
CHAPTER-2

Materials, Experimental design, And Characterization methods

2.1: TOXIC HEAVY METAL ANALYSIS IN CHOLIC ACID BY Q-ICP-MS

The Cholic acid of synthesis grade used in the study was obtained from Suvidhinath Laboratories. Merck in Darmstadt, Germany provided the 65% HNO₃, the 1000 mg/L certified reference metal stock standard solutions of V, Co, Ni, Cd, Hg, Pb, and As, and the analytical grade ethylene diamine tetra acetic acid sodium salt. The solutions were made in analytical grade HNO₃ at a concentration of 2-3%. To create deionized water, Millipore's Milli-Q plus water purification system—which is based in Bedford, Massachusetts, in the United States—was used. We used nitric acid with a concentration of less than 69.0%, Honeywell's TraceSELECTTM for trace analysis, a yttrium standard for ICP TraceCERT® (1000 mg/L Y in nitric acid), and a bismuth standard for ICP TraceCERT® (1000 mg/L Bi in nitric acid) for this experiment. Following a four-hour soaking period in an analytical grade solution containing 20% volume/volume HNO₃, all of the autosampler vials, centrifuge tubes, and plastic bottles were cleaned by washing them three times with deionized Milli-Q water. The element impurities were acquired from Sigma-Aldrich in accordance with ICH Q3D, Standard 1. 15 ppm of arsenic (As), 5 ppm of lead (Pb) and cadmium (Cd), 30 parts per million of mercury (Hg), 50 parts ppm of cobalt (Co), 100 ppm of vanadium (V), 200 parts per million of nickel (Ni), and three more elements, including 150 [1-3].

2.1.1: Sample preparation

A commercial sample of Cholic acid weighing about 100 mg was carefully weighed and transferred into a 15 mL calibrated plastic tube. After transferring 90 mL of HNO₃ with a concentration of 65% into a volumetric flask with 3000 mL capacity that also included 1000 mL of deionized water, the solution was well mixed, diluted with water up to the mark, and then shaken. After putting the sample in the sample tube and adding 3 mL of strong nitric acid to it, the material was allowed to digest while the tube was shaken intermittently during the process. After the sample has been digested, and the sample has become transparent and there are no longer any vapours of nitric acid emerging from the sample tube, the content is diluted with water until it reaches the 10 mL threshold.

2.1.2: Microwave Digestion

When it comes to microwave-assisted digestion, there are open-vessel and closed-vessel techniques. The vast majority of pharmaceutical processes may be carried out successfully using a closed vessel approach. The Mth 2018–001, STD 75 microwave digestion system, which was produced by PerkinElmer and included a 16-position unit size, was utilised for the digesting process. Accurately weighed 0.2 g of material into a volumetric flask holding 10 mL and then combined it with 7.0 mL of concentrated HNO₃. The sample was then digested after being moved into the digester vessel and the previously described digestion procedure being selected. After being brought down to room temperature, the liquid was put into a volumetric flask of 10 mL and then filled with sterile water. After transferring 5.0 mL by pipette into a volumetric flask containing 10 mL, dilute the solution with deionized water until it reaches the appropriate concentration. The table below provides further information on the digestion of the sample [4-6].

2.1.3: Standard stock solutions for calibration

A standard comprising 100 ppm of V, 50 ppm of Co, 200 ppm of Ni, 5 ppm of Cd, 30 ppm of Hg, 5 ppm of Pb, and 15 ppm of As was used to create the standard stock solutions for calibration. Next, using 2% HNO₃, the standard stock solutions were diluted to a volume of 20 mL. These stock solutions were then further diluted to provide various calibration standard levels (Table 1).

Table 1: Dilution of standards for calibration

Solution name (%)	Volume of standard stock solution (mL)	Make Up Volume (mL)	Concentration (µg/L)						
			V	Co	Ni	Cd	Hg	Pb	As
LOQ (30)	0.3	50	30	15	60	1.5	9.0	1.5	4.5
50	0.5	50	50	25	100	2.5	15	2.5	7.5
80	0.8	50	80	40	160	4.0	24	4.0	12
100	1.0	50	100	50	200	5.0	30	5.0	15
120	1.2	50	120	60	240	6.0	36	6.0	18
200	2.0	50	200	100	400	10	60	10	30

LOQ: Limit of quantification

The dilution of standards for calibration is described in Table 1, with an emphasis on a number of elements, including Co, Ni, Cd, Hg, Pb, and As. The table presents different solutions with varying concentrations expressed in $\mu\text{g/L}$ and the corresponding volumes of standard stock solution required to create these solutions. Additionally, it mentions the limit of quantification (LOQ) for each element, set at $30 \mu\text{g/L}$ [7].

The table is essential in analytical chemistry and quality control processes, particularly for assessing the accuracy and precision of analytical instruments, such as spectrophotometers or atomic absorption spectrometers. To guarantee that these equipment can precisely identify and measure the concentrations of the specified elements in diverse samples, calibration is essential. "Concentration ($\mu\text{g/L}$)" in the table denotes the intended concentration of every element in the calibration standard solutions. The "Volume of standard stock solution (mL)" shows how much of the stock solution, which presumably has a much higher concentration of the element, is required to achieve the desired concentration. The "Make up Volume (mL)" indicates the volume of a suitable solvent or buffer needed to dilute the stock solution to reach the desired concentration.

For example, to make a calibration standard containing $80 \mu\text{g/L}$ of Cobalt (Co), you would need to add 80 mL of diluent to 0.8 mL of stock solution in order to achieve the appropriate concentration. The instrument's capacity to identify and measure Co in test samples would subsequently be validated and calibrated using this calibration standard [8].

In analytical chemistry, the Limit of Quantification (LOQ) is a crucial parameter. It denotes the lowest concentration of an analyte (in this case, the items on the list) at which the analytical device can accurately quantify it. The limit of quantification (LOQ) for all elements in this table is set at $30 \mu\text{g/L}$. This implies that any quantity below this threshold may not be detectable by the instrument [9-13].

2.1.4: Spiked sample solution

Precisely weighed about 100 mg of the sample into a calibrated 15 mL polypropylene tube. Table 2 specifies how much standard stock solution 2 has to be applied. After adding 3 mL of concentrated HNO_3 , the sample was periodically shaken to aid in digestion. Following sample digestion, deionized water was added to the sample to complete the digestion when the sample turned clear and all nitric acid vapours stopped evaporating from the sample tube.

2.1.5: Criteria for validating the analytical method

The approach was validated by looking at and evaluating a number of factors, such as linear dynamic range, method linearity, accuracy, precision, LOD, LOQ, and uncertainty measurement. In compliance with ICH Q2 (R1), Q-ICP-MS was used to confirm the analytical method for the measurement of V, Co, Ni, Cd, Hg, Pd, and As [14, 15].

2.1.6: Q-ICP-MS analysis

A standard analysis method involves taking six duplicate readings and conducting 30 sweeps over the range of analyte masses, pausing for 40 microseconds for each mass during each sweep. Aspirating a sample was the first step in the process, which was followed by a 60-second rinse with 2% HNO₃. He gas was used to maintain the device in the KED state at a flow rate of 4.34 mL min⁻¹ [16, 17].

2.1.7: Characterization of Cholic acid

A Perkin Elmer spectrometer (Model: Spectrum GX) made in the United States of America was used to record the materials' Fourier transform infrared spectra at ambient temperature. The adsorbent was added to KBr after the air background was measured, and the sample was scanned 32 times over a frequency range of 400–4000 cm⁻¹. In order to acquire ¹H and ¹³C NMR spectra, a Bruker, Model: 400 MHz FT NMR, Avance III Spectrophotometer was utilised, and [CDCl₃] acetone was chosen to serve as the solvent. In relation to the TMS internal reference, the chemical shifts are expressed as ppm values in the reports. After applying a gold coating, the samples were subjected to SEM-EDX analysis using a device manufactured in the Netherlands by Philips and designated as an SEM EDX XL-30. The field electron source scan had a resolution of 2 nm and a voltage of 30 keV.

Table 2: Dilution for spiked sample preparation

Level of spiked sample preparation (%)	Amount of calibration standard stock solution to be added (mL)
LOQ (30%)	0.3
100%	1.0
150%	1.5

LOQ: Limit of quantification

Table 2 lists the dilution processes needed to prepare spiked samples at various concentrations, emphasising how much calibration standard stock solution needs to be added. The table includes three levels: the limit of quantification (LOQ) at 30%, 100%, and 150%. In the context of analytical chemistry and quality control, these levels represent different concentrations of an analyte, and they are essential for creating calibration curves and validating the performance of analytical instruments [18].

The volume of calibration standard required to reach the appropriate concentration for each level is indicated in the column labelled "Amount of calibration standard stock solution to be added (mL)". Since the LOQ level is set at 30%, it denotes the lowest analyte concentration at which the analytical device can detect it with accuracy. To get the sample's concentration to match the analyte's full concentration at the 100% level, you would need to add 1.0 mL of the calibration standard. Usually, this serves as a reference point to make sure the device can measure the analyte at its standard concentration with accuracy [19-20].

For the 150% level, 1.5 mL of the calibration standard is added to the sample. This level represents a higher concentration than the standard, which can be useful for assessing the instrument's ability to detect and quantify analytes above the standard range. It's important for evaluating the instrument's linearity and response to elevated concentrations. The LOQ, as mentioned earlier, serves as a crucial benchmark in analytical chemistry. A crucial factor in assessing the sensitivity of the instrument, it denotes the lowest concentration at which the device can accurately quantify the analyte. The ability to accurately measure concentrations at or above the LOQ is essential for the instrument's reliability in analytical applications.

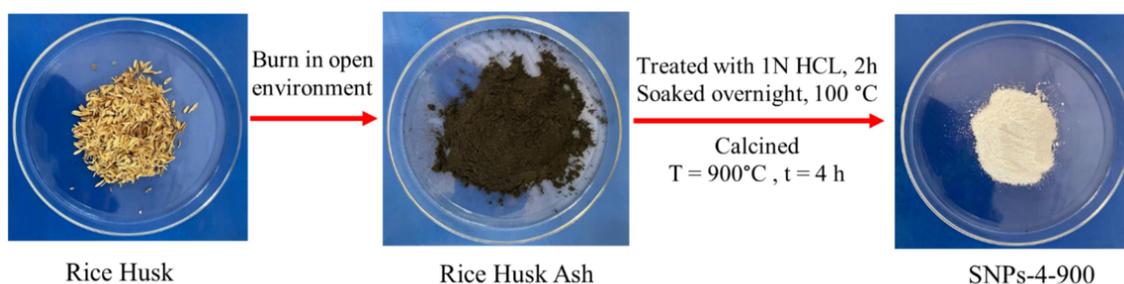
2.2: GREEN REMOVAL OF HEAVY METALS FROM LOSARTAN BY RICE HUSK ASH NANOPARTICLES

During the process of preparing silica nanoparticles, rice husk ash (also known as RHA) was utilised as a source of silica. The rice husk comes from the Rice research station at Kheda, which is located in Gujarat. The powdered version of the medication losartan potassium was provided by the pharmaceutical business. SDFCL was the vendor from whom we purchased HCl acid of the AR grade (35-38%). Deionized water was utilised all the way through the experiment.

2.2.1: Producing Silica Nanoparticles (SNPs)

Mixing 5.0 gm of rice husk ash with 30 mL of 1N hydrochloric acid and stirring at 100 °C for two hours yields SNPs. After that, we let the combination sit for a full day. RHA was dried in an oven set to 110 °C after being acid treated, filtered, rinsed with distilled water, and finally dried. Calcination in a muffle furnace of acid treated RHA yielded Nano silica [21, 22]. Temperature and calcination duration are two independent factors to think about while working with calcined silica nanoparticles. We started with 4h calcination at 900°C, and then continued with 8 h calcination at the same temperature. Which are shown in Fig. 1 below, respectively, and which we have subsequently described using a wide range of analytical techniques.

(a) SNPs-4-900



(b) SNPs-8-900



Fig.1: Synthesis of samples: (a) SNPs-4-900 (b) SNPs-8-900

2.2.2: Analytical procedure

The sorbent, which is a biological material (biomass), is able to aggregate and adsorb contaminating chemicals onto the functional groups that are located on its surface in accordance with well-known forces such as soft-soft and/or hard-hard forces via electrical charge (following the required pH regulation) or covalent bond. It is essential to take into consideration the fact that the adsorption technique contains a sub category known as bio-

sorption. The root SNPs from RHA was used in this technique as a bio sorbent to remove seven heavy and dangerous elements from the losartan potassium medicine [23, 24]. These elements include Hg, Pb, Cd, Co, Ni, and V. After that, the sample was prepared to withstand the high pressures and temperatures that would be encountered in the microwave digester (PerkinElmer, Titan MPS). ICP-MS (produced by PerkinElmer and referred to as the NexION 2000) was applied in order to ascertain the metal concentration inside the clear filtrate. By calculating the change in metal concentration from the solution's beginning to its end, we were able to calculate the amount of metal taken up by the plants.

2.2.3: Characterization

The thermal stability of a material, as well as the proportion of volatile components, were both determined with the use of TGA (EXSTAR TG/DTA 6300 TG). The covalent bonding information was characterised using FTIR (PerkinElmer Spectrum Two), and functional groups were defined using this technique. We opted for SEM (ZEISS-evo 18) analysis of the surface to determine its morphology. We performed an EDX analysis on a sample to determine its elemental make-up. We have collected data on the size range of nanoparticles with the use of a particle size analyser that was manufactured by Malvern and is known as the Master Sizer 2000. We examined the creation of layers as well as the components that make them up by using TEM analysis (JEOL, JEM 2100). We were able to determine the surface areas of nanoparticles as well as the pore size distributions of these particles by the use of BET analysis (Micromeritics, ASAP 2010). We investigated a three-dimensional topographic technique with a high atomic resolution using AFM (VEECO, United States of America). This enables us to quantify the roughness of a surface. We employed XRD (Sigaku Smart Lab) to look into the crystal structure of a substance).

2.3: NANO-REMEDICATION: RICE HUSK NANOPARTICLES FOR HEAVY METAL REMOVAL IN POTATOES

Rice Husk (RH) of the "GNR-3 (Gujarat Navsari Rice – 3)" rice variety was collected from the Navsari Agricultural University, which is located in Navsari. The term "Kufri Badshah" refers to the region in northern Gujarat where potatoes are grown. AR grade HCl from SDFCL, with a concentration of 35-38%. Deionized water was utilised all the way through the experiment.

2.3.1: Extraction of SNPs

The first thing that needed to be done in order to get the RH ready for use was to give it a comprehensive cleaning to get rid of any unwelcome particles such as dirt, dust, or sand. A spotless starting material was obtained through this cleaning operation, which allowed for more processing to take place. After that, the rice husk was dried in an oven at 100 °C for 24 hours, which was enough time to completely remove any remaining moisture. A synthesis method with 1 N HCl was applied to the RH so that the structure and composition of the husk could be modified. The material was subjected to a high temperature of 80 °C for a period of 2 h as part of this synthesis. This helped to facilitate the required chemical changes inside the substance. Following the completion of the synthesis process, the RH that had been treated with acid was painstakingly washed numerous times with distilled water. This was done to ensure that any traces of acid or other pollutants were removed. After going through a meticulous washing procedure, the RH that had been treated with acid was dried for a second time, this time at a temperature of 90 °C over the course of one night. The material was further prepared for the process of calcination at this stage that involved drying it. Before moving on to the essential phase of calcination, the dried rice husk was ground up into a powdery consistency. During the calcination process, a homogenous and uniform combustion was achieved thanks to the powdering process. The calcination was carried out in a box furnace that was carefully regulated and programmed, namely the Lindberg/Blue type, at a temperature that was raised to 650 °C for a period of time that lasted for 4 h. This final stage of calcination assisted in moulding the RH material into the proper shape, so setting the groundwork for its prospective uses in a variety of different industries (Fig. 2).



Fig. 2: Synthesis of the different stages of mesoporous SNPs

2.3.2: Analytical procedure

The root silica nanoparticle, which was generated from rice husk ash (RHA), played a significant function as a bio-sorbent in this novel and environmentally friendly strategy. It offered a sustainable option to remove toxic heavy and harmful materials from potatoes that were supplied from the north Gujarat area. Elements including Co, Ni, Pb and Cd were especially singled out because of the proven harm they do to humans and ecosystems. The material was subjected to intensive treatment in a microwave digester to kick off the purifying process. More specifically, the model used was the PerkinElmer Titan MPS, and it was run at high pressure and temperature. This process guaranteed that the desired metals could be removed and separated from the potato samples in an effective manner ICP-MS was utilised to analyse the clear filtrate that was collected during the extraction process, which is a relatively recent technological advancement. This was done so that the metal content could be accurately quantified. For the aim of accomplishing this goal, a PerkinElmer NexION 2000 instrument was utilised because of its remarkable sensitivity and precision in determining the amounts of trace metals. The quantity of metal that was absorbed by the plant biomass was assessed by comparing the concentrations of the metal in the aqueous solution at the beginning of the procedure to the concentrations at the conclusion of the process. This critical evaluation cleared the way for environmentally responsible and sustainable agricultural practises in the north Gujarat area by helping us understand the efficiency of the root silica nanoparticle bio-sorbent in the removal of heavy metals from the potatoes [25].

2.3.3: TGA

In order to get an understanding of the thermal characteristics of rice husk, sophisticated analytic methods were utilised. In order to accomplish this goal, an EXSTAR TG/DTA 6300 TG, which is capable of doing accurate thermal analysis, was utilised. The experiment required carefully placing samples weighing 1.0 mg each into a platinum crucible placed within the thermo balance. The samples were then subjected to a controlled heating procedure, commencing at RT and progressing up to 700 °C, at a pace of 10 °C / minute. To guarantee that the conditions for the analysis were constant, the experiments were carried out in an air environment with a continuous gas flow of 60 mL per minute (mL/min). The TGA Q500 thermo balance by TA Instruments was used to perform the thermo gravimetric analysis. This was done in order to process the thermal data that was generated. In depth

understanding of the thermal behaviour of the rice husk samples was achieved through additional analysis and interpretation of the data using the programme Universal Analysis 2000 TA. The maximum rate of the reaction might be estimated, in example, by using the peak temperature found in the DTG (Derivative Thermogravimetric) curve. This helped shed light on essential thermal properties as well as transformation processes that took place within the rice husk. This in depth examination adds to a greater comprehension of the material's behaviour and helps in the investigation of its possible uses in a variety of business sectors and research fields.

2.3.4: Spectroscopic analysis

An FT IR analysis was performed to verify the SNP's existence and identify its chemical make-up. The SNP's FT IR spectra were meticulously examined using the highly dependable PerkinElmer Spectrum Two instrument. To prepare the samples for analysis, the dry SNPs was cautiously mixed with potassium bromide (KBr). This amalgamation was then placed in a specialised sample container, which ensured that the FTIR measurement would take place under the best possible conditions. Because the FTIR study spanned such a large range, which ranged from 400 to 4000 cm^{-1} , we were able to get a complete spectrum of the vibrations and interactions that take place within the SNPs. We were able to further strengthen our understanding of the SNP's one of a kind features and possible applications through the use of this advanced technology, which allowed us to acquire vital insights into the molecular composition and structure of the SNPs.

2.3.5: Surface structure and morphology

The SNP's surface structure and chemical composition were painstakingly analysed using state of the art microscopy techniques. TEM and FESEM with EDX capabilities were chosen for this analysis. The FESEM examination was carried out with a magnification of 15.0 K X, 20.00 K X, and 30.0 K X, an electron high tension of 15 kV, and a zoom of 1 m and 2 m; the working distance varies across images. Before the photographs were taken, the specimens went through a procedure that coated them with gold particles for the purpose of improving image visibility. To better understand the formation and composition of the SNPs sample, we performed a TEM analysis using a JEOL JEM 2100 instrument at an accelerating voltage of 200 kV. A few mg of the substance were dispersed throughout a solvent consisting of ethanol and distilled water at a ratio of 2:3. Before collecting photographs using a TEM, the copper

grid was dried at room temperature after being briefly submerged in the solution that had been used previously.

2.3.6: Particle size analysis and surface charge

The particle size distribution was studied using a light scattering instrument (HORIBA SZ-100) with a resolution of 20 nm to a resolution of 80 m. This opened the door for the study of the SNP's size distribution and surface charge. The experiment was conducted with the back scattering angle set at 1700 and the temperature set at 25 °C. Both the refractive index and the viscosity of water were measured to be 1.3303 and 0.8903 mPa, respectively.

2.3.7: Surface area and pore structure analysis

We relied on cutting edge analytical methods in order to get an accurate reading on the characteristics of the material. An automated analyzer known as the Micromeritics ASAP 2010 was used, which is recognised for its precision and effectiveness. It was helpful in determining both the specific surface area and the pore size distribution. To better comprehend the material's characteristics, we used the Brunauer, Emmett, and Teller (BET) method. By using this technology, we are able to obtain insightful knowledge on the surface area and pore structure of the material. We used indirect molecular adsorption techniques, especially nonlocal density functional theory (NLDFT) and N₂ isotherms, in order to investigate the microscopic pore size distribution on a molecular level. These high level techniques, which were done at a temperature of -196.5 °C, offered a full information on the complicated pore network that the material had and what it meant for the material's general characteristics. By doing this in-depth investigation, we were able to get vital data that contributed to our advancement of our understanding of the possible uses and performance of the material in a variety of sectors.

2.3.8: Surface topography analysis

Atomic force microscopy (AFM), which provides an exhaustive three-dimensional analysis of topography, was utilised so that the surface morphology of the materials could be investigated in fine detail. For the purpose of this study, a scanning probe microscope manufactured in the United States by VEECO was used. During the course of the experiment, scanning pictures of the materials were painstakingly obtained using the AFM in contact air-mode, with the scanning probe fitted with nitrite tips. Using this method required a great deal

of attention to detail. Imaging of the material surfaces may be carried out with great precision and without causing any damage thanks to this set-up. During the process of scanning, the AFM instrument was optimised to function at its highest possible level by being set at a frequency of 300 kHz when it was in operation. As a direct consequence of this, topographical images were taken, which included essential aspects of the surface like height, width, and cross-section. This in-depth AFM research revealed important insights into the fine details of the surface structures of the materials, which assisted in gaining a better understanding of their physical characteristics as well as the possible uses for those characteristics.

2.3.9: Crystallization structure result

To conduct the X-ray diffraction (XRD) analysis, we used Rigaku's (Japanese) Smart Lab system. This was done in order to explore the crystallographic characteristics of SNPs (Root SNPs). In order to light the sample, the XRD experiment used a radiation with 40 kV of potential and 30 mA of current. During the course of the investigation, a thorough two-theta scanning range that extended from 5 to 1000 was utilised, and the scanning speed was established at 8.2551° per minute. This methodical methodology enabled us to collect accurate and comprehensive XRD spectra, which in turn provided us with invaluable insights into the crystalline structure of the SNPs as well as its chemical make-up. The information gleaned from this study is crucial, as it helps shed light on the unique properties of the RH-SNP and its potential applications in various scientific and technical fields.

2.4: SUSTAINABLE COFFEE REMEDIATION WITH RICE HUSK NANOPARTICLES

The Navsari Agricultural University in Navsari was the origin of rice husk (RH) that was generated from the 'GNR-3 (Gujarat Navsari Rice – 3)' rice variety. The "Robusta" kind of coffee was sourced from the province of Kerala in India. SDFCL was responsible for the delivery of HCl of AR grade and with a concentration of 35-38%. Throughout the entirety of the experiment, deionized water served as the medium of choice.

2.4.1: Extraction of SNPs

The initial step of rice husk preparation was a cleaning procedure to eliminate any undesired contaminants such as dirt, dust, and sand. This was the beginning of the rice husk preparation process. After that, the rice husk that had been cleansed was subjected to a drying process that was meticulously managed and took place in an oven that was precisely heated to 100 °C for a period of 24 h [16]. During this process, any traces of moisture that may have been present were efficiently extracted, which allowed the husk to become prepared for the future transformation. In order to bring about the desired alterations in its structure and content, the RH was subjected to a synthesis process that was carried out with the aid of 1 N HCl. During the course of this synthesis, the component was heated to 80 °C in order to facilitate the necessary chemical transformations [17,18]. This step lasted for a total of 2 h. Following the completion of this synthesis, the RH that had been treated with acid was given a thorough washing in water that had been distilled to verify that all of the acid and any other pollutants had been properly eliminated. A thorough cleaning procedure was required in order to maintain the cloth's pristine state. After a complete washing, the rice husk that had been treated with acid went through a second drying phase; this time, it was done at a little higher temperature of 90 °C, and it was left for the night. Thorough drying was utilised to ready the material for the next, crucial phase in its transformation, the calcination process. [19]. In preparation for the crucial calcination stage, the RH that had been dried was crushed into a powder using a very fine grinding mechanism. This essential powdering process was implemented in order to achieve and sustain a consistent and homogeneous combustion throughout the calcination process, which is an essential component in the following development of the material. The final and most crucial stage was the precision controlled calcination process. This stage took place in a programmed box furnace, namely of the Lindberg/Blue type. The process of calcination required a high temperature of 650 °C and took place over the course of four hours. During this final step of calcination, the rice husk material was moulded into the appropriate shape, which set the framework for future usage of the material in a range of industries [20,21]. During this stage, the rice husk material was also calcinated. (Fig. 2).



Fig. 2: Outward manifestations of mesoporous SNPs formation at various stages

2.4.2: Analytical procedure

In this investigation, we used 400, 1000, and 2000 mg of coffee powder biomass together with 200 mg of SNPs contained inside a conical flask. The total amount of SNPs was 200 mg. These mixtures were shaken in a rotary shaker at 250 rotations per minute (rpm) while the temperature was kept at RT for a total of 6 h [22, 23]. During the course of our investigations, the biomass weights were measured to a high degree of accuracy. Our primary focus was on the removal of metals that are damaging to the environment. More specifically, we wanted to get rid of V, Co, Ni, Cd, Hg, Pb and As, which are all known for having negative impacts on human health as well as the environment [24-26]. We began the process of purification by putting the samples through severe conditions inside of a high-pressure and high-temperature microwave digester known as the PerkinElmer Titan MPS. This allowed us to get the process started. This guaranteed that the desired metals could be successfully extracted from the coffee powder samples and separated from one another. The pure filtrate that resulted from the extraction process was analysed using ICP-MS, a cutting-edge technology. We utilised the PerkinElmer NexION 2000 instrument, which is well-known for its high sensitivity and accuracy in detecting trace metal concentrations, in order to obtain exact quantification of the metal content. This was accomplished by using a flame photometer. The amount of metal taken up by the plant biomass was calculated by comparing the pre- and post-operation concentrations of metal in the aqueous solution. Because of this in-depth analysis, we were able to determine whether or not the root SNPs bio-sorbent was effective in eliminating heavy metals from potatoes. This discovery paves the door for agricultural practises in the North Gujarat area that are ecologically benign and sustainable, leading to an ecosystem that is safer and healthier [27, 29].

2.5: REFERENCES

- [1] Carr GP, Wahlich JC. A practical approach to method validation in pharmaceutical analysis. *J Pharm Biomed Anal* 1990;8:613-8.
- [2] Lecomte F, Hubert C, Demarche S, De Bleys C, Dispas A, Jost M, *et al.* Comparison of the quantitative performances and measurement uncertainty estimates obtained during method validation versus routine applications of a novel hydrophilic interaction chromatography method for the determination of cidofovir in human plasma. *J Pharm Biomed Anal* 2012;57:153-65.
- [3] Shabir GA, Lough WJ, Arain SA, Bradshaw TK. Evaluation and application of best practice in analytical method validation. *J Liquid Chromatogr Relat Technol* 2007;30:311-33.
- [4] Bruce P, Minkinen P, Riekkola ML. Practical method validation: Validation sufficient for an analysis method. *Mikrochim Acta* 1998;128:93-106.
- [5] Wilschefski SC, Baxter MR. Inductively coupled plasma mass spectrometry: Introduction to analytical aspects. *Clin Biochem Rev* 2019;40:115-33.
- [6] Shafiei, Nasrin, Mahmoud Nasrollahzadeh, and Siavash Irvani, Green synthesis of silica and silicon nanoparticles and their biomedical and catalytic applications, *Comments on Inorganic Chemistry* 41 no. 6 (2021) 317-372.
- [7] Sharma, Sanjeev K., Gaurav Sharma, Abhishek Sharma, Kirti Bhardwaj, Km Preeti, K. Singh, Anirudh Kumar et al, Synthesis of silica and carbon-based nanomaterials from rice husk ash by ambient fiery and furnace sweltering using a chemical method, *Applied Surface Science Advances* 8 (2022) 100225.
- [8] Sachan, Deepa, Aiyagari Ramesh, and Gopal Das, Green synthesis of silica nanoparticles from leaf biomass and its application to remove heavy metals from synthetic wastewater: A comparative analysis, *Environmental Nanotechnology, Monitoring & Management* 16 (2021) 100467.
- [9] Sekhar, K. Chandra, C. T. Kamala, N. S. Chary, and Y. Anjaneyulu, Removal of heavy metals using a plant biomass with reference to environmental control, *International Journal of Mineral Processing* 68 no. 1-4 (2003) 37-45.
- [16] Snehal, Sikha, and Pushpa Lohani. "Silica nanoparticles: Its green synthesis and importance in agriculture." *Journal of Pharmacognosy and Phytochemistry* 7, no. 5 (2018): 3383-3393.

-
- [17] Ali, Marwa, and AA-Ali Drea. "Green synthesis and characterization of antibiotic amorphous nano silicon oxide powder extracted from rice husk ash." *International Journal of Current Research and Review* 13, no. 24 (2021): 94-99.
- [18] Alhadhrami, A., Gehad G. Mohamed, Ahmed H. Sadek, Sameh H. Ismail, A. A. Ebnalwaled, and Abdulraheem SA Almalki. "Behavior of silica nanoparticles synthesized from rice husk ash by the sol-gel method as a photocatalytic and antibacterial agent." *Materials* 15, no. 22 (2022): 8211.
- [19] Nayak, P. P., and A. K. Datta. "Synthesis of SiO₂-nanoparticles from rice husk ash and its comparison with commercial amorphous silica through material characterization." *Silicon* 13, no. 4 (2021): 1209-1214.
- [20] Zhang, Zhiliang, Wenxiu He, Jianzhong Zheng, Guangquan Wang, and Jianbing Ji. "Rice husk ash-derived silica nanofluids: synthesis and stability study." *Nanoscale research letters* 11 (2016): 1-8.
- [21] Yadav, Virendra Kumar, and M. H. Fulekar. "Green synthesis and characterization of amorphous silica nanoparticles from fly ash." *Materials Today: Proceedings* 18 (2019): 4351-4359.
- [22] Sachan, Deepa, Aiyagari Ramesh, and Gopal Das. "Green synthesis of silica nanoparticles from leaf biomass and its application to remove heavy metals from synthetic wastewater: A comparative analysis." *Environmental Nanotechnology, Monitoring & Management* 16 (2021): 100467.
- [23] Rajendran, Saravanan, A. K. Priya, P. Senthil Kumar, Tuan KA Hoang, Karthikeyan Sekar, Kar Yeen Chong, Kuan Shiong Khoo, Hui Suan Ng, and Pau Loke Show. "A critical and recent developments on adsorption technique for removal of heavy metals from wastewater-A review." *Chemosphere* 303 (2022): 135146.
- [24] Naseem, K., Q. Imran, M. Z. Ur Rehman, M. H. Tahir, and J. Najeeb. "Adsorptive removal of heavy metals and dyes from wastewater using *Azadirachta indica* biomass." *International Journal of Environmental Science and Technology* 20, no. 5 (2023): 5799-5822.

[25] Sekhar, K. Chandra, C. T. Kamala, N. S. Chary, and Y. Anjaneyulu. "Removal of heavy metals using a plant biomass with reference to environmental control." *International Journal of Mineral Processing* 68, no. 1-4 (2003): 37-45.

[26] Usgodaarachchi, Leshan, Charitha Thambiliyagodage, Ramanee Wijesekera, and Martin G. Bakker. "Synthesis of mesoporous silica nanoparticles derived from rice husk and surface-controlled amine functionalization for efficient adsorption of methylene blue from aqueous solution." *Current Research in Green and Sustainable Chemistry* 4 (2021): 100116.

[27] Nguyen, Tan Tai. "Effective Removal of Phosphate from Waste Water Based on Silica Nanoparticles." *Journal of Chemistry* 2022 (2022).

[28] De la Calle, Inmaculada, Mathieu Menta, Marlène Klein, and Fabienne Séby. "Screening of TiO₂ and Au nanoparticles in cosmetics and determination of elemental impurities by multiple techniques (DLS, SP-ICP-MS, ICP-MS and ICP-OES)." *Talanta* 171 (2017): 291-306.

[29] Punia, Pinki, Manish Kumar Bharti, Rakesh Dhar, Preeti Thakur, and Atul Thakur. "Recent advances in detection and removal of heavy metals from contaminated water." *ChemBioEng Reviews* 9, no. 4 (2022): 351-369.