Spectroscopic and Chemical Evaluation of Oriental Medicine (Oystershell Pearl and Coral)

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Summary: The results of determination of trace elements aluminum, arsenic, cobalt chromium, copper, Iron, magnesium, manganese, nickel, phosphorous, lead, sulfur and zinc in oyster shell, pearls and coral used as traditional medicine using two types of digestion methods, a microwave assisted total digestion and Tappi method are reported. The results have been validated by the determination of relative standard deviation (RSD). The RSD values for the elements for the microwave method were acceptably low in most of the cases. The RSD values for the determined of the elements for the block digestion were not appreciably different from the RSD values obtained for microwave digestion. The RSD percentage obtained for the microwave digestion of shell, pearl and coral are almost less than five or about five percent with the exception of iron (0.3 µg /g in pearl which has highest RSD value 43. RSD value obtained by the block digestion (Tappi Method) matrix (Shell, pearl and Coral) were usually lower than five percent reaching upto 47 % for nickel (0.744 µg /g in coral). Other highest is 43 for chromium (0.536 µg / g in shell). It was observed that the higher RSD values are observed for those elements which are present in quantity less than 1µg/g. The chemical analysis was also carried out.

Introduction

Calcium is of great importance in blood coagulation; to give firmness and rigidity to bones and teeth and in acid –base balance. It is essential to lactation; for activating enzymes; for the function of the nerves; muscles including the myocardium and for maintaining the permeability of the membranes. In addition to that the calcium mediates the intercellular actions of many drug substances [1].

Calcium deficiency symptoms are brittle bones, poor development of bones and teeth, dental caries, rickets, tetany, heart stony, hyperirritability, excessive bleeding. In diagnosis, normal content of calcium in blood serum is 9 to 10.5 mg. per 100 ml. of blood. In evaluating the calcium level, laboratory error and variation may be the causes of inaccurate or inconsistent values [1].

Calcium deficiency increases the quantity of Staphylococcus and Enter-Ococcus bacteria in the body and the use of calcium decrease their quantity in the body. Calcium hydroxide water solution stops the growth of bacteria and it also has antimicrobial properties. It has been found by experiments that calcium reduces the blood pressure of the body. It is also anti hypertension and reduces the cardiac contractility [2].

In allopathic system of treatment of diseases calcium gluconate, calcium lactate and calcium carbonate are used for calcium deficiency treatment. All the chemicals used for medical purposes are governed by the medical jurispedence.

In traditional method for treatment of deficiency of calcium oyster shells, pearls and corals are used. [2].

It is claimed that medicinal powder of shell pearl and coral taken internally detoxify the system, cleans and clear the skin; removes age and liver spots on the skin; give the skin a younger appearance; adjust the body energy; calm the liver; relieves stress from the mind; treat sore throat; adjust the nerve system; regulate the metabolism; improve eyesight; treat insomnia; and energize the body. When pregnant women and breast feeding women take it, pearl powder is believed to speed up bone development and promote intelligence in babies. When used topically, it is famous for keeping skin smooth, soft,

and lustrous looking; it is also used in treatment of open sores burns, and cuts; as well as homeopathic cure for fever [3].

Clinical study has confirmed the bio availability of calcium from oyster Shell, pearl and coral is superior to the calcium salt being used in allopathic medicine [4].

In addition to that these natural materials are rich in trace metals. A number of researchers have investigated the presence of trace metals like B, Sr, Mg, Rb, Ba, Pb, Cd, Ni, Cr, Zn and U etc in fresh water samples or in tissues [5-9]. These trace elements were very important for the determination of metabolic pathways of coral and their mode of feeding.

In present study we have investigated the presence of trace metals in bone dried samples of oyster shell, pearl and coral used in oriental medicine by using inductively coupled Plasma–Optical Emission Spectrometer. We have also investigated the Microwave digestion method and Block digestion method for the analysis of oyster shell, pearl and coral.

Results and Discussion

Samples digestion was carried out by mainly two methods block digestion and microwave digestion. The block digestion was completed in approx 2.5 hrs. In an effort to decrease the sample processing time, microwave assisted digestion was also evaluated. Microwave digestion was completed in approx 30 minutes. The results of the replicate reading are recorded in Table-3.

There are different heating systems that can be used for digestion such as, sand-bath, heating plate, pressure digestion bombs and aluminum blocks. The introduction of microwaves, with both open and closed pressurized systems, has allowed a considerable reduction in the total time of analyses as well as in the risk of sample contamination [10]. Whereas the use of closed digestion systems is mandatory for total determination of volatile elements to avoid losses (eg. As, Se, Hg, Cr, mg Pb and S) the use of open systems allows a higher sample intake and facilitates the acid evaporation to dryness, reducing in that way the analytical problems associated with high acid concentration.

Nitric acid (bp 122 °C) is an oxidizing acid that will dissolve most metals to form soluble metal nitrates. Most metals form soluble metal chlorides with several notable exceptions: AgCl, $HgCl_2$, and $TiCl_4$ which are insoluble and $PbCl_2$ which is only slightly soluble. Hydrochloric acid (bp 109 °C) is often used in combination with nitric acid for dissolution. Selenium, Mercury, Vanadium, Titanium and Antimony chlorides have low melting and boiling points. Hydrofluoric Acid (bp 112 °C) is most commonly used in inorganic analysis because it is one of the few acids that can dissolve silicates. Hydrofluoric acid's strong complexation capabilities prevent the formation of sparingly soluble products of several metals, increasing the solubility and stability of those elements. Arsenic, chromium, selenium, sulfur, silicon, antimony and vanadium fluorides have reasonably lower boiling point and sublimation point than digestion temperature. Concentrated Sulfuric acid is capable of oxidizing many substances. It has a boiling point of 339 °C. Which is greater than the working range of Teflon.

Concentrated perchloric acid (60 – 72 %) and Hydrogen peroxide are also used along with the mineral acids. Perchloric acid is a powerful oxidizing agent when warm.

The combination of hydrogen peroxide and sulfuric acid forms monoperoxosulphuric acid (H_2SO_5), a very strong oxidizing reagent. The melting and boiling points of the potentially volatile compounds of these elements formed during acid digestion are listed in Table-1 [10-12].

In this study we have used Nitric Acid and hydrogen peroxide for microwave digestion and nitric acid and perchloric acid for block digestion. In both the methods samples were pre digested with nitric acid after that it was cooled and hydrogen peroxide or perchloric acid was added for further digestion. Nitrate and perchlorates of all almost all the elements are soluble in water. Hydrofluoric acid is not recommended for digestion [12]. Similarly sulfuric acid cannot be used for microwave digestion. Aqua regia also has limited use because some of the chlorides are insoluble in water (Table-1).

Chemical Analysis

Results of chemical analysis of the Oyster shell, pearls and coral are given in Table-2. The percentage of moisture is 0.1% to 0.4% it means the

Table-1: Melting and Boiling Points of Potentially Volatile Species [8, 9, 10]

Element	Compound	Melting Point °c	Boiling Point	Solubility
Arsenic	Arsenic Triflouride	-5.95 / -8.50	57.8	
	Arsenic Trichloride	-16.0 °C	130.2 °C	
Chromium	Chromyl Flouride		117.0 °C	
	Chromyl Chloride	-96.5 °C	117.0 °C	
Selenium	Selenium Hexaflouride	-50.8 °C	Sublimes -63.8 °C	
	Selenium chloride	-85 °C	129 °C	
	Selenium Tetrachloride		Sublimes when heated	
	Selenium Tetraflouride	-9.5 °C	106 °C	
Magnesium	Magnesium Flouride	1248 °C	2260 °C	
	Magnesium Chloride	712 °C (rapid Heating)	Releases chlorine at 300 °C when slow heated	
Lead	Lead Flouride	824 °C	1293 °C	
	Lead Chloride	501 °C	950 °C	Soly in water (g/100 ml): 0.057 (0degrees); 0.065 (20degrees). The soly increases in the presence of HNO 3 or nitrates.
Sulphur	Sulfur chloride	-77 °C	138 °C	
	SF6	-50.8 °C	63.8 °C	
Mercury	$HgCl_2$	277 °C	Volatalizes at 300 °C	
	645	<650 °C		
Aluminum	$AlCl_3$			
	AlF_3		Sublimes at 1272 °C	
Silicon	$SiCl_4$	-70°C	59°C	
	SiF_4	-90.2°C	Sublimes -95.7°C	
Antimony	SbCl5	3.5 °C	68 °C	
	SbF5	8.3 °C	141 °C	
Titanium	TiCl4	-24.1 °C	136.4 °C	Sol in water
	TiF4	<400 °C	Sublimes at 284 °C	
Vanadium	VF_4		Sublimes at 100~120 °C	
	VF_5	19.5 °C	47.9 °C	
Sliver	AgCl	457°C		52 x 10-6g/100g water at 50°C
	AgF	435 °C	1150 °C	

Table -2: Chemical Analysis of Shell, Pearl and Coral

Sr No	Elements	Shell %	Pearl %	Coral %
1	Moisture	0.08	0.40	0.18
2	Loss on Ignition	44.00	46.00	42.44
3	SiO_2	3.80	3.90	5.74
4	Al_2O_3	0.63	0.60	0.53
5	Fe_2O_3	0.37	0.30	0.37
6	CaO	40.83	39.84	42.08
7	MgO	3.82	ND	6.07
8	Na_2O	3.93	7.97	3.88
9	K_2O	2.3	1.12	0.18

samples are bone dry. Ignition loss of all the three materials is almost within the acceptable range (less than 10 %). SiO_2 is higher in coral (5.7%) than pearl and shell.Al_2O_3 and Fe_2O_3 are also similar. The quantities are also the same. The variation in the results of CaO(40-42 %) is within 5%. Magnesium is not detected in pearl but coral has almost double the quantity than that of shell. The Sodium and Potassium were determined by flame photometer. Shell and coral have almost the same quantity of sodium oxide but the pearl have almost double the quantity present in shell and coral, Percentage of Potassium is higher in the shell than the pearl but the coral have only 0.2 % potassium.

Validation of Results

Validation of results was carried out by determination of relative standard deviation. The relative standard deviation (RSD = S.D/Mean) [13] when expressed as a percentage is also known as coefficient of variation. This gives an indication of how good or poor the precision is relative to the magnitude of the measured value. The RSD values are given in the table-3 along with the results. The number of the replicates was three or higher. RSD for elements determined by microwave digestion method were acceptably low in most of the cases or not appreciably different from the RSD observed for the metal determination for block digestion.

The RSD percentages obtained for the microwave digestion (Shell, Coral and Pearl) are almost less than five or about five percent with the exception of Iron (3.91µg/ g) which has the highest RSD value 43 in pearl. It has been reported that this type of results are obtained for the elements which are present in small quantities in the sample. The other high RSD is 8.74 for manganese in shell. It is within the acceptable limit. The RSD values for

Table-3: Validation of microwave-assisted total digestion and Block digestion Results

ELEMENT	SHELL		PEARL		CORAL	
	MICROWAVE	BLOCK	MICROWAVE	BLOCK	MICROWAVE	BLOCK
Aluminum	23.42 ± 3.06	25.956 ±12.24	8..51±3.0	.2605± 4.11	74.661 ± 2.50	85.1005 ± 3.94
Arsenic	N.D	3.61 ± 4.39	N.D	.559±3.071	N.D	9.63 ± 4.95
Calcium	87856.5 ± 0.0384	182728.8 ±2.68	202949.4 ± 2.25	08989±2.12	186422.9 ± 0.65	193528.4 ±1.79
Cadmium	N.D	N.D	N.D	I.D	N.D	N.D
Cobalt	0.737 ±5.208	0.343± 2.68	0.533 ±4.25	.468 ±0.601	0.493 ± 1.72	0.495 ± 0.421
Chromium	N.D	0.536 ±43.2	0.53±1.600	.492 ± 9.58	1.972 ± 1.69	1.981 ± 0.453
Copper	0.737 ±5.21	0.352 ±0.9041	N.D	.45592 ±3.07	1.4787 ±1.68	1.731 ±17
Iron	21.694 ± 2.933	14.99 ± 14.0	3.91 ±43	.04 ± 8.83	256.68 ±5.11	298.2 ± 10.45
Magnesium	14.6785 ± 1.6427	17.909±3.9048	35.1833 ± 8.2269	0.199 ±19.20	12096.94 ± 0.4996	12522.94 ±1.1668
Managnese	275.08 ± 8.74	247.58 ±6.49	449.5 ± 3.86	65.74± 6.56	4.927 ± 1.622	5.684 ±5.24
Nickel	N.D	N.D	0.534 ±3.0	.467 ±1.061	2.015 ± 1.404	0.744 ± 47.65
Phosphorous	47.04±2.61	49.867 ±6.72	76.0 ± 4.36	3.95 ± 2.42	84.50 ± 2.57	106.50 ± 5.92
Lead	N.D	N.D	N.D	I.D	1.727±0.022	0.495 ± 0.421
Sulfur	142.83 ±4.50	170.6 ± 8.34	246.5 ± 2.27	59.61 ± 5.18	2726.7 ± 1.58	2836.64 ±1.84
Zinc	3.5016 ±0.224071	3.103768 ± 0.4468527	N.D	I.D	4.865 ±3.59	9.904 ± 0.453

Results expressed in µg/g, Mean Value ± R.S.D. of replicates (n = 3 for microwave digestion and n = 4 for block digestion)

microwave digestion of less than five percent has been reported by other workers [12,14].

The RSD values obtained (Shell, pearl and coral) are usually lower than 5 percent reaching up to 47 percent for Nickel (0.744 µg/g) in Coral. Other highest is 43.2 for Chromium (0.536 µg/g) in shell. Both these materials are present in quantities less than 1mg/g in the sample. Other RSD values which are higher than 10 percent are Aluminum (12.24 µg /g), and Iron (14.0 µg/g) percent in shell. Magnesium (19.20 µg/g) in pearl, and Chromium (1.73 µg /g) in coral 17.0 percent.

The Arsenic is not detected in microwave digestion (Shell, pearl and coral). But is detected in block digestion. Cadmium is not detected by microwave digestion and block digestion.

Shell, pearl and coral contain almost equivalent amount of calcium (200 mg/g). The quantity of magnesium is much higher in coral (12 mg/g) than shell (16 µg/g) and pearl (32 µg/ g). Manganese present in Shell (260 µg/ g) and pearl (560 µg/ g) is in much higher quantity than in coral (5 µg/ g). Lead is not detected in shell and pearls while coral contains1 µg/ g of lead. Coral contains Iron 270 µg/g as compared to shell 17 ug/ g and pearl 4ug/ g. Coral also contain higher quantity of copper (about 1.5 ug / g) as compared to 0.456 ug/ g in shell and 0.5 ug /g in pearl. Nickel is not detected in shell but Pearl contains 0.5 ug/ g as compared to coral which contain 1.4 ug/g of Nickel. Coral also contain higher amount of sulfur (2.8 mg/ g) as compared to pearl which contain 0.25 mg/g and shell (0.15 mg/ g). Zinc is not detected in pearl but shell contains 3 µg/g and 7.5 µg /g in coral.

In most of the literature analysis is based on the fresh water coral along with tissue. They only determined B, Ca, Mg, Sf, U, Cd, Cu, Cr, Ni and Zn [5-9,13-17]. These trace elements were very important for the determination of metabolic pathways of coral and their mode of feeding.

In our study we used dried samples of shell, pearl and coral for analysis used in the preparation in traditional and oriental medicine. The moisture determined for these materials is less than 0.4 percent. Their consumption is in tons in Pakistan. The toxic trace metal cadmium is not detected in any samples of oyster shell, pearl and coral. Lead is not detected in pearl and shell. Also the lead detected in coral is far less than (1.7µg/ g) the standard specification set for medicinal clay(13µg/ g). Arsenic quantity varies from 3.6 to 9 µg/g in oyster shell pearl and coral samples. Which is much higher for the standard set for medicinal clay (ie < 1µg/ g). Arsenic is a constant element of cell life and is present in eggs. Poisoning limit is 5 to 50 mg per kg body weight [1]. Oyster shell, pearl are used as a medicine. There daily doses are not more than 2 to 4 gms [2] so that the quantity of arsenic would be much lower than the poisoning limit. The quantity of trace elements cobalt, chromium, copper, iron, manganese, nickel, phosphorus and zinc is well within the limits which are being used presently in multivitamin composi-tions such as centrum. The major elements calcium, magnesium, sodium and potassium are essential for good and long health.

In Pakistan the citrate of these compounds is marketed for the treatment of respiratory depression, cardiotonic, melancholia, Digestive Stimulant, parlaysis, anti-hemorrhagic, thromobo-emboloism [2]. In America Coral Calcium is very poplar for good and long health and it is claimed that coral calcium supplemented with vitamins has cured arthritis, colon cancer, Fibromylogia and prostate cancer [15]. Clinical evaluation has demonstrated that the calcium bio-availability is better than that of calcium carbonate and calcium lactate when conducted on the normal 4 elderly subjects aged from 66 to 86 [4]. It is also demonstrated that oyster shell have more potent phosphate binding capacity than $CaCO_3$, despite indistinguishable immediate effects on the increment of the serum calcium and supperssion of serum PTh. OSE may therefore represent a new candidate as potent phosphate binder, with a prospect for replacing $CaCO_3$ currently used in renal failure [16].

Experimental

Samples

Bone dried powder samples of oyster shell, pearl, coral were kindly provided by Dr K. M Janjua, Qarshi International (Pvt) Ltd.

Block Digestion

Acid digested samples were prepared following the procedure described by the Technical Association of Pulp and Paper Industries (TAPPI) standard methods (1996–97). An accurately weighed, dried, ground sample (approximately 2 g) was added to a digestion tube followed by 10 ml HNO_3 (63% v/v). The sample was refluxed for 1 h then permitted to cool. Subsequently, 3 ml H_2O_2 was added and the digestion was continued for a further 1 hr. On cooling, the resulting clear digest was diluted to 100 ml with distilled deionised water [14].

Microwave digestion

HNO_3 (9 ml, 63% v/v) was combined with approximately 0.1 g of an accurately weighed, dried, ground sample in a Teflon PVA digestion vessel. When the reaction became less vigorous, 1 ml H_2O_2 was added and the vessel was sealed using the capping station of the Microwave Laboratory System (Milestone, Monroe, CT, USA). The vessel was transferred to the microwave cavity of the instrument, heated gradually (power increase to 1000 W over 5 min) and digested at this setting for a further 15 min. After cooling to ambient temperature, the vessel was vented and then opened. The resulting digest was transferred to a 50 ml volumetric flask and diluted to the mark with distilled deionised water [14].

The samples digested with microwave and by block digestion both were analyzed by the Varion Vista-MPX CCD simultaneous ICP-OES model 1998. The ICP was first standardized by running standard solutions of 1, 10, 20, 30, 50 ppm of the elements to be analyzed.

Chemical analysis was carried out by the standard chemical method [17]. Results are given in Table-2. The sodium and potassium was analyzed using Genway flame photometer Model No: PFP 7.

The standard deviation for small measurements is calculated by the following formula [13].

$$S.D = \sqrt{\frac{\sum_{i=1}^{N}(xi - \bar{x}}{N-1}}$$

The Relative standard deviation is given by

$$RSD = \frac{S.D}{Mean} x100 \quad [13].$$

All the calculated values for the ICP results are given in Table-3.

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