International Journal of Enhanced Research in Science Technology & Engineering, ISSN: 2319-7463
Vol. 2 Issue 11, November-2013, pp: (71-76), Available online at: www.erpublications.com

A Novel Method for Conversion of Eggshell Hydroxyapatite Particles to Nano-size Using Microwave Irradiation

Nadira A Hatim, Zeina M Ahmad
University of Mosul, College of Dentistry, Department of Prosthodontics, IRAQ

Abstract: Hydroxyapatite (HA) is one of the most widely used biomaterials with osteoconductive properties. The properties of HA can be enhanced when the material is synthesized in the Nano scale range. The aim of this work was to synthesis HA macroparticle from egg-shell and to convert it to nanosize using microwave irradiation. Materials and methods: The raw Eggshell was cleaned , crushed and calcinated at 900^{0}C to transformation of calcium carbonate into calcium-oxide. HA formed by addition of Phosphoric acid to aqueous suspension of calcium oxide, HA precipitate was filtered, washed and sintered. HA a powder obtained was grinded using a domestic blender. The next stage is the size reduction of the HA powder to produce micro sized HA by using Micronizer. Microwave was adjusted on high power to irradiate HA for 30 and 60 minutes. Laser Diffraction Particle Size Distribution Analyzer was used to estimate particle size and surface area of HA. Results: Grinding HA with domestic blender produce 35.9737µm mean particle size. The surface area of the powder was 25108 cm^2/cm^3.With microwave irradiation for 30 minutes, the particle size drastically decrease to nano size, (0.1583 µm),. The surface area of the particle was greatly increased (3.9179E+5cm^2/cm^3). Conclusions: Grinding HA by domestic blender and Micronizer followed by microwave irradiation for 30 minutes produce HA with nano size particle.

Keyword: Eggshell, Hydroxyapatite, Micronizer, Microwave, Nanoparticles, Surface area.

Introduction

Naturally occurring hydroxyapatite (HA) is a mineral with a hexagonal structure that is composed of calcium phosphate groups with a general formula of $Ca_{10}(PO4)_6(OH)_2$) for the unit cell. Synthetic HA is similar to the natural occurring inorganic component found in the bone matrix and teeth. Because of this close similarity, its synthetic form is one of the most widely used biomaterials for reconstruction of the skeleton due to the lack of local or systemic toxicity together with its osteoconductive properties[(1,2)], it has good biocompatibility and bioactivity properties with respect to bone cells and other body tissues[(3-5)].These properties are very important because bone tissue constantly undergoes remodeling, it will support bone ingrowth and osseointegration when used in orthopaedic, dental and maxillofacial applications.

Unfortunately, due to its low mechanical strength, the use of pure HA ceramics is restricted to low load bearing clinical applications. In some cases, combining HA with other materials, such as polymers and/or glasses to form a composite, can alleviate these deficiencies [(6-8)]. Many researches in nanotechnology has highlighted the need to investigate the formation of HA in the nanometer size range, because matter at the nanometer scale can have significantly different physicochemical properties[(9-11)]. Significant improvements in the properties of HA can be seen when the material is synthesized in the Nano scale range [(12)]. Microwave radiation plays an important role in the synthesis of HA nano rods. One advantage of microwave heating is rapid volumetric heating which result in higher reaction rate, selectivity and reduction of time, as a result a microwave heating open up the possibility of fast preparation of material in a short time with low cost, energy saving and high efficiency for material production[(13,14)].

Precipitation of nano sized HA using microwave irradiation from citrate-phosphatesolutions has been reported [(15)]. This research present a novel method for conversion of HA macro particle that's synthesis from natural source (Eggshell) to nano- size using Micronizer and microwave irradiation and to characterize it by laser diffraction and SEM.

Materials & Methods

* Synthesis of Hydroxyapatite powder

Hydroxyapatite (HA) was successfully produced by using recycled eggshell in an earlier work [16], the raw eggshell was collected and their surface was mechanically cleaned to remove the internal crust lining the shell. The eggshell was then crushed and calcinated in an air atmosphere at 900^0C. The thermal treatment leads to transformation of eggshell from calcium carbonate into calcium-oxide. The chemical reaction is given by an equation:

$$CaCo_3 \longrightarrow CaO + CO_2$$

Then, by slow addition of Phosphoric acid to aqueous suspension of calcium oxide (according to molar ratio) under a constant stirring, HA formed according to the following equation:

$$10Ca(OH)_2+6H_3PO_4 \longrightarrow Ca_{10}(PO4)_6(OH)_2+18H_2O$$

The suspension was left to cool to room temperature(22 ± 2^0C) to complete the reaction process for 24 hours ,then HA precipitate (that is, in slurry form) was filtered via Buchner funnel with washing several time with distilled water then sintered in an oven at temperature of $100C^0$ for 1 hour[8].

*Conversion of HA powder to nano-sized particles

For production of HA nano particles, the following steps was carried out:

1- Grinding with domestic blender

HA powders obtained after sintering were then ground down using a domestic blender (Bruon N-999A) in order to eliminate the agglomerates that had formed during the sintering process .Grinding time was 1 minute on speed no.1 followed by 2 minutes on no.3 speed.

2- Grinding with Micronizer

The next stage in the HA powder processing is often the size reduction of the HA powder to produce micro sized HA by using Micronizer (Air Pac,India). Micronizer is an air jet mill that is widely utilized and incorporated within the pharmaceutical industries to produce offline powders, it's ideal for almost any material that requires ultra-fine grinding.Typically the jet mill will grind friable or crystalline materials down to the 1 to 10 micron average particle size range. Inside the Micronizer, precisely aligned jets create a vortex. Material is fed into this vortex along an engineered tangent circle and accelerated [17, 18].

3- Microwave irradiation

Microwave (Panasonic NN-MX 36 WF, JAPAN) was adjusted on high power to irradiate HA for 30 minute and 60 minutes. So for production of HA nanoparticle the following treatment strategies were carried out:
1- HA grinded by domestic blender (group B)
2-HA grinded by domestic blender and Micronized (group M)
3- HA grinded by domestic blender and Micronizer followed by microwave irradiation for 30 minutes (group M1)
4- HA grinded by domestic blender and Micronizer followed by microwave irradiation for 60 minutes (groupM2)
5- HA grinded by domestic blender followed by microwave irradiation for 30 minutes (group B1)
6- HA grinded by domestic blender followed by microwave irradiation for 60 minutes (group B2)

*Measurements of particles size and surface area

HORIBA's LA-300 Laser Diffraction Particle Size Distribution Analyzer was used to estimate particle size and surface area of HA. Laser diffraction technology is now the standard in most particle size analysis processing industries. In fact, the analyzer itself does not measure particle size, it measures the angle and intensity of light scattered from the particles. Light scattering has long been used to investigate the size of various objects. HORIBA's LA-300 Analyzer

measures the particle size Range from 0.1 to 600µm matching the widest range of applications with a performance equivalent to most competitors' full range systems [19].

Result and Discussions

The mean particle size of the HA powder subjected to different treatments are shown in the table (1), median, geographic mean and surface area of HA particle are also presented.

Through figure (1) to figure (6), the distributions of particle size of HA powder after treatments are shown. In figure (1), which represent the HA produced by grinding HA powder by domestic blender, the graph demonstrates a broad range of particle sizes, its appears to have a bimodal distribution. The mean particle size was found to be 35.9737µm while the median was 21.167 µm. The surface area of the particle was 25108 cm^2/cm^3.

After grinding the HA powder with Micronizer, the particle size was greatly decrease .The graph in figure (4) shows bimodal submicron and over micron size distribution. The mean particle size was 2.4357µm.The geographic mean , which is a type of mean or average, indicates the central tendency or typical value of a set of numbers by using the product of their values (as opposed to the arithmetic mean which uses their sum)[20], it was 0.8752 µm and the surface area was increased to 1.6406 E+5 cm^2/cm^3. Grinding with Micronizer produced particles less than one micron. This attributed to high-speed rotation of the rotating part of Micronizer, which subjects the material to particle-on-particle impact, creating increasingly smaller fines, while centrifugal force drives large particles toward the perimeter, fine particles move toward the center where they exit through the vortex finder [18].

The third step of treatment of the HA powder, after using domestic blender and Micronizer , was irradiation with microwave for 30 minutes ,here the particle size drastically decrease to nano size (Figure 3), the graph show unimodal distribution with mean particle size was 0.1583 µm , the median was 21.167µm. The surface area of the powder was3.9179E+5cm^2/cm^3 .When the microwave irradiation increased to 60 minutes, the particle size increased (Figure 4), this was attributed to the growth of the HA grains at longer heating time, this was in line with other researchers [21, 22], who found that the grain size increases slowly with sintering temperature of HA and at higher temperatures grain growth continues at an accelerated rate.

However, in our microwave-irradiation experiments, HA grinded with domestic blender and subjected to microwave irradiation for 30 minutes causing growth of grain size while size reduction started after irradiation for 60 minutes (Figure 5 and 6).

The PO_3^- ions of the HA particles possess strong polarizability and thus are excellent microwave-absorbing agents that can heat the reagents to the desired temperature in very short time. During microwave heating, polar molecules try to orient with electric field, the heat is generated by rotation friction and collision of molecules, causing rapid volumetric heating, and high reaction rates, leading to products with small particle sizes, narrow size distribution, and high purity. Hence, microwave irradiation is a break through over classical synthesis methods [23, 24].

Conclusions

Grinding HA by domestic blender followed by Micronizer produce micro size particle, while the nano size particle was obtained after microwave irradiation for 30 minutes. Microwave irradiation for 60 minutes lead to growth of HA particle. Size reduction of HA grinded with domestic blender was started after irradiation for 60 minutes.

Acknowledgement

A special word of thanks also goes to members of General Company for the manufacture of medicines and medical supplies in Nineveh fortheir help provided through grinding with Micronizer and the powder characterization by Laser Diffraction Particle Size Distribution Analyzer .The authors would like to thank Professor Dr. Taqa AA for his help and advice during synthesis of HA.

References

[1]. Hutmacher DW, Schantz JT, Lam CXF, Tan KC, Lim TC. State of the art and future directions of scaffold-based bone engineering from a biomaterials perspective. J Tissue EngRegen Med. 2007;1:245–260. [PubMed].

[2]. Habraken WJ, Wolke JG, Jansen JA. Ceramic composites as matrices and scaffolds for drug delivery in tissue engineering. Adv Drug Deliver Rev. 2007;59(4–5):234–248. [PubMed].
[3]. Blom A. Which scaffold for which application? CurrOrthopaed. 2007;21(4):280–287.
[4]. Habibovic P, de Groot K. Osteoinductive biomaterials-properties and relevance in bone repair. J Tissue Eng and Regen Med. 2007;1(1):25–32. [PubMed].
[5]. Kalita SJ, Bhardwaj A, Bhatt HA. Nanocrystalline calcium phosphate ceramics in biomedical engineering. Materials Science and Engineering C. 2007;27(3):441–449.
[6]. Ono I, Tateshita T, Nakajima T. Evaluation of a high density polyethylene fixing system for hydroxyapatite ceramic implants. Biomaterials. 2000;21(2):143–151. [PubMed].
[7]. Bonner M, Ward IM. Hydroyapatite/polypropylene composite: a novel bone substitute material. J Mater SciLett. 2001;20(22):2049–2051.
[8]. AL-Bahar ZJH, Hatim NA, Taqa AA .Evaluation of the effect of incorporating hydroxyapatite (dried egg shell)on some properties of relined denture base. Master thesis (2012) ,College of Dentistry-.Mosul University.
[9]. Ozine GA, Arsenault AC. Nanochemistry: A Chemical Approach to Nanomaterials. Cambridge, UK: Royal Society of Chemistry; 2005.
[10]. Guozhong C. Nanostructures and Nanomaterials: synthesis, properties and applications. London, UK: Imperial College Press; 2004.
[11]. Christenson EM, Anseth KS, van den Beucken JJ, et al. Nanobiomaterial applications in orthopaedics. J Orthop Res. 2007;25(1):11–22. [PubMed].
[12]. Vollmer M. Physics of the microwave oven. Phys Ed. 2004;39(1):74–81.
[13]. Siddharthan A, Seshadri SK, Sampath-Kumar TS. Influence of microwave power on nanosized hydroxyapatite particles. Scripta Mater. 2006;55(2):175–178.
[14]. CAO JM, FENG J, DENG SG, CHANG X, WANG J, et al: Microwave-assisted solid-state synthesis of hydroxyapatite nanorods at room temperature. J Mater Science. 2005;40: 6311–6313 LETTERS.
[15]. Arami H, Mohajerani M, Mazloumia M, Khalifehzadeh R, et al. Rapid formation of hydroxyapatite nanostrips via microwave irradiation . Journal of Alloys and Compounds. 2009, 469 : 391–394.
[16]. Gre´taGergely, FerencWe´ber, Istva´nLuka´cs, Attila L. To´th, Zsolt E. Horva´thb,JuditMiha´ly, CsabaBala´zsi. Preparation and characterization of hydroxyapatite from eggshell.Ceramics International. 2010; 36: 803–806.
[17]. http://www.indiamart.com/concastrefractories/mining-machinery.html.
[18]. http://sturtevantinc.com/micronizer.php#
[19]. (http://www.horiba.com/scientific/products/particle-haracterization/technology/laser-diffraction/)
[20]. http://en.wikipedia.org/wiki/Geometric_mean
[21]. Muralithran, S. Ramesh. The effects of sintering temperature on the properties of hydroxyapatite. Ceramics International. 2000;26: 221-230.
[22]. Nath S, Basu B, Sinha A, A Comparative Study of Conventional Sintering with Microwave Sintering of Hydroxyapatite Synthesized by Chemical Route. Trends In Biomater and Artificial Organ. 2006;19(2) 93-98.
[23]. Ying-Jie zhu. Microwave heating; A promising method for rapid preparation of inorganic nano structure, Nano technology researches .editor: Xiaohua Huang .ISNB 978-1-60021-902-3, pp:3-8 2008 Nova science puplisher, Inc.
[24]. Pushpakanth S, Srinivasan B, Sreedhar B, Sastry TP, "An in situ approach to prepare nanorods of titania-hydroxyapatite (TiO_2-HAp) nanocomposite by microwave hydrothermal technique", Mater Chem Phys. 2008;107(2–3): 492–498.

Table (1): Particle size and surface area for different HA groups

HA	Mean (µm)	Median (µm)	Geographic mean/(µm)	Surface area (cm2/cm3)
B	35.9737	21.1674	15.2247	25108
M	2.4357	0.3670	0.8752	1.6406 E+5
M1	0.1563	0.1583	0.1548	3.9179E+5
M2	2.9763	2.7743	2.2417	40981
B1	89.3069	46.2849	35.8679	18481
B2	66.5133	31.6820	28.6978	17010

B: HA grinded by domestic blender
M: HA grinded by domestic blender and Micronizer
M1: HA grinded by domestic blender and Micronizer followed by microwave irradiation for 30 minutes
M2: HA grinded by domestic blender and Micronizer followed by microwave irradiation for 60 minutes
B1: HA grinded by domestic blender followed by Microwave irradiation for 30 minutes
B2: HA grinded by domestic blender followed by Microwave irradiation for 60 minutes

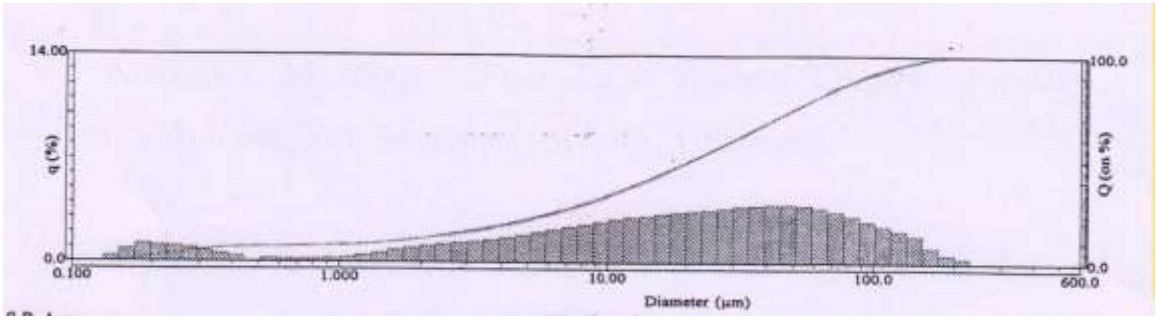

Figure (1): Particle size distribution for HA grinded by domestic blender

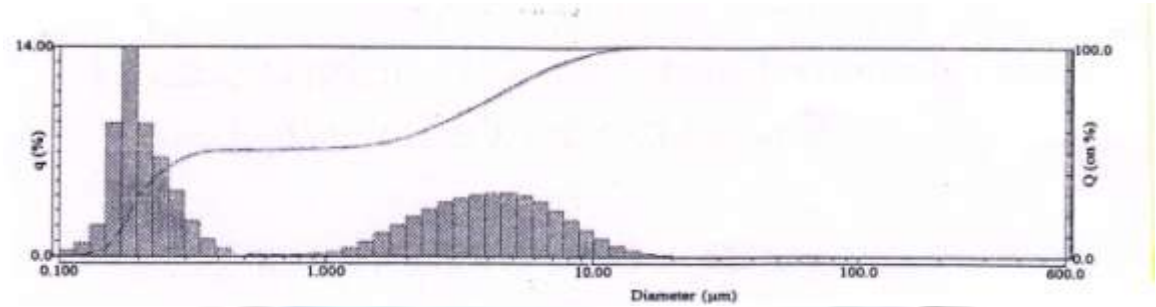

Figure (2): Particle size distribution for HA grinded by domestic blender and Micronizer.

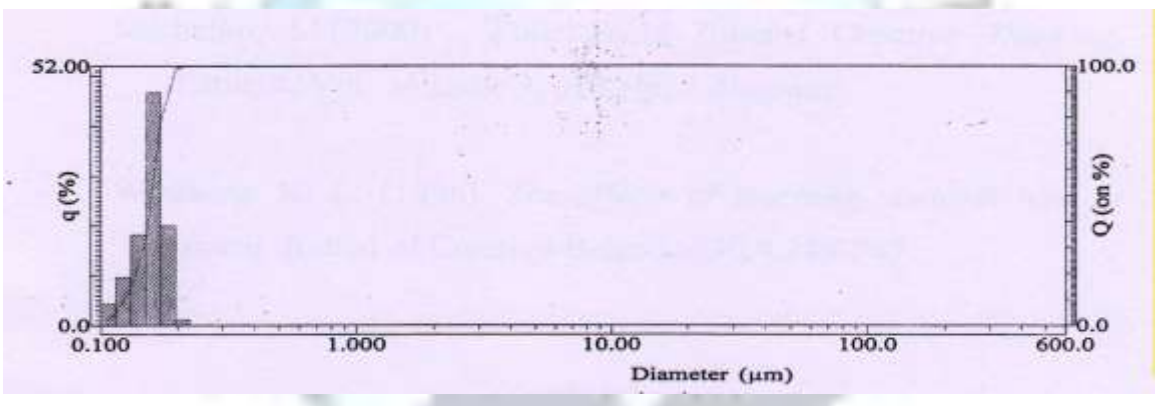

Figure (3): Particle size distribution for HA grinded by domestic blender and Micronizer followed by microwave irradiation for 30 minutes.

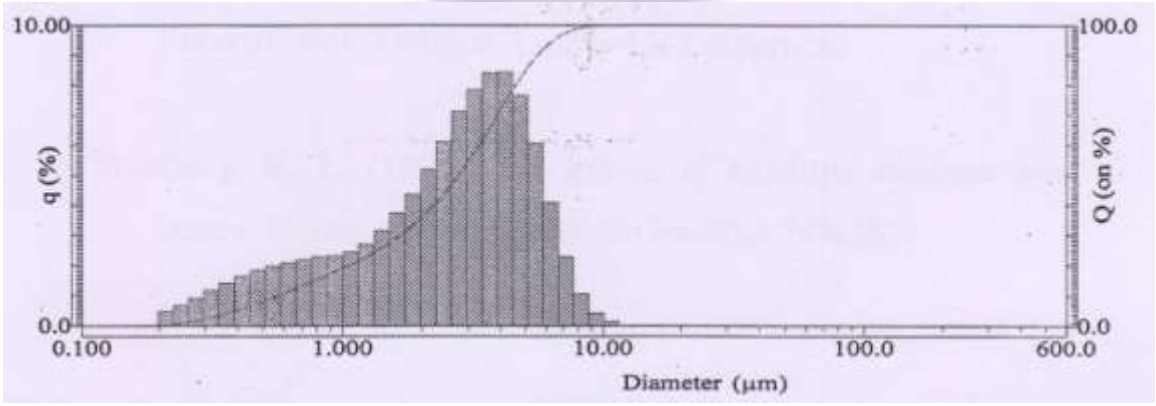

Figure (4): Particle size distribution for HA grinded by domestic blender and Micronizer followed by microwave irradiation for 60 minutes.

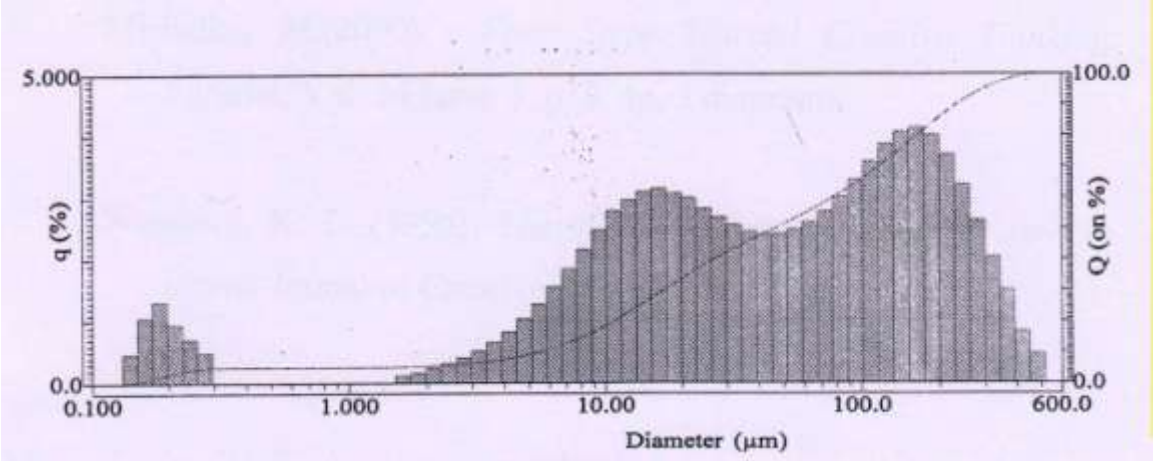

Figure (5): Particle size distribution for HA grinded by domestic blender followed by microwave irradiation for 30 minutes.

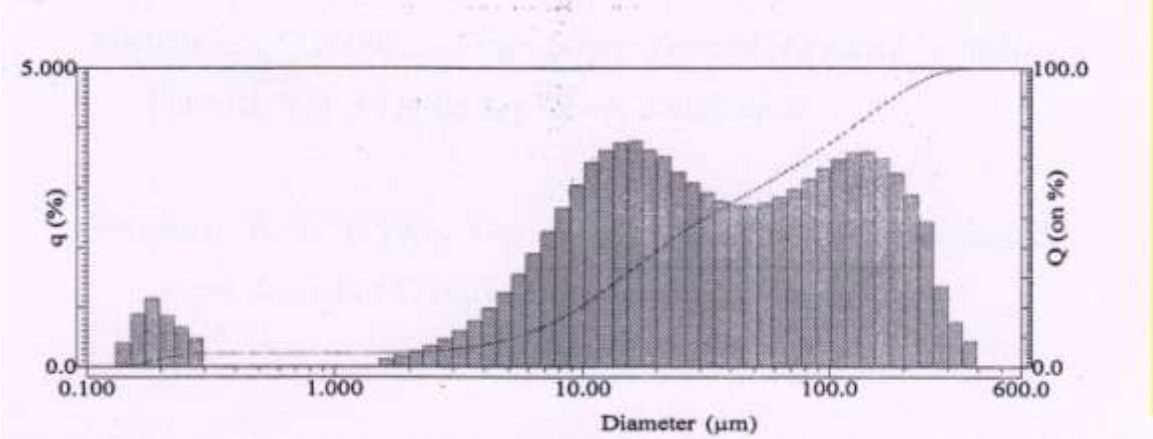

Figure (6): Particle size distribution for HA grinded by domestic blender followed by microwave irradiation for 60 minutes.