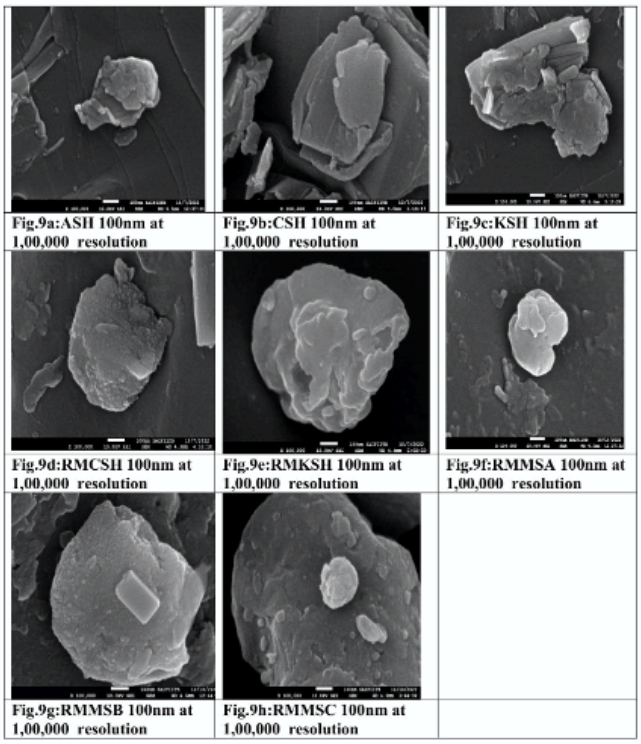

Fig.9a:ASH 100nm at 1,00,000 resolution | Fig.9b:CSH 100nm at 1,00,000 resolution | Fig.9c:KSH 100nm at 1,00,000 resolution

Fig.9d:RMCSH 100nm at 1,00,000 resolution | Fig.9e:RMKSH 100nm at 1,00,000 resolution | Fig.9f:RMMSA 100nm at 1,00,000 resolution

Fig.9g:RMMSB 100nm at 1,00,000 resolution | Fig.9h:RMMSC 100nm at 1,00,000 resolution

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