Synthesis of hydrokxyapatite based duck egg shells using precipitation method

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Abstract. The need for implants increases with the high number of fracture sufferers resulting from accidents both on the road and workplace accidents. Bioceramics is a ceramic product or component used in the medical and dental industry, especially as an implant or organ replacement. Hydroxyapatite is one type of bioceramics that is widely used because it has osteoconductive properties (can stimulate bone growth), bioactive and biocompatible. This study aims to reduce and add value to the use of duck egg shells waste. This research method consists of three stages, namely: calcination of duck egg shells, HA synthesis with the wise drop precipitation method and Characterization using XRD and SEM to identify the phase, lattice parameters, and crystal size and determine the morphology of the sample which has been synthesized. HAp synthesis was carried out by reacting CaO from duck eggshells with phosphate source (NH_4) $2HPO_4$ using precipitation method at 1000^{o}C sintering temperature for 5 hours and calcination time for 4 hours. Based on the results of the XRD analysis showed the purity of the Hap sample. While the SEM test shows the morphology of the HAp sample.

1. Introduction

Damage to the tissue system in living things, especially humans, is a driving force in the study of the development of biomaterials for the purposes of replacing damaged tissue. The increasing need for biomaterials is a factor that triggers the search for alternative biomaterials that can replace lost tissue structures without causing adverse effects and are affordable by the community. One of the biomaterial synthesis that is being developed is hydroxyapatite bioceramics [1].

Hydroxyapatite compound is a bioceramic compound formed from the main elements of calcium and phosphorus with the formula $Ca_{10}(PO_4)_6(OH)_2$. This bone replacement technology from bioceramic hydroxyapatite is biocompatible and it will be integrated with bone so that it does not need to be removed. Within three weeks, bioceramics began to coalesce with bone. Muscle tissue begins to stick and new bone tissue grows around it. This shows that hydroxyapatite is accepted by the body. Not only fractures can be cured with this bioceramic hydroxyapatite, but also bone loss due to bone cancer [2].

In medical applications, it is known that the body's response to implantants in the form of calcium phosphate compounds is related to the Ca-P ratio and the crystallinity of its compounds. Therefore, the selection of hydroxyapatite making technology needs to consider whether the products produced from the technology are the closest to the required specifications. For implants, the desired hydroxyapatite specification is one that has a Ca-P ratio of 1.67 and has the same crystal structure as the hydroxyapatite in animal / human bones [3].

The potential development of biomaterials for bone replacement is considered necessary because of the high number of cases of bone surgery. At Dr Soetomo's hospital it was found that at least 300-400 cases of bone surgery were performed every month. The number of cases of bone surgery will increase with the increasing number of elderly people and traffic accidents. The use of bioceramics is also more advantageous than the use of bone cement from PMA plastic polymers used in bone surgery technology. By using bioceramics, surgery is only done once because the active substance hydroxyapatite is integrated with bone.

Based on Hydroxyapatite import data from BPS, from 2009 to 2012 showed a significant increase, in 2009 Apatite group import data was recorded at 5 kg per year, in 2010 it was 58.5 tons / year, in 2011 it was 80 tons per year, whereas in 2012 it experienced a significant increase of 1330 tons per year. From these data it can be concluded if the growth needs of Apatite groups representing more than 35% per year hydroxyapatite. Hydroxyapatite needs in Indonesia are met by import markets from other countries such as China. According to BPPT, the price of 1 gram of HA can reach Rp 1,000,000.00 [4]. Therefore, the synthesis process needs to be done as an alternative to produce HA with the same quality as commercial HA.

HA can be made from synthetic and natural calcium sources [5]. Sources of synthetic calcium that are generally used for the synthesis of HA are CaO [6], Ca $(NO_3)_2$, Ca $(OH)_2$, $CaCO_3$[7], and $CaCl_2$ (Windarti, Tri & Yayuk Astuti, 2006). Natural calcium sources used for the synthesis of HA generally have high levels of calcium including, cow bones [8], egg shells and free-range chicken [9], eggshell shells [10], and crab shells [11]. Making HA from natural ingredients is better than synthesis results because the material comes from nature so that it will improve bioactive and biocompatible properties. In this study, the source of calcium used in the synthesis of HA is a duck eggshell. Duck eggshell has a calcium carbonate content of 92.57%, this shows that the duck eggshell is possible as a source of calcium in the production of hydroxyapatite.

2. Method

The tools used are hammer, crussible, furnace, mortar, digital balance, erlenmeyer tube, magnetic stirrer, measuring flask, stative, aluminum foil, filter paper, funnel, burrete, furnace, petri dish, plastic sample, label paper, spatula, X-ray diffractometer (XRD) and scanning electron microscope (SEM). The materials used include duck eggshell shells, diamonium hydrogen phosphate $(NH_4)_2HPO_4$, and aquades.

2.1. Calcination of Eggshell

The process of treating eggshells includes cleaning, drying and calcining. The treatment begins with cleaning the duck's eggshell using aquades. Then dried for 24 hours in the open air.

Figure 1. The calcination process for 6 hours.

Calcination of the duck eggshell was carried out with furnace at a temperature of 700°C and 1000°C respectively for 6 hours. Provision of high temperatures in the calcination process aims to produce calcium oxide compounds and so that impurities attached to the duck eggshell disappear so that pure calcium oxide compounds are obtained. The choice of calcination temperature is 700 °C and 1000 °C because the precise calcination temperature is not yet known to produce pure calcium oxide. From the two calcination temperatures, the maximum results will be seen. These results will later be used as a basis for hydroxyapatite synthesis.The result is then crushed using a mortar until smooth.

2.2. Synthesis of HA with the Wise drop Method

HAp powder is synthesized by the precipitation wise drop method, which is the process of slowly mixing the phosphate precursor solution into a calcium hydroxide ($Ca(OH)_2$) suspension. Calcium oxide produced through the calcination process of duck shells made $Ca(OH)_2$ and $(NH_4)_2HPO_4$ suspensions with a Ca / P ratio of 1.67: 1. The manufacturing process is by taking 2.96 grams of CaO duck powder and 3.96 grams of $(NH_4)_2HPO_4$ weighed using a digital balance.

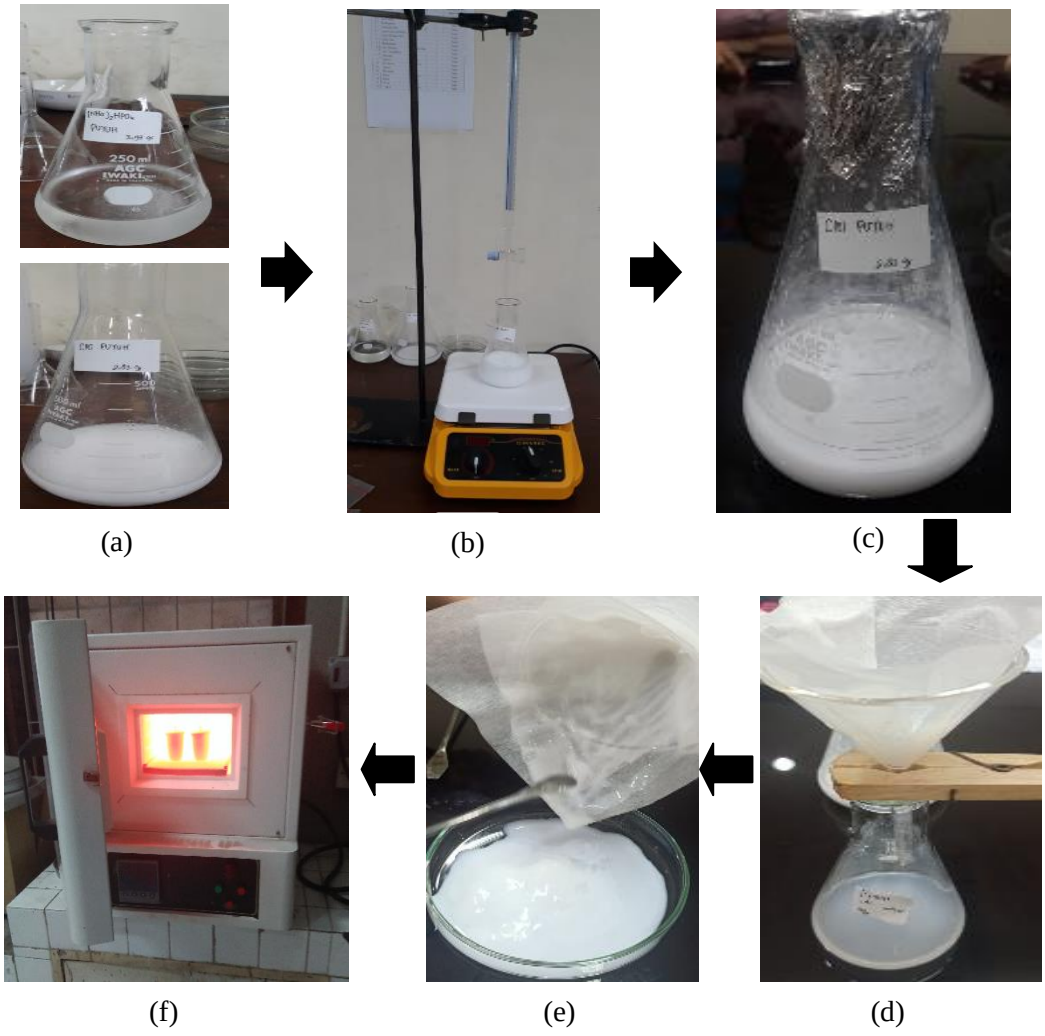

Figure 2. HA synthesis process (a) CaO and $(NH_4)_2HPO_4$ solutions (b) CaO drops $(NH_4)_2HPO_4$ (c) Precipitated one night (d) Washed and filtered (e) Oven dried (f) Sintering for 5 hours.

Calcined CaO powder was dissolved with 100 mL of distilled water solution into the erlenmeyer for the first solution. $(NH_4)_2HPO_4$ is dissolved with a 100 mL distilled water solution in a measuring flask for the second solution. The precipitation method is carried out by dripping the second solution into the first solution at a flow rate of 10 ml / min for 100 minutes using a burret and stirring at 350 rpm using a magnetic stirrer so that it is homogeneous. After the $(NH_4)_2HPO_4$ solution is completely mixed, then the mixture is closed using aluminum foil and aging for one night. The resulting precipitate is then filtered using competitive paper and washed three times using aqudes, then dried at a temperature of 110 ^oC for 3 hours. The sintering process is carried out at 900 ^oC for 5 hours.

2.3. HA characterization

2.3.1. Phase Analysis (Characterization Using XRD). The HAp powder produced in this study was placed in a metal holder and characterized by x-ray diffraction patterns in the range of 2θ. This characterization is done to identify the crystalline phase formed, determine the lattice parameters and crystal size, and determine the degree of crystallinity of the sample. The characterization results are matched with data contained in the joint committee on powder diffraction standards (JCPDS).

2.3.2. Morphological Analysis (Characterization Using SEM). The morphological analysis of the samples produced in this study was carried out using a scanning electron microscope (SEM). Scans are carried out at magnifications of 500x, 1000x, and 5000x. This analysis was conducted to determine the porosity structure and pore size formed and the percentage of elements contained in the sample.

3. Result and Discussion

3.1. Results of Calcination of Duck Eggshell

The results of calcination of duck egg shells and quail eggs using furnaces with temperature variations of 700 ^oC and 1000 ^oC respectively for 6 hours are in the form of powder as shown in table 1.

Table 1. The characteristics of the calcined powder for 6 hours of duck shell at 700 ^oC and 1000 ^oC.

Calcination Temperature	Powder Color	Powder Size
700 ^oC	Blackish gray	Rough and large powder sizes
1000 ^oC	Bright white	Smooth and small powder size

From table 1 it can be seen that the results of calcination with a temperature of 700 ^oC for 6 hours for duck eggshells have not produced CaO compounds but are still in the form of $CaCO_3$ compounds. This can be seen from the color of the resulting powder which is blackish gray, and the size of the resulting powder is still relatively large.

As for the results of calcination with a temperature of 1000 ^oC for 6 hours for a duck eggshell has produced CaO compounds, this can be seen from the color of the powder that is bright white and the size of the powder is very fine or small. The compound which was $CaCO_3$ then decays into CaO through the addition of calcination temperature to 1000 ^oC. The decay occurs due to the combustion process with very high temperatures, which causes the release of carbon. Following is the decay reaction of $CaCO_3$ compounds to CaO compounds [12].

3.2. The Result of Duck Eggshell Synthesis

The efficiency of the Hap compound synthesis from CaO and $(NH_4)_2HPO_4$ compounds can be shown in table 2. The mass of HA compound is much smaller than the total mass of compound CaO and $(NH_4)_2HPO_4$.

This is because in the process of mixing the two compounds to powder it requires heating using an oven, resulting in the evaporation process, which is a compound in the form of liquid that evaporates into gas. So the mass of HA produced is smaller than the combined mass of the two compounds of CaO and $(NH_4)_2HPO_4$. Besides the Hap compound that has been deposited before drying is washed first, during the washing process there is also some mass wasted by the distilled water in the washing process.

Table 2. The efficiency of HA samples formed from CaO as a base material.

Mass (gram)			Efficiency (%)
CaO	$(NH_4)_2HPO_4$	HAp	
2.83	3.97	4.66	68.53

Based on the equation of the reaction that occurs between the suspension of Ca $(OH)_2$ and solution $(NH_4)_2HPO_4$ produces a product in the form of compound $Ca_{10}(PO_4)_6(OH)_2$ in the solid phase and NH_4OH and H_2O in the liquid phase. Therefore, the mass efficiency of the HA compound produced by the synthesized sample is 68.53%. The reaction equation for the formation of HA compounds, is chemically illustrated in the following reaction equation:

$$10Ca(OH)_2 + 6(NH_4)_2HPO_{4(s)} + 2H_2O_{(aq)} \rightarrow Ca_{10}(PO_4)_6(OH)_{2(s)} + 12\ NH_4OH_{(aq)} + 6H_2O_{(aq)}$$

3.3. The results of the characterization using XRD

HA results obtained using the precipitation method with the concentration ratio used refers to one indicator of the formation of HAp, the Ca / P ratio of 1.6718. HA is formed from 2.83 grams of CaO compounds and $(NH_4)_2HPO_4$ of 3.97 grams to produce HAp compounds.

The mass of CaO and mass (NH4) 2HPO4 are determined based on the conditions for the formation of HA compounds. HA compound formed from the combination of calcium solution and phosphoric acid solution with a ratio of calcium solution and phosphoric acid solution is 1.67: 1. This means that the mass of CaO with a ratio of 1 and mass (NH4) of 2HPO4 with a ratio of 1.67. If a stoichiometric calculation is used in a 100 ml aquades solution, the CaO mass is 2.83 grams and the mass (NH4) 2HPO4 is 3.97 grams. So that hydroxyapatite is formed. HA was then tested using the XRD test. The XRD results can be shown in figure 3. The purpose of being tested using XRD is to see the phases formed and determine the lattice parameters and determine the degree of crystallinity.

Based on Figure 3 it can be seen that the purity of HAp produced from duck eggshells is around 87.3% while 12.7% are impurities which are hydrogen compounds. From the level of purity of HA formed we can conclude that HA from duck eggshells has sufficient purity of 87%. This is because the type of shell used has a high CaO content of around 90%. The crystal peaks produced by HA from duck eggshells with the highest intensity were found at an angle of 2θ: 10.26 oC, 25.85 oC, 31.75 oC, 32.16 oC, 34.03 oC, 46.66 oC, 49, 44 oC. Based on the XRD diffraction pattern in Figure 3, overall the peaks of the diffraction intensity of the sample are the peaks of the HAp phase. The hexagonal HA crystal structure has a lattice parameter a = b = 9,423 Å and c = 6,8801 Å. This result is the same as the commercial HA crystal structure.

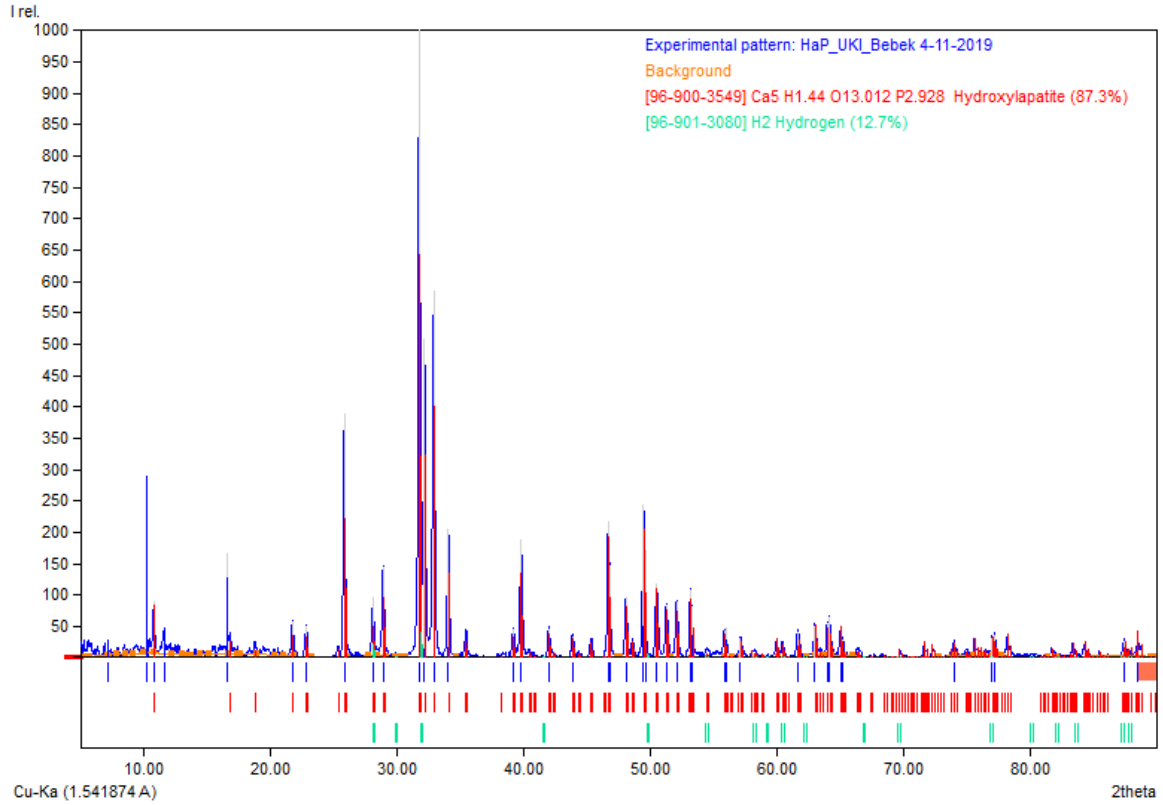

Figure 3. The XRD diffraction pattern of duck eggshells resulted from sintering at 900 °C for 5 hours.

3.4. The results of the characterization using SEM

The results of the analysis conducted using SEM showed several things such as the size of the HAp particles produced, the uniformity of the HAp particles as well as the smallest and largest sizes of the HAp produced. In samples with CaO sources, duck shells are shown in Figure 4 with a magnification of 500x, Figure 5 with a magnification of 1000x and Figure 6 with a magnification of 5000x.

These SEM results show that the morphology of the HAp formed is still in the form of coarse particles and the size between the particles is also not homogeneous meaning that the size of the particles is not uniform, some are small and there are still large ones. The smallest and large particle sizes are shown in Figure 4 with a magnification of 500x. The smallest HAp particle size that can still be found is 2,150 µm while the largest HAp particle that can be observed is 35.94 µm. In figure 6 with a magnification of 5000x, the morphological results that can be observed that show fine grains of apatite compounds that join each other only the size is still not uniform, there are still particles that have different sizes. This might be due to the uneven size of the duck shell because it uses the manual method that uses mortar and pestle.

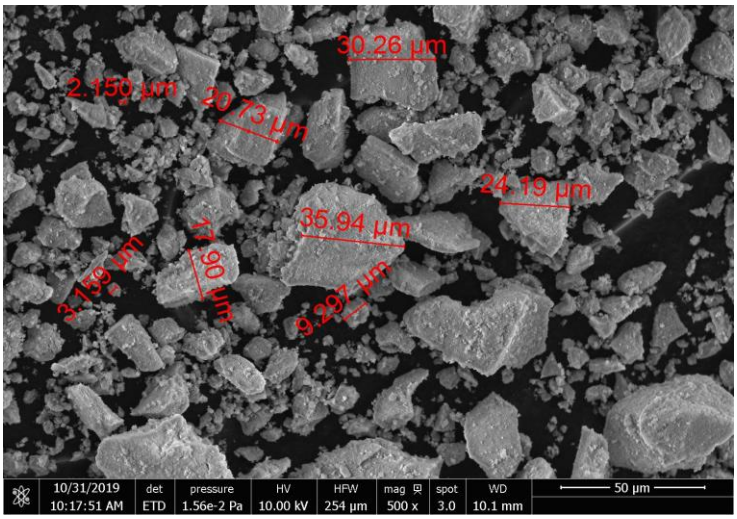

Figure 4. The results of SEM HA with a magnification of 500x.

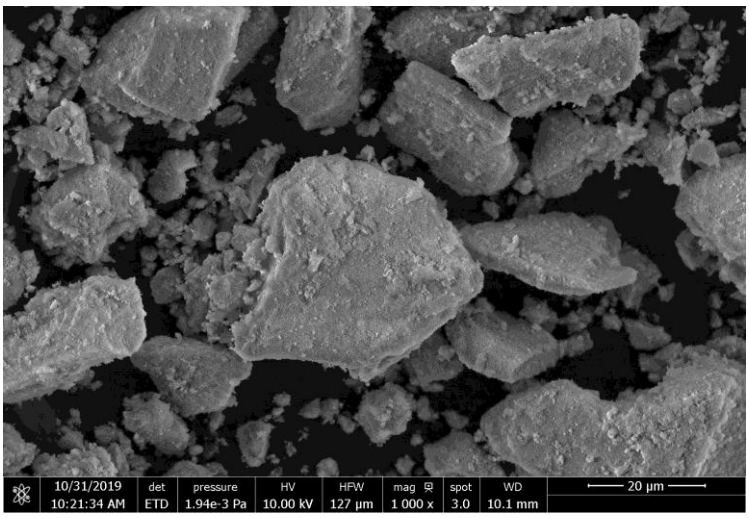

Figure 5. The results of SEM HA with a magnification of 1000x.

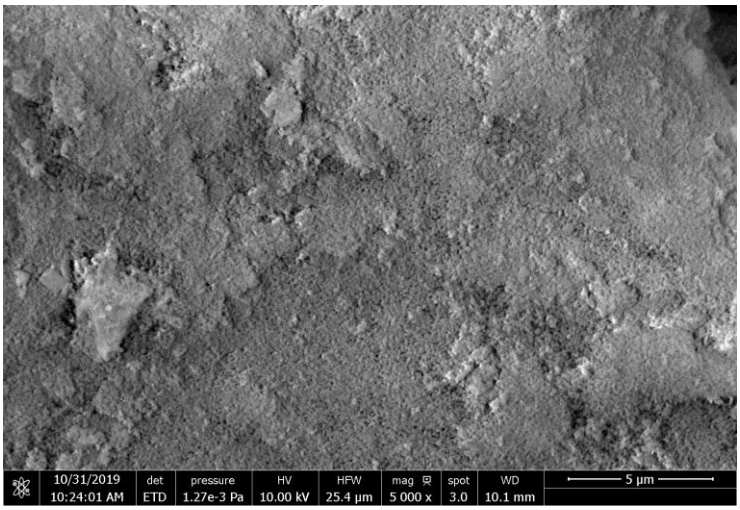

Figure 6. The results of SEM HA with a magnification of 5000x.

4. Conclusion

Duck eggshells are able to produce HAp with a calcination time of 6 hours using the precipitation method. The mass efficiency of the HAp sintering results was 68.53%. The XRD diffraction pattern shows that the synthesis HA compound has 85% similarity with HA database. Crystal peaks produced by HA from duck eggshell with the highest intensity are found at an angle of 2θ: 10.26 ^{o}C, 25.85 ^{o}C, 31.75 ^{o}C, 32.16 ^{o}C, 34.03 ^{o}C, 46.66 ^{o}C, 49.44 ^{o}C. SEM analysis test results show the morphology of the particle surface and its size. The smallest HAp particle size is 2,150 μm while the largest HA particle is 35.94 μm. Particles that are formed are still not uniformly visible, and are not homogeneous. Particle size is not homogeneous due to uneven shell grinding process, because it still uses the manual method (by hand). So that further research needs to be done by using a better and more stable scouring, so HA will be obtained with a homogeneous or uniform size. In addition, for further research it is expected that the HA particle size resulting from the synthesis is smaller than the results obtained, namely in the nanoscale, so that its application is more optimal.

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