

Characterization of Bio-Active Nanoparticles – Bhasma an Indian Ayurvedic Drug

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Submission Date: 14-8-2013; Review completed: 19-9-2013; Received Revised: 28-09-2013; Accepted Date: 21-1-2014

ABSTRACT

Bhasmas are the multi elemental drugs derived from natural resources. Physicochemical analysis of various bhasmas supplied by different manufacturers has been reported. Particle size analysis has been done using dynamic light scattering technique. The size of bhasma particle is in the of the order of 1000nm. Chemical analysis of soluble matter of bhasma has been done using inductively coupled plasma – atomic emission spectroscopy technique. Elemental composition of bhasma differs from supplier to supplier. Inductively coupled plasma – atomic emission spectroscopy results show 91.87%, 83.81 and 80.77% soluble matter in tamra bhasma by dhoot, tambra bhasma by Ajit Joshi and swarn makshika bhasma respectively.

Keywords: Bhasma, ICP-AES analysis, Dynamic Light Scattering, Nano-particles.

INTRODUCTION

According to ayurveda, all the things which exit in this world can be used as medicine. The combination of ayurveda and Greek medicine is called unani medicine. In the south India, it is known as siddha medicine. Metals, minerals, jewels, pearls are used for bhasma preparation. All of these elements are turned to calxes popularly known as bhasmas in ayurdeva terminology. In Ayurvedic terminology tissues are called as dhatus. The maintenance of the body equilibrium is called health and disorder is called disease.

Bhasma is calcined product of minerals. This calcination is done in a special kind of pot called pits filled with cow dung cakes. Produced powder after roasting is called bhasma. Bhasmas are prepared either from pure metals or from ores. Bhasmas are generally prepared from the natural sources of minerals. During their preparations some juices are added for giving unique properties to the bhasma. Final bhasma which is

received after purification and calcination does not have metallic luster. Inorganic substances are in atomic form in the medicine to make the medicine effective. Atomic and ionic forms are easily absorbed by the body.

There are several steps involved for bhasma preparation. Like sodhna - purification, bhawna - heating with some juices, and marana - to get fine powder etc. Following steps are to be followed to prepare bhasma from the minerals and ores:

Sodhna: Harmful substances from the mineral are removed during the sodhna process. Some kind of juices are added to the mineral powder in a Kadhai - pan. Juice level is adjusted just above the level of powder. Heating is to be continued for some time until lime juice had reacted and the powder is subjected for drying. This step is repeated for several times to get purified powder.

Bhawna: The powder recovered from Shodhna is transferred to the mortar and the agan juices are added to the powder to

DOI: 10.5530/ijper.48.1.10

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make the powder wet. Wet grinding is done until entire water goes off from the powder.

Puti: The grounded material from Bhawna is transferred to the disc and is covered with inverted disc of similar kind. The joint is wrapped with wet cloth smeared with clay. The entire assembly is placed inside a hearth containing 12kg of cow dung in the earthen vessel which is 3 feet in depth, 3 feet in width and 3 feet in length. The earthen vessel is filled up to half of it with cow dung cakes and then grounded powder assembly is placed on it. Again, rest of vessel is filled with cow dung cakes. The dung is set on fire. The fire continues and keeps the pot red hot for about 4 to 5 hours and then starts cooling. The cooling is allowed overnight and then the material is removed and grinded again for homogenization. This step is repeated several times depending on requirement.

Bhasmas are the mutlielemental drugs prepared from natural mineral, ores and herbs. Literature shows that bhasmas are very cost effective without any side effect and permanent cure of diseases. Very fine bhasmas give very significant effect as compared to coarse one. In the literature elemental analyses of some Bhasmas have been reported as given in following section .

Vasanth et al. (1971a) have reported that talakam bhasma contains 56 to 60% arsenic in the form of sulfide. Vasanth et al. (1971b) have investigated lead is the main ingredient of naga bhasma which is in the form of lead oxide. They have reported that lead is 65–68%, As (5.5–6.5%), antimony (0.1–0.3%), aluminum (0.7–0.8%), and sulfate (9–14%). It comes out to be 80.3–89.6% of the total mass.

Sondhi and Janani (1994) have reported total material balance 49.22% in virat bhasma, 57.12% in mukta shukti bhasma, 55.69% in kukkutandatvak bhasma, 52.67% in shankha bhasma, 87.65% in godanti bhasma, 75.19% in yashad bhasma, and 26.59% in bang bhasma. Sondhi et al. (1995) have reported total mass balance 72.10% in tribang bhasma, 82.89% in naga bhasma, 46.88% in mandur bhasma, 30.23% in akik bhasma, 35.74% in kajjali, 69.50% in loha bhasma, 86.13% sangeshva bhasma, and 45.68% in rajat bhasma.

Bajaj and Vohora (2000) have reported that swarna bhasma consists of 46.9% gold and 0.15ppm mercury. Mahaptra and Jha (2010) have prepared swarna makshika bhasma and characterized it using x ray diffraction and scanning electron microscope. Scanning electron microscope results show uniform agglomerate of size 1–2 microns. Swarna makshika bhasma prepared contains ferric oxide, ferrus sulfide, copper sulfide and silicon oxide. However, raw swarna

makshika contains CuFeS_2 . During bhasma preparation the complex compound got converted into simple compound of very small size.

Goel and Sairam (2002) have reported that tamra bhasma contains copper oxide between 44.45 to 66.13%, ferric oxide less than 6.03% , and less than 2.75% sulfur. Wadekar et al. (2005) prepared and characterized the tamra bhasma. X-ray diffraction result shows the crystalline nature of bhasma and bhasma particle were of 32.2nm size. Bhasma does not have grain boundaries as tested by scanning electron microscopy. Their EDAX analysis gives 60% presence of copper and large percentage of carbon in bhasma.

Reddy et al. (2003) have reported that praval bhasma has more than 5000ppm magnesium, iron is 500ppm, and Nickel, tin, and lead are less than 50ppm. Praval bhasma was prepared from coral calx. Coral calx contains maximum amount of the calcium carbonate and they have not reported anything about carbon, sodium, and oxygen content of praval bhasma.

Bhowmick et al. (2009) studied physicochemical analysis of jasad bhasma using ICP-AES and EDAX analysis techniques. They have reported 88.32 wt% soluble matter in the jasada bhasma and remaining 11.70% oxygen–analyzed by EDAX-SEM. Their x-ray diffraction result shows the absence of crystalline zinc metal in jasada bhasma. There was shortage of oxygen at the surface of the bhasma which was supported by X-ray photon spectroscopy analysis.

Singh et al. (2010) has synthesized and characterized naga bhasma. Their x ray diffraction analysis shows the presence of nano crystalline structure of drug. Sub-micron size particles are also detected in transmission electron microscope analysis of the prepared bhasma. These submicron particles are formed due to agglomeration of nano size crystalline particles. Nano size crystalline particles get agglomerated due organic materials being used from the herbal sources during preparation of bhasma. Presence of lead, sulfur, carbon and oxygen were detected at the surface of bhasma using X ray photon spectroscopy analysis. Element analysis was 99.20% including oxygen.

Umrani and Paknikar (2011) have characterized zinc bhasma and tested in vitro. There was no adverse effect of the bhasma. Spherical shape and crystalline nature of bhasma was detected using high resolution transmission electron microscope and x-ray diffraction analysis. Presence of 320nm size particles was detected using diffuse reflectance spectroscopy. Atomic absorption spectroscopy results show 70.7% zinc content in the bhasma. No major cytotoxicity was observed during in vitro testing.

Vadnere et al. (2013) has prepared and characterized muktashukti bhasma. They suggested that repeated calcination is required to stabilize the particle to a minimum size. Their results show very high crystallinity in the final bhasma. It was also reported that small particles with high crystallinity increase easy absorption, distribution, metabolism and excretion which are very important characteristics of drug for potential use. Their results shows only qualitative analysis of particle size and no information was reported regarding chemical analysis.

None of the elemental analysis reported by earlier researcher is complete except Bhowmick et al. (2009). Hence, it is require to develop a sequence of the experiments that can give 100% mass balance, phase analysis, surface area measurement, and surface chemical analysis of the bhasma. All the particle of greater than one mm is called macro scale, 100–0.1mm is microscale, and 100–1nm in nanoscale. Nanoparticle has properties that can improve the drug delivery. Finer size particles may be taken by cells more efficiently than larger one. Fine particles get cleared from the body easily. To deliver the particles to deep tissues of lungs aerosol is the best way. These can be absorbed by body easily. Van et al. (2003) reported that efficiency of drug can be improved by proper optimization of size and density. Metal ions are used as antimicrobial agents such as mercury. Arsenic compounds can also be used against syphilis. Zinc, cadmium, zirconium, and tin salt with polymers can also be used as antimicrobial. Soluble form of silver is very effective against bacteria.

It is critical job to find right target for drug delivery due to cost factor. All protein-protein interaction can be described by proteomics (Pillutla et al., 2002). Differential techniques are used in genomic approach such as differential display and subtractive hybridization. On the other hand biological interaction technology allows prediction of function role protein in a specified pathway. Both the techniques are sufficient to drug delivery validation.

The aim of control drug delivery is to control the rate of drug for extended period of time. So far, drugs are given in two ways oral and intravenous. But recent developments are done in drug delivery such transdermal which are skin, nasal, ocular, and pulmonary. These methods are called non conventional. It is totally depends on mass transfer, reaction kinetics, thermodynamics, and transport phenomena. The study of absorption, distribution, metabolism, and excretion of drugs in human is done in pharmacokinetics. To make the tablets water soluble, hydrophobic matrix is to be mixed with the drug during formation of it. Farrell et al.

(2004) has reported that drug release follows a square root of time for a tablet. A drug can be filled in the coatings which work as membrane to get the control release of the drug. Extended time release is the characteristic of membrane systems. Sometimes drug become ineffective due to degradation before time. Such a drug could yield toxic by-products to the life.

Body needs many minerals to be remaining in healthy condition. But we can not take minerals as such. In bhasmas, the minerals are there and they have better bioactivity. Bhasmas are not size specific. It may be of nano size particle or micron size. Bhasma contains so many elements and it necessary to find out the element which is necessary for a particular disease. If only that element can cure that disease then there is no necessity to give multi elemental bhasmas. So, it becomes important to see the biological activity of bhasmas and synthetic drugs. Secondly, so far size of particle is not known. Literature shows that bhasmas give better effect than other medicines. Therefore, it becomes necessary to see the effect of particle size on the biological activity. This paper deals only the particle size and chemical characterization of bhasmas.

EXPERIMENTATION

Particle size analysis of bhasma

Particle size analysis has been done using dynamic light scattering (DLS) technique. Particles in the suspension undergo random motion that is called the Brownian motion. When beam of monochromatic light from source hits the particles under random motion then some portion of light get scattered. Analysis of random intervals of scattered photons reaching to the detector is done using dynamic light scattering. Zeta Plus Analyzer is used for particle size analysis. It is manufactured by Broke Haven Corporation, UK. An argon laser is there to produce 660nm laser light for analysis. This instrument measures the change in intensity of the laser light with time at a particular angle after interaction with spherical shape particles suspended in aqueous media. The hydrodynamic diameter of the spheres was measured using inbuilt auto correlation function. The Intensity of the scattered light will be same for the particles of the size smaller than the wavelength of the incident light. Such a scattering is called Raleigh scattering. Based on Mire theory the intensity of scattered light depends on angle of scattering for particles greater than 250nm.

Time dependent fluctuation in the intensity of scattering light can also be measured by a suitable detector – photomultiplier. Photomultiplier transmits signal to the

digital correlator, this give the time dependant intensity correlation function after normalizing it. Fluctuations in the scattered light intensity come from the particles that are small enough to undergo Brownian motion and their distance constantly vary with time. Total intensity which reaches to the detector fluctuates due to constructive and destructive interference of the light scattered from the adjacent particles within the same illumination zone. Light reaches to detector contains the information about the motion because it comes from the particles under that motion. Fluctuation of intensity with time gives the diffusion coefficient of the particles. Using that diffusion coefficient and viscosity of the medium, the hydrodynamic radius of particle is calculated using Stokes Einstein equation.

Elemental analysis of the bhasma

Inductively Coupled Plasma – Atomic Emission Spectroscopy (ICP-AES) has been used for elemental analysis of the bhasma. ICP-AES has been supplied by Plasmalab, 8440, GBC, Scientific Equipment, Dandenong, Australia. Frequency: 27.12Mhz, power RF:1.2Kw; Plasma gas flow rate: 18L/min; auxiliary gas flow rate: 6L/min; sample uptake: 2.5ml/min; integration time: 5.0s; nebulizer: GMK nebulizer; spray chamber: cyclonic chamber. Ionized gases which consist of free electrons, ions, and excited neutral is called plasma. The energy is transported to external electromagnetic field by these charge particles. Thermal plasma is in the temperature range of 2000–20000K. Temperature of ions and electrons may be different within same plasma. Plasma torch consist of copper anode and tungsten cathode. The gas passes through the gap between these two electrodes. A direct current arc is produced between the electrodes which generate high temperature and velocity flame. Plasma produced gets the temperature of 20000K. Generally argon, helium, nitrogen gases are used for plasma generation.

Each element emits light at its characteristic wavelength, and the light intensity is considered as the concentration of the element. The solution under analysis is pumped to the nebulizer it converts it to aerosol by the flow of argon gas. The finest droplets go to ICP-AES torch on the other hand larger droplets fall down to the drainage. The inductively coupled plasma is circulated in the two turn coil that carry high frequency current. These high frequency currents give generation for varying magnetic field. When charge particle cuts the magnetic line of that varying magnetic field that create ohmic heating. ICP-AES flame temperature is maintained at 6000K to 10000K by inductive heating effect. No chemical bond can stand up to this temperature. This high temperature completely atomized the solution entering the ICP.

Many atoms are converted to ionized state at such a high temperature produced strong emission lines and there are large number of lines for particular atom.

ICP-AES sample preparation

Two step procedure is used for ICP sample preparation.

Step 1

In this step, acid digestion of bhasma sample have been done. Half a gram of bhasma for analysis is taken in a 100ml beaker and then 10ml mixture of perchloric acid and nitric acid (1:1) was added to the sample. The sample is heated to reduce the volume to 5ml using hot plate. Again, 10ml acid mixture added and heated till 5 ml volume is received in the beaker. After cooling it, the recipe was filtered using Whatman filter No. 41 into the volumetric flask of 100ml. It is called filtrate one for ICP-AES analysis.

Step 2

The residue which was received from step one is to be dried and get the amount of the residue. This residue is mixed with lithium meta borate. The ratio of lithium meta borate to residue is 3:1. This total is kept in the platinum crucible covered with the lid. The crucible is then heated in the furnace at 1000°C for an hour. After an hour, it was taken out and immediately quenched the crucible into the beaker of 250ml which contains 10ml nitric acid and 70ml distilled water. This solution is subjected to stirring for 24 hrs and filtered in a flask. Distilled water is added to make the total volume 100ml. It is filtrate two for ICP-AES analysis.

RESULTS AND DISCUSSION

Particle size analysis

Three bhasma samples jasad bhasma, and tamra bhasma, and swarna makshika bhasma are analyzed using dynamic light scattering apparatus.

Jasada bhasma (Zn 030614 AJS–FIN)

Table 1 shows the particle size analysis of jasad bhasma supplied by Ajit Joshi (AJ). First and second rows in Table 1 have data for 100% intensity. There is variation in particle size for same sample and same measurement time. Sample without sonication gives the particle size 1439.2nm and with sonication it is 931.5nm. It gives the possibility of loose aggregates in the suspension and 8min sonication is able to break those aggregates. Doubling the sonication time (16min) does not affect the particle size of bhasma as shown in third row. Hence, it is concluded that there is no need to go for higher sonication time. Eight min sonication is enough to break

Table 1: Particle size distribution of Jasad Bhasma (Zn 030614 AJS – FIN). Medium: Aqueous, Conc.: 2.5mg/ml, Refractive Index: 1.397, Temperature: 25°C, Angle of Scattering: 90°, *: Filtrate from 0.7 micron filter, and **: MeOH as medium (40 Vol/vol), Conc.: 9.92mg/ml

S. No.	Time of Measurement, Min	pH	Sonication Time, min	Settling time min	Effective Diameter nm	Mode 1			Mode 2				
						Dia, nm	Intensity	Dia, nm	Intensity	Dia, nm	Intensity		
1	2	6.68	0	0	35056.5	1439.2	100	1661.5	35	9307.2	16	10000.0	16
2	2	6.68	8	0	28694.3	931.5	100	1110.5	15	9158.4	22	10000.0	22
3	2	6.68	16	0	37221.5	1033.3	100	1222.5	30	9193.7	7	10000.0	7
4	60	6.68	0	0	130369.3	970.8	100	1092.9	44				
5	2*	6.68	8	0	41922.0	293.9	46	381.7	13	8775.4	100	10000.0	100
6	2	2.39	16	0	1902.2	982.1	84	1174.0	21	9146.1	100	10000.0	73
7	2	12.14	0	0	1182.0	568.6	100	703.1	30	8992.5	28	10000.0	24
8	2**		0	0	23083.8	705.8	100	865.5	55	9030.6	31	10000.0	31

the loose aggregates. In second mode, size of particle is in the order of 10000.0nm in all the cases i.e. with sonication and without sonication both. It may be only because of the some higher size particle that does not get separated by sonication.

Same sample has also been analyzed by filtering it through 0.7micron size filter as given in Table 1 row five. In this case result shows 10000nm particles which implies that there are some chains in the suspension which have at least one dimension lower than 0.7micron and got passed through the 0.7micron size filter. On the other hand, first mode shows that filtration gives the particle of 293.9nm size.

Table 1 row 4 shows that large chains are got settled for longer duration of measurements which is 60min in this case. It is clearly seen from the absence of second mode and the majority of 970nm size particles remain in the suspension.

Change in pH dose not affect these chains however only 12pH is able to separate the some more tightly packed aggregates that do not get separated by means of sonication as shown in Table 1 rows 2 and 7 respectively.

Jasad Bhasma (Zn 030307 AJ)

Table 2 shows that this jasad bhasma sample have particles in the range of 100 to 500nm. There is no such kind loose aggregates as in the case of earlier jasad bhasma sample. Most of the particles are of the 400nm size. Glycerin which has higher viscosity than water when used as medium gives enhancement in the particle size. It may be possible that particles are trying to form aggregate of higher size due to viscous effect of medium as shown in Table 2 rows 3 and 1 respectively.

Tamra Bhasma (Cu 030614 AJS-FIN)

Some loose aggregates of size 900nm are present in tamra bhasma which get separated by 16min sonication as shown in Table 3 rows 1 and 2 respectively. Most of the particles are of 550nm which give 100% intensity. Some higher order particles are also in suspension which get reduced by means of settling as shown in Table 3 row 3. There is no such higher order particle in the suspension after 12hr settling. Only 400nm size particles are present in the suspension as shown in Table 3 row 4. These higher order may be some tightly packed particles which get separated by 12pH as shown in Table 3 row 6 because change in pH only affecting the second mode and there is no effect in first mode as shown in Table 3 rows 6 and 2 respectively.

Table 2: Particle Size Distribution of Jasad Bhasma (Zn 030307 AJ). Conc.: 10mg/ml, Refractive Index: 1.59, Temperature: 25°C , and Measurement Time: 2min per run

S. No.	Medium	Sonication Time min	Effective Diameter nm	Mode 1				Mode 2			
				Dia, nm	Intensity	Dia, nm	Intensity	Dia, nm	Intensity	Dia, nm	Intensity
1	Aqueous	0	4258.8	103.5	34	122.3	5	393.5	100	505.5	10
2	-do-	24	3174	166.6	17	190.1	3	623.6	100	711.6	35
3	Glycerin (40% Vol/ Vol)	0	583.1	488.1	100	682.7	14	5111.6	19	6393.0	15
4	-do-	32	408.2	203.2	59	244.1	32	556.8	100	668.9	22

Table 3 : Particle Size Distribution of Tamra Bhasma (Cu 0303614 AJS-FIN), Conc.: 10mg/ml, Refractive Index: 1.397, Temperature: 25°C

S. No.	Sonication Time, min	Settling Time, hr	pH	Effective Diameter, nm	Mode 1				Mode 2			
					Dia, nm	Intensity	Dia, nm	Intensity	Dia, nm	Intensity	Dia, nm	Intensity
1	0	0	7.03	2149.9	987.8	100	1188.8	29	9115.6	6	10000.0	6
2	16	0	7.03	11148.0	542.3	100	684.6	12	8899.6	53	10000.0	53
3	0	2	7.03	16275.2	480.7	100	550.8	6	1245.4	8	1346.8	4
4	0	12	7.03	3078.2	397.8	100	417.9	13				
5	16	0	2.03	10366.1	705.8	100	958.4	6	9030.6	52	10000.0	51
6	16	0	11.98	4074.6	496.7	30	615.5	16	1052.1	100	1303.8	48

Table 4: Particle Size Distribution of Swarna Bhasma(SM 030708 BAG), Medium: Aqueous, Conc.: 5.1mg/ml, Refractive Index: 1.000, Temperature: 25°C, Measurement Time: 2min per run, and *: Filtered with 0.7 micron size filter

S. No.	Sonication Time, min	Settling Time, hr	pH	Effective Diameter nm	Mode 1				Mode 2			
					Dia, nm	Intensity	Dia, nm	Intensity	Dia, nm	Intensity	Dia, nm	Intensity
1	0	0	4.34	22515.5	1033.3	100	1222.5	19	9193.7	16	10000.0	15
2	8	0	4.34	11785.1	931.5	100	1110.5	23	9158.4	18	10000.0	17
3	0*	0	4.34	16549.7	438.6	100	557.9	40	8866.3	77	10000.0	76
4	8*	0	4.34	12758.1	438.6	100	557.9	46	8866.9	56	10000.0	55
5	0*	0	2.01	75362.9	9989.7	100	10000.0	5				
6	8*	0	2.01	46274.7	257.9	26	293.9	24	8775.4	100	10000.0	100

Swarna makshika bhasma (SM 030708 BAG)

It has the particles of 931.4nm size which give 100% intensity. Loose aggregates of 1033.5nm are present in the suspension which get separated by 8 min sonication as shown in Table 4 rows 1 and 2 respectively. Some chains of 10000nm size are also in suspension which were unaffected by sonication and have at least one dimension smaller than 0.7micron that is why it does not get separated by means of filtration as shown in Table 4 row 3. These were unaffected during the change

in pH as shown in Table 4 rows 5 and 6 respectively. The filtrate of 0.7micron size filter does not have any loose aggregate which can be separated by means of sonication as shown in Table 4 rows 3 and 4 respectively. Filtrate contains mostly 438nm size particles in suspension.

Elemental analysis of bhasmas

Elemental analysis of tamra bhasma (Cu 030614 AJS-FIN and Cu 030520 Dhoot) and swarna makshika

Table 5: Percentage ICP-AES analysis of Tamra Bhasma (Cu 030520 Dhoot and Cu 030614 AJ S–FIN), and Swarna Makshika Bhasma (SM 030708 BAG)

Element	Bhasma									
	Tamra Dhoot		Tamra AJ Fin				Swarna Makshika			
	Acid Digestion	Fusion	Glass Filter		Whatman No. 41		Glass Filter		Whatman No. 41	
			Acid Digestion	Fusion	Acid Digestion	Fusion	Acid Digestion	Fusion	Acid Digestion	Fusion
Na	0.0362	0.0477	0.3848	0.9848	0.2367	0.0429	0.3673	0.2595	0.2965	0.0713
Mg	0.091	0.8507	0.1849	1.7229	0.0757	0.4302	0.2388	0.7628	0.1019	0.8656
Al	0.1483	0.4763	0.3125	1.1418	0.2346	0.3104	0.2922	0.6117	0.1966	0.5516
Si	0.0497	10.2722	0.0339	29.2299	0.0489	5.2404	0.0679	26.6821	0.0432	11.1086
P	0	0	0	0.0884	0	0	1.1914	0.0682	1.3431	0.1986
S	21.144	0.1826	0.3554	0.1016	0.2424	0.0260	0.9444	0.0866	0.5523	0.1521
K	0.6215	0.6094	0	0	0	0.3463	0.4662	0.2622	0.4328	0.6528
Ca	0.2235	1.3861	0.3089	1.2428	0.2768	0.6054	0.1735	0.5809	0.1632	1.3568
Cr	0.0301	0.0031	0.0815	0.0985	0	0	0.0697	0.0598	0.0096	0
Mn	0.0153	0.0195	0.0214	0.0395	0.0202	0.0152	0.013	0.0159	0.1354	0.0170
Fe	13.559	0.5293	0.5078	3.9257	0.5019	0.1429	58.009	2.8088	51.357	10.3808
Cu	28.412	0.3685	71.44	0.0604	73.273	1.3589	0.9279	0.0052	0.4921	0.0194
Zn	0.0892	0.0476	0.1548	4.4010	0.1605	0.0474	0.0496	0.0430	0.0417	0.0468
As	0	0	0	0	0	0	0	0	0	0
Sn	0.0773	0.0774	0.1464	0.7077	0.0894	0.0893	0	0	0.0743	0.0743
Hg	12.455	0.0476	0	0	0	0	0	0	0.0243	0.0159
Pb	0	0	0	0	0	0	0	0	0	0
Total wt%	76.953	14.918	73.932	43.4451	75.16	8.6554	62.811	32.2468	55.264	25.5116
Grand Total (Acid digestion and fusion) wt%	91.871		117.3771		83.8154		90.0578		80.7756	

bhasma (SM 030708 BAG) have been done using ICP-AES technique. Bhasmas are multi elemental powder drugs as shown in Table 5. Acid digestion gives only around 70% of mass balance and 15% mass is obtained from the fusion. Earlier researchers have only reported the acid digestion part that is why their analysis is not complete up to 100%. A two step method has been developed which gives 90% mass balance as the soluble part. There is some material balance violation in case of tamra bhasma AJ Fin sample. This violation may be due to the addition of silicon oxide from the sand filter during scrapping of the residue. Remaining 10% may be considered as hydrogen and oxygen that can be analyzed by EDAX analysis.

CONCLUSIONS

Average particle size in the bhasma is in the order of 1000nm. Sonication is helpful in breaking the loose aggregates present in the bhasma. Two minutes sonication time is sufficient to break these loose aggregates. There is no effect of sonication time beyond 2 minutes. Bhasma samples have varying composition from supplier to supplier. Almost 90% matters in the bhasma is soluble and can be analyzed using ICP-AES analysis. ICP-AES analysis shows that bhasmas are multielemental drugs which can be used to cure many diseases. Remaining undissolved matter is to be analyzed using EDAX.

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