# Development and evaluation of egg shell powder calcium: Quantitative and qualitative approach

**Anasuya Patil[1], Mangirish Deshpande[2], Pearl Dighe[2], Himansu Bhusan Samal[3], Manoj kumar[4], Sachinkumar Dnyaneshwar Gunjal[5] and Trupti Pratik Durgawale[6*]**

[1]Department of Pharmaceutics, KLE College of Pharmacy, 2nd Block, Rajajinagar, Bengaluru, Karnataka 560010

[2]PES Rajaram and Tarabai Bandekar College of Pharmacy, Farmagudi Ponda Goa, 403401

[3]Department of Pharmaceutics, School of Pharmacy and Life Sciences, Centurion University of Technology and Management, Odisha- 752050

[4]R V Institute of Pharmacy, Bijnor, Uttar Pradesh

[5]Department of Pharmaceutics, Amrutvahini College of Pharmacy, Sangamner, Maharashtra State, India. Savitribai Phule Pune University, 422605

[6]Krishna institute of pharmacy, KVV'S, Karad, India

***Corresponding author:**

**Dr Trupti Pratik Durgawale,**

**Assistant Professor,**

**Krishna institute of pharmacy,**

**KVV'S, Karad, India**

**Abstract**

Calcium is one of the major components in the functioning of human body. Various researches proved that; egg shell is the rich source of calcium which can be taken as supplements in case of calcium deficiency. Our aim of the project was to determine various qualitative and analytical estimation of egg shell (ES) powder. ES powder was prepared and characterized by micromeritic studies i.e., bulk density, tapped density, compressibility index, hauser's ratio etc. Qualitative estimation, ash value determination and analytical characterization i.e., FTIR, SEM, XRD, DSC, TGA. Results of the above-mentioned studies revealed that the ES powder lies in the range of passable to good flow property with sound quantity of calcium present in it. Particle size of ES powder is 2-5 µm, crystalline in nature with melting point of $780^0$ C. Also possess wide range of functional groups i.e., -OH group at 3700.55 $cm^{-1}$, carboxylic acid at 1435.93 $cm^{-1}$ etc. which was determined by various peaks in FTIR. So, from the results we can conclude that ES powder can be used in the formulation of calcium supplements which is beneficial for the patient suffering from any kind of calcium deficiency.

**Keywords:** Egg shell powder, micromeritic study, qualitative and quantitative estimation etc.

## Introduction

Calcium is a mineral that is most frequently linked to strong bones and teeth, but it also plays a critical role in blood clotting, assisting with muscular contraction, and regulating regular heartbeats and nerve activity. A poorer bone structure and brittle bones are additional

consequences of low calcium intake. The body's needs for calcium can be satisfied through dietary calcium consumption. These facts on calcium deficiency show that supplementation is the most effective treatment for this problem. Calcium compounds, such as calcium carbonate (40% elemental calcium), calcium citrate (21%), calcium gluconate (9%), and calcium lactate (13%), can be used as supplements. The pharmaceutical industry uses ESs in a variety of supplements to strengthen bone structures and eliminate radioactive materials [12]. Calcium carbonate makes up about 96 % of the mineralized shell. The remaining components are the organic matrix (2%), magnesium, phosphorus, and various trace elements. The ES matrix precursors, which are later absorbed into the calcifying shell, are present in the acellular uterine fluid [13]. 94 % calcium carbonate, 1% calcium phosphate, 1% magnesium carbonate, and 4% organic material make up the ES. ES calcium powder derived from egg wastes that are calcinated during the production of egg products. It has a high calcium concentration and is used as a raw ingredient in foods, medications, and cosmetics because calcium carbonate is its main element. It has been investigated whether natural calcium-based alternatives to phosphates in cooked pork products exist, including milk calcium, oyster shell calcium, ES calcium, and calcium from marine algae. Since it decreased cooking loss and enhanced the product's textural features, they discovered that products containing ES calcium were the best candidates for phosphate substitution [2]. Because ES are a significant source of calcium, researchers have been exploring for creative methods to include them into the human diet to meet their calcium needs. It has been demonstrated through clinical investigations that giving some animals calcium from ES enhanced their bone mineral density and had anthracitic effects. ES calcium has a high bioavailability and has been shown to help with brittle nails, hair, constipation, and asthma in both children and adults.

## Materials and methods

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Good quality Chicken eggs were purchased from Gere Poultry Farm in Rajajinagar, Bengaluru, Karnataka. Solvents used in the study were of analytical grade like glacial acetic acid, ammonium oxalate and ammonium hydroxide.

### Preparation of ES powder

After being sanitized in boiling water for 24 hours, ESs were allowed to dry. After that, it was ground in a ball mill with the necessary amount of distilled water. In order for the heavy ES powder to settle in the bottom and the supernatant to separate, it was let to stand for the following 24 to 42 hours. To further remove extra liquid from ES, the supernatant was removed, and the concentrate was left to dry. Hot air oven was used to partially dry the ES powder, and it was then continuously heated for two to three days after that. Granules are allowed to come down to room temperature after drying in a hot air oven (Figure 1). ES powder was achieved by using a pestle and mortar to crush the granules. These procedures will produce an ES powder that will be employed in additional tests [14].

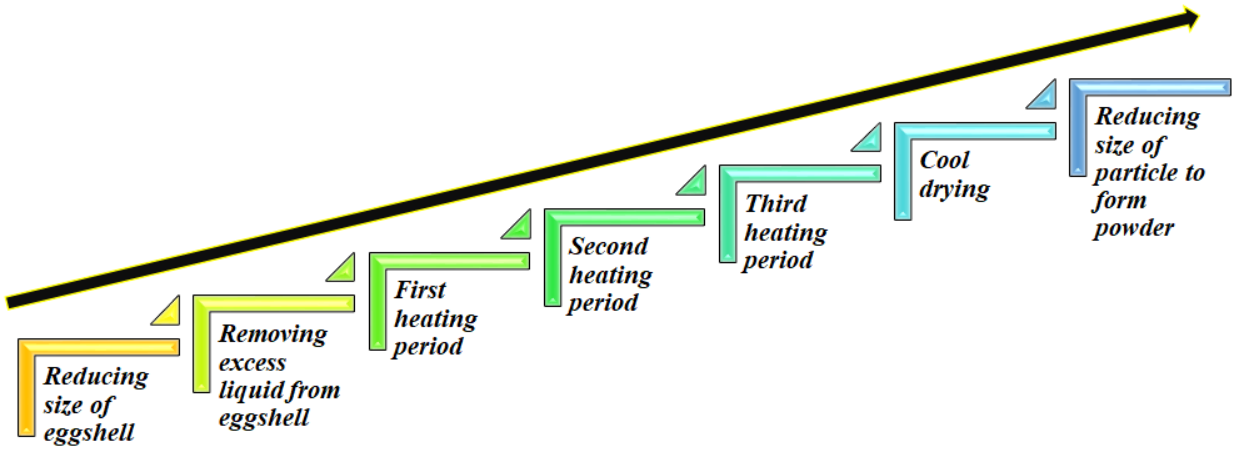



**Figure 1: Procedure for preparation of ES powder**

**Qualitative analysis of calcium in ES powder [15]**

To the solution, a small amount of $NH_4Cl$ was added. A white precipitate was produced when freshly made $(NH_4)_2CO_3$ solution was added after adding extra $NH_4OH$ to make the solution alkaline [10].

$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3$

*Confirmatory Test*

Acetic acid was added to solubilize the precipitate.

$CaCO_3 + 2CH_3COOH \rightarrow Ca(CH_3COO)_2 + H_2O$

A white precipitate of calcium oxalate was formed on adding ammonium oxalate to above solution.

$Ca(CH_3COO)_2 + (NH_4)_2C_2O_4 \rightarrow CaC_2O_4$

**Quantitative analysis of calcium in ES powder**

Quantitative analysis was done volumetrically by using a characteristic reaction of carbonate compounds with acids. Calcium carbonate is readily soluble in acid as per following equation:

$2HCl\ (aq) + CaCO_3\ (s) \rightarrow Ca^{2+} + CO_2\ (gas) + H_2O\ (l) + 2Cl^-$

Above reaction could not be used directly to titrate $CaCO_3$ because it is very slow when the reaction is closed to endpoint. Instead, the determination is achieved by adding an excess of acid to dissolve all of the $CaCO_3$ and then titrating the remaining $H_3O^+$ with NaOH solution to determine the amount of acid which has not reacted with $CaCO_3$. The difference between the amount of acid (HCl) initially added and amount left over after the reaction is equal to the amount used by $CaCO_3$. Following reaction was used to determine remaining acid:

$HCl\ (aq) + NaOH\ (aq) \rightarrow H_2O\ (l) + Na^+\ (aq) + Cl^-\ (aq)$

4.6 gm ES powder was transferred to beaker and 30-35 ml 6M HCl was added, filtered and content was transferred to 250 ml volumetric flask and volume was made up the volume with distilled water. 25 ml sample solution was then transferred to 250 ml volumetric flask and made up the volume with distilled water. 25 ml sample solution was transferred to conical flask and 25 ml distilled water, 15 ml 4.5 M NaOH solution and 10 drops color indicator were added. The solution was titrated with 0.05 M EDTA [16,17]. Percentage of $CaCO_3$ in weighed amount of ES powder was calculated using following formula:

$$\% \text{ of CaCO3} = \frac{\text{Weight of CaCO3}}{\text{Weight of ES}} \text{ x } 100$$

***Ash Value Determination***

Silica crucible was ignited and tarred. Then four grams egg shell powder was spread in an even layer and ignited by gradually increasing the heat to a temperature of 500–600°C until it was white. The material was cooled in a desiccator and weighed. The content of total ash was calculated in mg/g of air-dried material [18].

$$\% \, Ash = \frac{(Ash \; weight - crucible \; weight)}{[(crucible + sample \; weight) - crucible \; weight]} \; x \; 100$$

**Micromeritic study of ES powder [19-25]**

***Tapped Density***

The tapped density is an elevated bulk density that was produced by mechanically tapping a container holding a sample of ES powder. A graduated measuring cylinder or jar containing a sample of ES powder is mechanically tapped to get the tapped density. ES powder was added to a 50 ml measuring cylinder that was clean and dry. The cylinder was then struck 100 times from a fixed height, and the volume struck was recorded. It is expressed in gm/ml.

$$Tapped \; density = \frac{M}{Vt}$$

Where, M = mass of the ES powder

Vt = final volume of tapped ES powder

***Bulk density***

It measures the mass of ES powder to the bulk volume. The particle size distribution, shape, and cohesiveness, all affect the bulk density. Initial bulk volume was estimated by carefully pouring an accurately weighed amount of ES powder into a graduated measuring cylinder using a large funnel. It is given by the following formula, which is represented in gm/ml:

$$Bulk \; density = \frac{M}{Vo}$$

Where, M = mass of the ES powder

$V_o$ = bulk volume of the ES powder

***Hausner's Ratio***

The flowability of the ES powders was predicted using Hausner's ratio. Equation represents the Hausner's ratio.

$$Hausner's \; ratio = \frac{Tapped \; density}{Bulk \; density}$$

***Carr's Index/ Compressibility Index***

The flow behavior of the ES powder was determined using the compressibility index as a key parameter. It is inextricably linked to cohesion, relative flow property rate, and particle size. It is an efficient, quick, and widely used approach for estimating flow properties. Equation is a representation of Carr's index.

$$Carr's \; index = \frac{Tapped \; density - Bulk \; density}{Tapped \; density} \; x \; 100$$

***Angle of repose***

The maximum angle that can be formed between the surface of the ES powder pile and the horizontal plane is what is meant by this term. This approach employed a fixed funnel. The tip of the funnel was positioned at a predetermined height (h) above the graph paper-covered, flat horizontal surface. As the pinnacle of the conical pile barely touched the tip of the funnel, ES powder was gently poured through it. Using the following equation, the angle of repose was then determined:

$$Angle\ of\ repose = tan^{-1}(h/r)$$

Where, h=height of the pile

r=radius of the pile

**Charaterization of ES powder**

***FT-IR Spectrophotometric Study***

FTIR studies were performed which identified the presence of functional groups in the prepared ES powder. IR results have been registered using FTIR spectrophotometer (Model No. 234, Perkin Elmer) and image of ES powder has been registered in the 4000- 400 $cm^{-1}$ wavelength region [26].

***XRD Diffraction Pattern Studies***

When monochromatic X-rays interact constructively with a crystalline material, XRD can detect the results. When electrically charged particles are accelerated with enough energy, they decelerate and emit shorter wavelength electromagnetic radiation, or X-rays. The produced X-rays were collimated and directed at the sample. The sample interacts with the incident rays to produce a diffracted ray, which is then recognized, processed, and counted. The intensity of diffracted rays dispersed at various angles of material are plotted to produce a diffraction pattern. The XPERT-3 diffractometer equipment, based on Cu- K radiation at 45 kV and 40 mA, and a divergence fixed slit with a height of 1.52 mm, were utilized to acquire the XRD patterns. The sample was recorded with the following parameters: continuous scan type, duration per step, diffraction angle 2θ and minimum step size 0.001 [27].

**Field Emission Scanning Electron Microscopy (FESEM)**

TESCAN (Model MIRA-3 LMH) from the Indian Institute of Technology, Kanpur was used to evaluate the surface morphology of the ES powder using FESEM. Images were captured at various magnifications, including 500x, 2.00kx, 10.0kx, 20.0kx, and 30.0kx. FESEM is an electron-based imaging technique. The incoming electron beam is raster-scanned across the sample's surface to find any secondary or backscattered electrons. A cathode is known to as a field emitter-FESEM, which generates better images, when it emits electrons in response to an exceptionally strong electric field [28].

**Differential Scanning Calorimetry (DSC)**

DSC was a system that assessed temperature-dependent differences in the amount of heat required to raise the temperature of a sample. Almost constant temperature was maintained for the sample throughout the experiment. DSC was used to investigate the ES powder's thermal characteristics (Thermogravimetric Analyzers, Q600, TA Instruments, US). The samples placed on a standard alumina pan were heated at 300C/min from $10^0$C to $80^0$C with a purge flow rate of 10 ml/min for the DSC measurements, which were carried out in a nitrogen environment with a weight equivalent to 1 to 5.15 mg [29].

**Thermogravimetric Analysis (TGA)**

TGA was a method of thermal analysis that measured a sample's mass over time as the temperature changed. This measurement gave information on both physical phenomena, such as phase transitions, adsorption, desorption, and absorption, as well as chemical phenomena, such as thermal breakdown, chemisorption, and solid-gas reactions (such as reduction or oxidation). TA Instruments were used to perform TGA (Model SDTQ 600). The ramp was 100C/ml, and the flow rate was 100ml/min [30]. The sample was sealed into a standard alumina pan and heated to the set point temperature of $24.84^0$C at a purge flow rate of 99.96 ml/min for TGA measurements. The weight of the sample used was 4.89 mg.

**Results and discussion**

**Qualitative and quantitative estimation of calcium in ES powder**

Appearance of white precipitate after addition of ammonium oxalate confirmed that calcium was present in ES powder. Volume of standard EDTA solution consumed against $Ca^{2++}$ using color indicator was 10.8 ml. Mol of $Ca^{2++}$ present in sample solution of ES powder was calculated as follows:

1000 ml of standard EDTA solution contains 0.051 mol of EDTA

10.8 ml of standard EDTA solution contains (0.051x 10.8)/1000 = 5.412 x $10^{-4}$ mol of EDTA

1 EDTA = 1 mol of $Ca^{2++}$ i.e., 5.412 x $10^{-4}$ mol of $Ca^{2++}$

25 ml of sample solution contains 5.412 x $10^{-4}$ mol of $Ca^{2++}$

250 ml of sample solution contains (5.412 x $10^{-4}$ x 250)/25 = 5.412 x $10^{-3}$ mol of $Ca^{2++}$

Weight of $CaCO_3$ = mol of $CaCO_3$ x molar mass of $CaCO_3$

Weight of $CaCO_3$ = 5.412 x $10^{-3}$ x 100 = 0.5412 gram per 250 ml

It was measured that in 4.6 gram of ES powder, 11.78% of calcium was present.

**Ash Value**

A silicon crucible that had already been lit and tared received one gramme of the ES powdery substance. The substance was evenly distributed, and then it was heated up until it reached a temperature of 500–600°C and ignited, becoming white to show that there was no carbon present. As a result, a value of 0.6 w/v for Ash was discovered.

**Micromeritic characterization**

The ability of ES powder sample to pack under taps gives a measure of the ES powder cohesiveness which can be linked to its flowability. The tapped density of each ES powder was determined using Stampf Volumeter (model STAV 2003, JEF Germany). 42.74 gm of ES powder sample was poured through $45^0$ angle into a 50 ml glass measuring cylinder and the heap of the ES powder levelled off horizontally with a spatula and the bulk volume Vo was read. The volume, V500, of the ES powder column was then calculated by mechanically tapping the cylinder 500 times. Tapped density was determined using V500 to be 1.61 gm/ml. The tapped density is always greater than the apparent free flow density. The degree of ES powder packing that takes place in the container upon tapping is measured in industrial applications by tap density. Tap density depends on ES powder properties, including particle porosity, particle shape, and particle size distribution. The key element among them that alters considerably when bulk density is converted into tap density is the particle shape of ES powder. Lower bulk density often increases the possibility of tap density [31,32].

By adding 42.74 g of ES powder to a 50 ml glass measuring cylinder, the bulk volume ($V_o$) of the sample was calculated, and the bulk density was found to be 37 ml. A 1.15 g/ml bulk density was discovered. The bulk ES powder with a strong structural bonding will provide a low bulk density since it will be difficult to pour or dispense it into the hopper or bin because resistance will develop when the packing of the particles is rearranged. On the other hand, bulk ES powder has a greater bulk density because to the weak structural connections between the particles. When allowed to settle, the structurally frail ES powder easily dispersed, which was mostly noticed under tapping conditions. Reduced frictional and interparticle forces result in a particle's ability to reorganize itself in a more straightforward manner, which increases packing friction and bulk density [33,18].

Poor flowability of ES powder was indicated by a greater Hausner's ratio value, and the opposite was also true. This ratio reveals the capacity of the bulk ES powder to reorganize interparticulate spaces created by external forces like vibration or tapping. The ability of ES powder to reorganize its particles into interparticulate space is influenced by its cohesive strength, which is shown by Hausner's ratio. Despite this, there are other independent variables that might affect rearrangement, such as particle size distribution and the wall friction effect that a container applies. Hausner's ratio cannot thus be regarded as a basic characteristic. Hausner's ratio was found to be 1.4 which lies in the range of excellent flow property which shows that particles are able to rearrange themselves irrespective of the interparticle forces. A volume of ES powder was filled into a graduated glass cylinder and repeatedly tapped for a known duration. The volume of ES powder after tapping was measured and compressibility index was found to be 28.57. Therefore, from the results it was observed that the ES powder lies in the range of poor properties. When the cohesive force of the bulk ES powder is stronger than the force that is promoting movement, ES powder will have poor flowability. Cohesive forces between particles are known as "interparticle interactions" (e.g., gravity for silo discharge and compressed air pressure for pneumatic transport). The kinds and severity of interparticle interactions rely on (i) the bulk composition of the ES powder, (ii) the physical characteristics of the ES powder, and (iii) the environmental and processing conditions that the ES powder is exposed to. Actions must be done to decrease the frequency and intensity of the aforementioned interparticle interactions from happening at the bulk level in order to enhance the flowability of ES powder [34,35].

The angle at which the granular material changes between its various phases is known as the angle of repose. The steepest slope of the unconfined material, measured from the horizontal plane on which the material may be piled without collapsing, is one of the most often used definitions of the angle of repose. The average radius and height of the ES powder sample were determined to be 3.73 cm and 2.5 cm, respectively. As a result, angle of repose was determined to be $34^0$. As a result, it is discovered that the range of favourable flow properties for ES powder, or $31–35^0$, is applicable [36,37]. When needed, other glidants can be added to it to improve its qualities.

**Spectral analysis**

Typically, absorbance bands were grouped within two types: Group frequencies and fingerprint frequencies. Group frequencies are characteristic of small groups of atoms or functional groups such as $CH_2OH$ and C=O. These types of bands are typically seen above

1500 $cm^{-1}$ in the infrared spectrum and they're usually unique to a specific functional group, making them a reliable means of identifying functional groups in a molecule. Fingerprint frequencies, these are highly characteristic of the molecule as a whole; they tell what is going on within the molecule. These type of absorbances were typically seen below 1500 $cm^{-1}$ (Figure 2) in the infrared spectrum; however, some functional groups will absorb in this region as well. As a result, but the absence of a band is often more indicative than the presence of a band in this region.

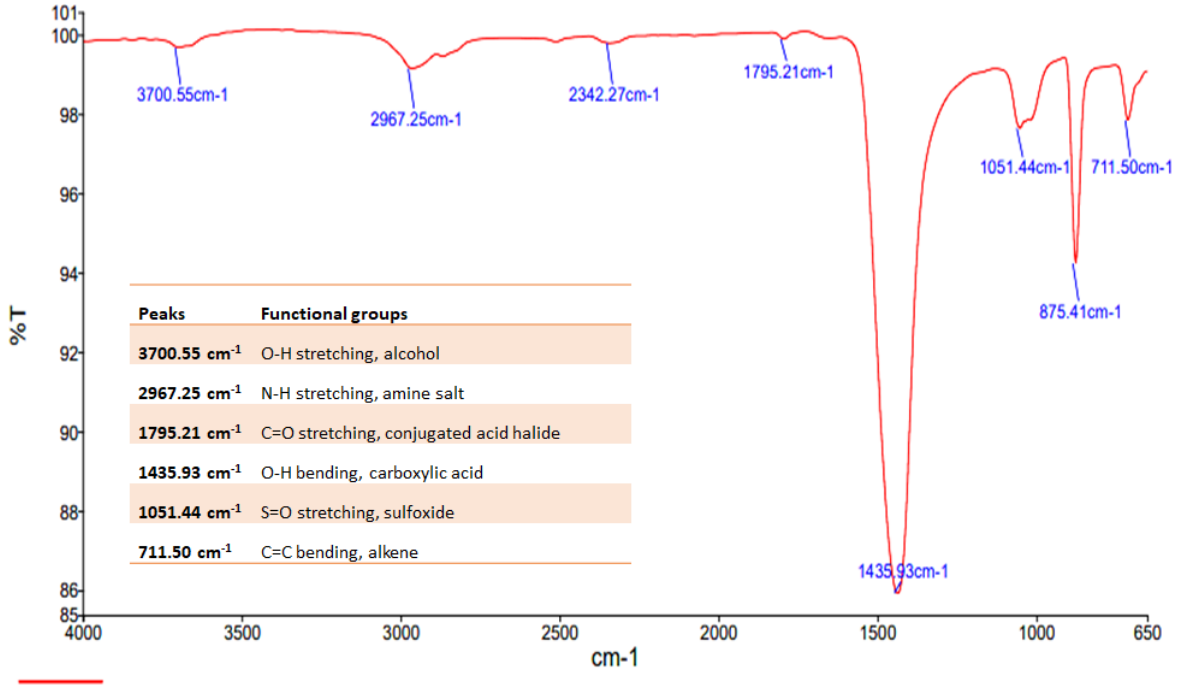



**Figure 2: FTIR spectra of ES powder**

XRD image of ES powder is shown in figure 3. The image revealed higher and lower intensity of peaks respectively which revealed that the ES powder exist in crystalline nature. SEM images of ES powder revealed that the particles exhibited smooth surface, rhomboidal shape with particle size between 2-5 $\mu$m (Figure 4).

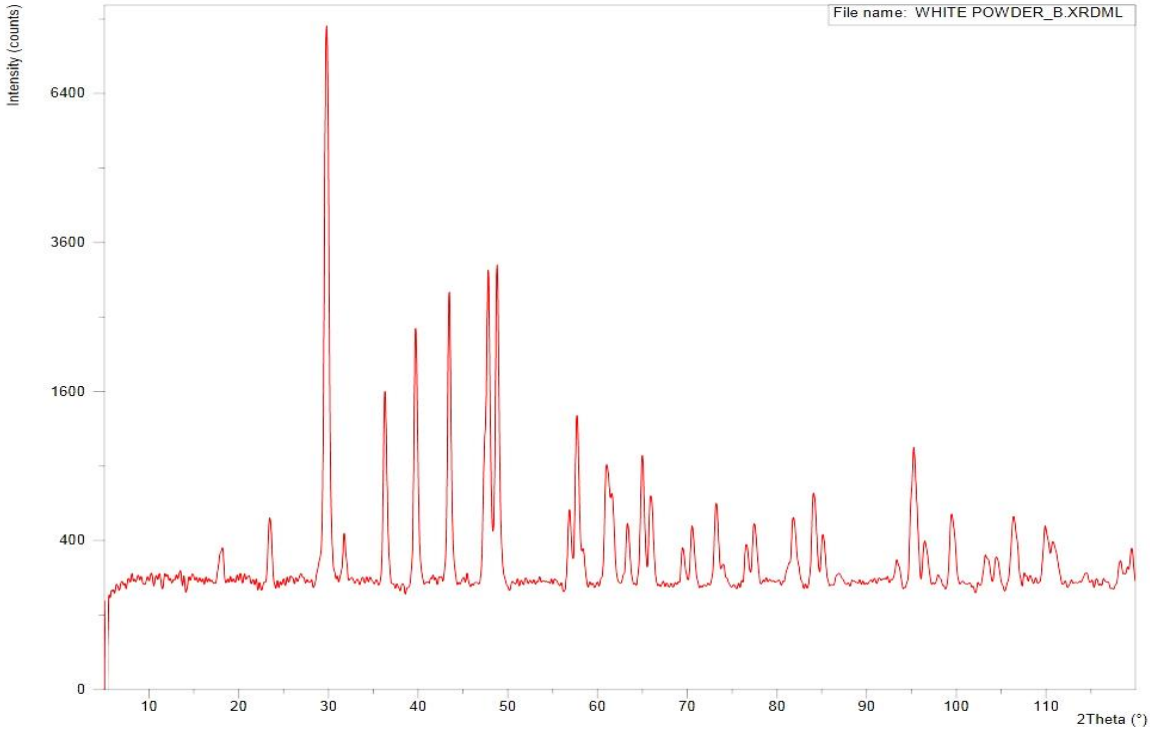



**Figure 3: Figure showing XRD of Egg Shell power**

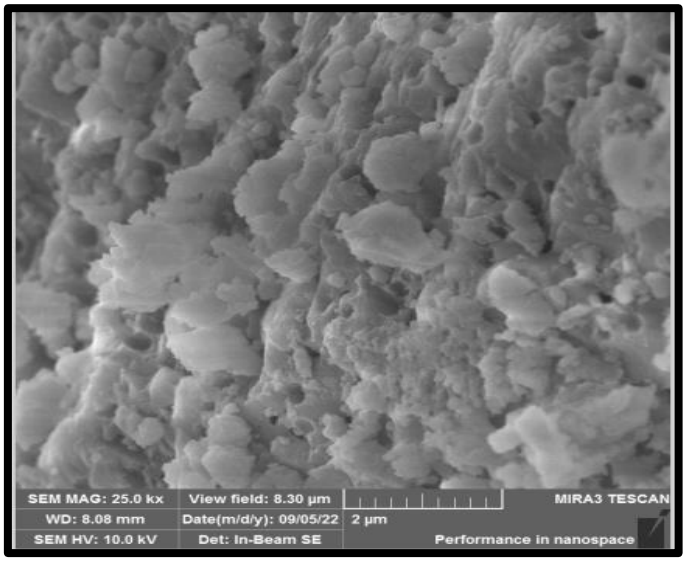
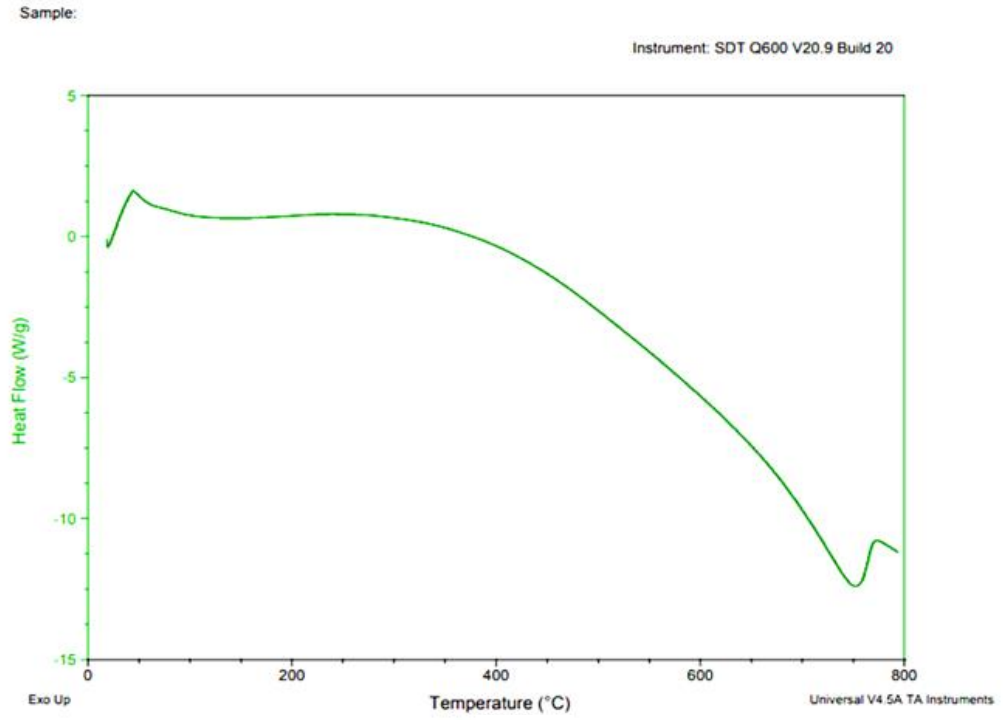
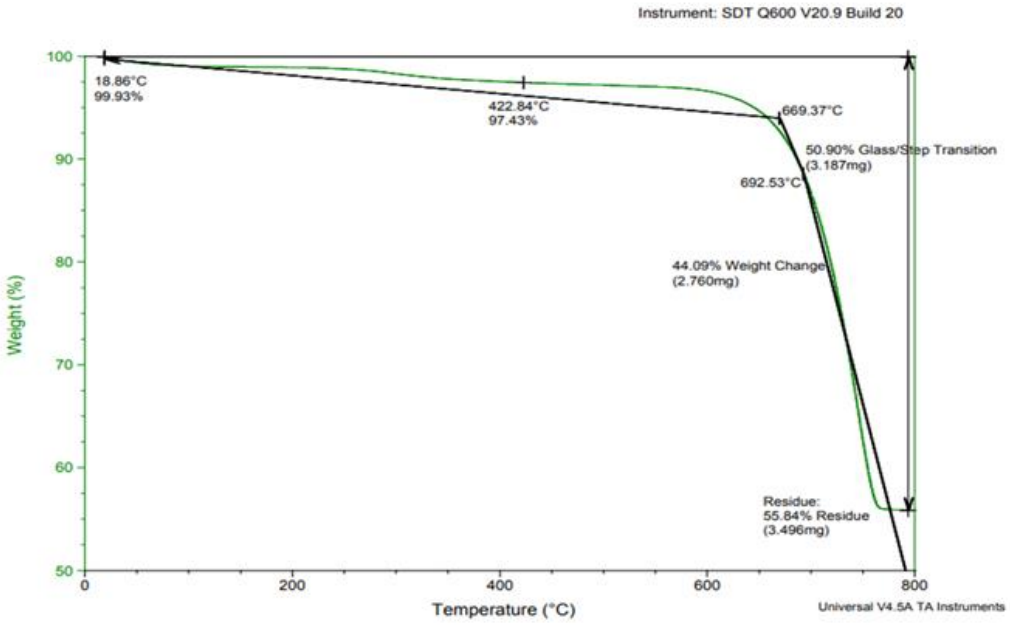



**Figure 4: Figure showing FESEM of ES powder**

A curve showing heat flux against temperature or against time is the output of a DSC experiment. According to the type of technology employed in the experiment, there are two basic conventions: exothermic reactions in the sample are displayed with a positive or negative peak. It was discovered that the melting point was 780°C (Figure 5A).




**(A)**




**(B)**

**Figure 5: Figure showing (A) DSC and (B) TGA of ES powder**

TGA analysis disclosed the weight loss and melting point at about 669.37℃ due to evaporation of water content as well as combustion. The graph showed that at 18.86℃ the sample weighed 99.93%. then with increasing in temperature the weight of sample start decreasing at about 97.43% at glass transition temperature (422.84℃). At third stage, the sudden loss of weight is observed at 692.53℃ with 50.90%. at the final stage the weight is loosed up to 55.84%. therefore, the total weight change observed was 44.09% i.e., 2.760 mg residue (Figure 5B).

## Conclusion

This study found that adding egg shell to traditional foods or as a dietary supplement could be safe, practical and acceptable method of increasing dietary calcium intake as bone deformities, reduction in bone density, osteoporosis, and various calcium deficiencies are common nowadays. Egg shell which is considered as waste can also be utilized for the wellbeing of humans as it is the rich source of calcium and as we have studied calcium plays an important role in maintaining calcium homeostasis in Alzheimer's disease, inflammation, stroke, wound healing bone reabsorption etc. it also plays an important role in reducing the adverse effect of fluoride. Other than calcium, boron, copper, iron, molybdenum, Sulphur, silicon, and zinc are microelements that are discovered in ES. On an average adults aged between 19 to 60 need 700 mg- 1000 mg of calcium a day. ES powder can be prepared easily, it is economical and easily available therefore supplements formulated from egg shell are of no or minimum side effects and approachable to patients who can't afford costly medication for calcium deficiencies. More research has been needed in this direction for effective dosage form including ES powder for the benefit of patient suffering from calcium deficiency.

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