Muga Sericin mediated Synthesis of Hydroxyapatite from Egg shell

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Abstract

Hydroxyapatite (HA) is one of the most versatile materials used for implantation due to its biomimic nature to natural bone. Silk fibers are used as a template for mimicking biomineralization. Sericin and natural silk fiber has the potentiality to facilitate apatite deposition and can be useful as a polymer material in the fabrication of hybrid materials analogous to bone through biomimetic processes. Egg shell is a rich source of biological Calcium. Production of hydroxyapatite was characterized by UV spectroscopy and X ray diffraction analysis.

Key Words: Hydroxyapatite (HA), Muga sericin, Egg Shell,

Introduction:

Hydroxyapatite (HA) is one of the most versatile materials used for implantation due to its biomimic nature to natural bone. It is highly biocompatible, little usage due to low sinter ability. Natural bone minerals are nano-structured non-stoechiometric HA of dimension 20 nm in diameter and 50 nm long with substitution of ions like Magnesium, fluoride and carbonate in minor concentrations. Synthetic apatite that are to be used for repairing damaged hard tissues, are expected to have characteristics close to those of biological appetitie in both composition and structure. Nano crystaline HA has proved to be of greater biological efficacy in terms of osteoblast adhesion, proliferation, osmoregulation and formation of new bone on its surface. It has been used for 30 years as implant in different parts of body. Body has synthetic apatites that are to be used for repairing damaged hard tissue. That is expected to have characteristics close to those of apatite in composition and structure. Some researchers also claimed that macroporus hydroxyapatite ceramic with rough pore wall containing abundant macroporus has the capability of osteoinduction.

Although the HA/Collagen nano composites for artificial bones have been developed by biomimetic processing which could mimic the nanostructure of real bone to some extents because of the ability of collagen to induce mineralization. Silk fibers are used as a template for mimicking biomineralization. Sericin is a natural silk protein and has the potentiality to facilitate apatite deposition and can be useful as a polymer material in the fabrication of hybrid materials analogous to bone through biomimetic processes. Silk fibroin also was capable to induce mineralization, soluble fibroin was used to fabricate HA/SF Composites. HA/ SF composites was developed with a co-precipitation method. It was demonstrated that silk fibers could induce apatite deposition on the surface of protein in solution mimicking body fluid. Silk powder / HA nano composite material was synthesized by via wet-mechano-chemical method at room temperature. Silk Sericin tend to induce the apatite deposition on its surface because Sericin has more carboxyl groups to induce mineralization (2).

Microwave synthesis is fast, simple & efficient method to prepare nano sized inorganic material compared with conventional methods. Microwave synthesis has the advantages of rapid growth, small particle size and narrow particle size distribution due to fast homogenous nucleation. Microwaves play an important role in reactions in aquous media and have been used for preparing HA in less than 45 min. Precipitation of nano sized HA using microwave irradiation has also been reported. The thermal stability of microwave synthesized HA increases with increases in the aging time, microwave irradiation time and power. (5).

India currently ranks 4th in the world with all annual production of (17,32, Co_3) tons of eggshell. By taking 11% weight nearly it comes around 1, 90,000 tons of eggshell waste is washed. The eggshell represents

the 11% of total weight of egg and is composed by calcium carbonate (94%), calcium phosphate (1%), organic matter (4%) and magnesium carbonate (1%) (3).This material goes as a waste and leads to pollution since it favours microbiological action. (4).

An eggshell is constituted by a three layer structure namely cuticle, the spongeous layer, i.e. cuticle, the spongeous layer and the lamellar layer. Scanning electron microscope studies have demonstrated that the matrix fiber do not only surrounded by the calcite crystals but pass through the crystals.

There is possibility of producing hydroxyapatite with formula $Ca_{1o}(PO_4)_6$ $(OH)_2$, a biomaterial, which forms part of the isomorphs family of apatites. In biological System HAP represents the main inorganic component of the structure of bones and teeth.

There are Several other methods of preparing HA crystals reported in the literature including Wet-Chemical deposition, biomimetic deposition, sol gel and electro deposition HA nanoparticles synthesized at low temperature are nanocrystaline. A transition temperature (T=60°c) can be defined as a limit for the HA nanocrystal Synthesis, above this critical temperature nanocrystals become polycrystalline (1).

Materials and methods:

Muga Silk worm, Antheraea *assama* ,w/w cocoons were collected from sericulture extension farm , Central Silk Board at Cooch Behar. About 1.2 gm of this cocoon were cut into pieces and gently washed in 100 volumes (w/v) of water at 65^0c for 3 min. To eliminate the pigment and inorganic compounds, the pieces were immersed into 70% (v/v) methanol at 25^0c for 10 days. Thereafter they were boiled in the presence of o.5% Na_2CO_3 (50 volumes) at 98°C for 30 to 60 min ,then kept at 25°C for 2 days, filtered through No.1 filter paper. The filtrate was dialyzed through Membrane 60 (HIMEDIA) for 3 days. Materials retained after the dialysis was lyophilized and kept at - 20^0c. (6).

Uncrushed eggshells were collected and boiled in water for 30 min. Then it is placed in hot air oven for 60 min to remove the water and moisture content of the shells (4). Dried shells were crushed to fine powder using kitchen blender. (FIG.1)

After blending of the egg shell to fine powder, 5 gm of it was taken. Then 50 ml of double distilled water was added and then 10 ml of concentrated HCL was added. It results in forth formation. After mild heating solution was turned into clean calcium chloride solution and then filtered. In each half of the solution 16 ml of 0.025(M) Na_2HPO_4 was added to maintain Ca/P ratio at 1.67. In one set of experiment 1(M) NaOH is added drop by drop to maintain pH8. In another set along with this all reagents, 0.1% sericin was added before dropping 1(M) NaOH to adjust pH. As soon as pH was maintained at desired value, (2) white precipitate appeared in one set (FIG 2). The precipitate paste form was subjected to microwave irradiation in a domestic microwave oven (LG. made) (4).

Result:

Absorption spectra revealed that HAP and HAP with Muga Sericin both starts to absorb from 227nm instead of 230 nm i.e. there is a red shift in absorption spectra which indicate the synthesis of nano HAP particle which will be further characterized by XRD analysis (FIG 3).

X-Ray Power Diffraction (XRD) analysis of the synthesized HAP samples was done in reflection mode CuKá (ë=1.541 8A°) radiation. The data were analyzed in 2è range from 10° to 80° with a scanning step of 2° per min.FIG4andFIG5 shows the XRD patterns HAP and HAP with Muga Sericin. The main similarities between FIG 4 and FIG 5 is that there is no change in phase composition .Phase of HAP crystal and HAP with Muga Sericin crystal is 100 at 32.02°(2è) in case of HAP and 31.9°(2è) in case of HAP with Muga Sericin. But peak at 45.72°shows 221 phase of crystal .In both occasion peak at 75.54° also shows 212 phase of crystal. The main similarity between FIG 4 and FIG 5 is the phase composition of HAP crystal both in absence and presence of Muga Sericin . Peak at 31.09°(2è) in case of HAP and 32.02°(2è) in case of HAP with Muga Sericin represent a crystal with 100 phase .Where as peak at

45.72°(2è) in HAP and at 45.70°(2è) HAP with Muga Sericin represent crystal characteristic 211 phase .Peak appear at 75.54°(2è) in HAP and HAP with Muga Sericin represent a crystal with 111 phase .There is no specific band for Calcium Carbonate and Calcium Chloride.

The mean grain size of HAP powder was determined by Debye-Scherrer formula = 0.9ë **D** B Cos è, where D represents mean grain of HAP size. B strands for full width at half maximum peak, ë is the diffraction of wave length (ë=1.54A°).The analysis of results showed that average particular diameter is 31.7 nm .And the particle diameter is distributed between 17.8 nm to 51.75 nm .In presence of Muga Sericin particle average diameter 60.05nm and particle diameter is distributed from 41.62 nm to 86.62 nm which is statistically significant. ANOVA Studies also confirm that there is no significant change in size of characteristic HAP crystal having 211 phases in absence and presence of Muga Sericin (Table I).Size of particles depends on the relative velocity of nucleation and crystal growth. If nucleation reaction is faster, the crystal growth rate is slower and even close to zero, the nano HAP is obtained, otherwise the particle diameter would become larger. On the other hand aggregation of small particles may cause the forming of larger particle size. Additionally, the process of preparation is under room temperature, which avoided agglutination under high temperature.

FIGURE 1 showing the fine powder of crushed eggshell

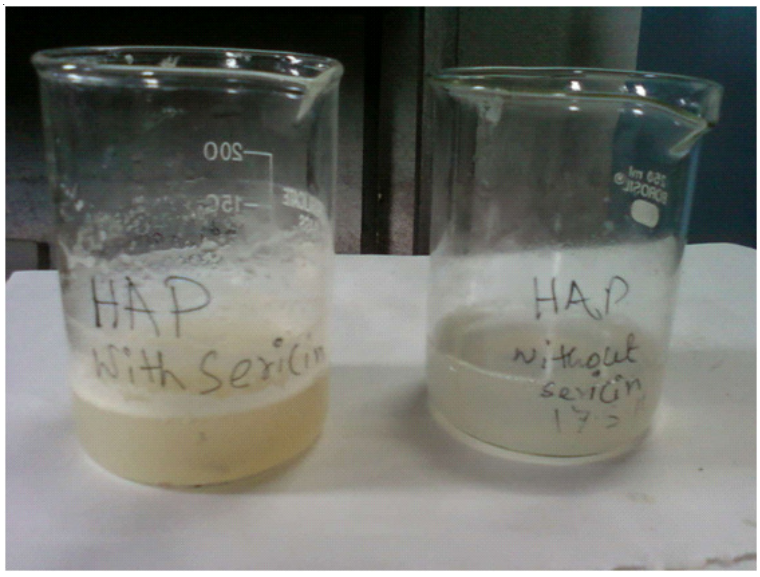

FIGURE 2 showing white precipitate of HAP & creamy precipitate of HAP with Muga Sericin

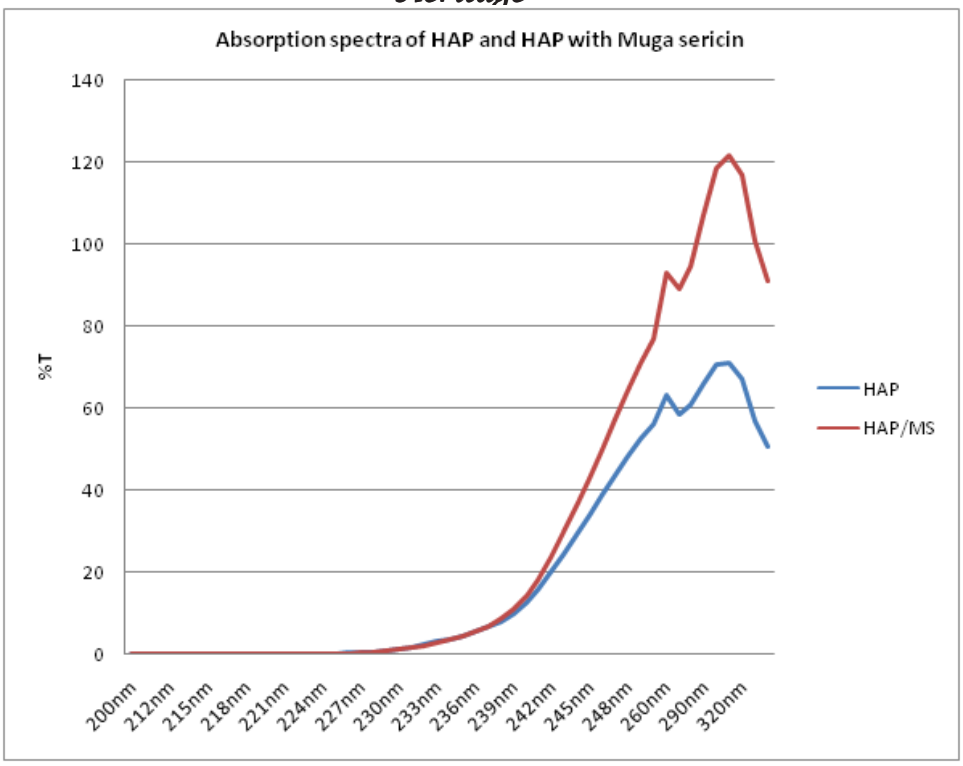

FIG. 3 showing absorption spectra of HAP and HAP with Muga Sericin.

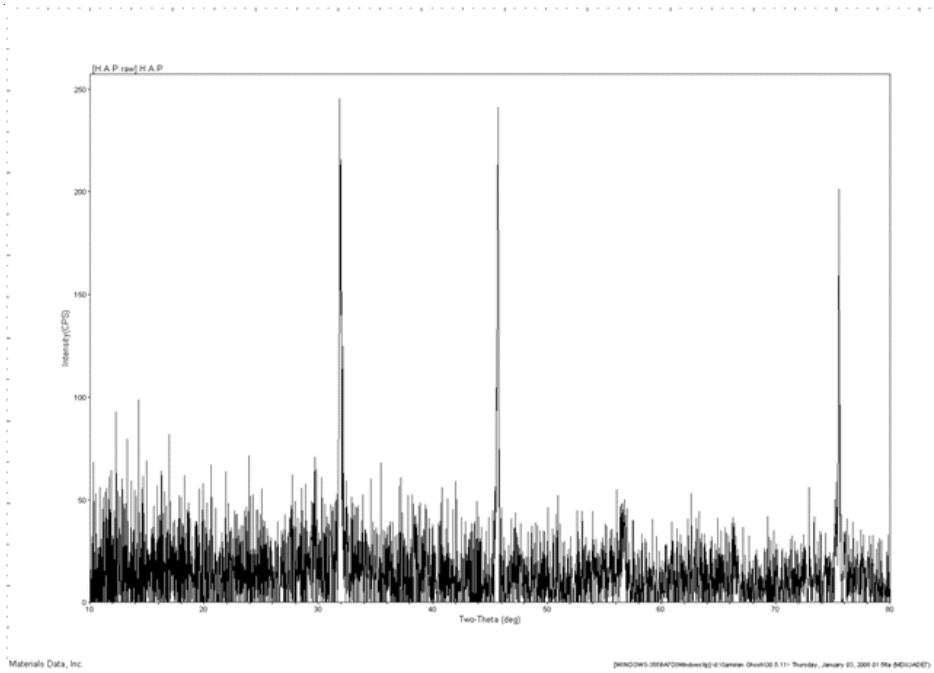

FIG 4 showing XRD spectra of HAP

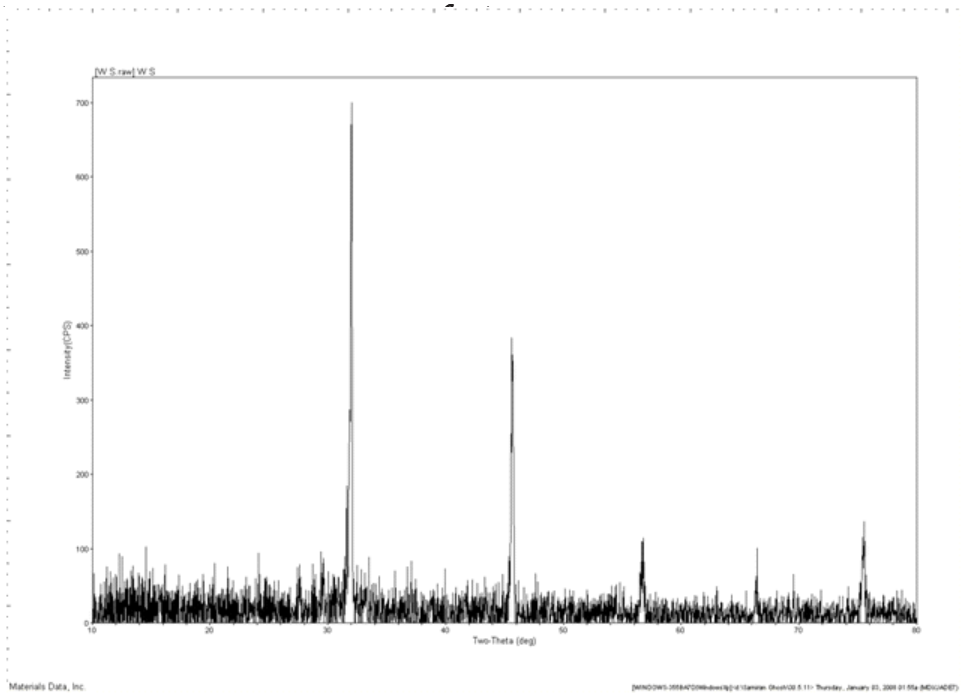

FIG. 5 showing XRD spectra of HAP with Muga Sericin

PEAK	SIZE OF CRYSTAL IN NM	
	HAP	HAP-MS
Peak 6	49.6	86.62
Peak 8	51.75	51.91
Peak 11	17.78	41.62
Average	51.75	60.05

Table1 showing size of crystals (in nm) of HAP and HAP with Muga Sericin.

Discussion:

Silk is composed of two major proteins named Fibroin (70-80% wt) and Sericin (20-30% wt.). Sericin contains much more acidic Amino acids (Asp and Glu) than Fibroin does. Sericin has the ability to induce heterogeneous nucleation of apetite in a solution that mimics physiological conditions. Our procedure of synthesis of HAP starting from egg shells in a phosphate solution at a room temperature represents a novel way for producing a useful biomedical material. Synthesized HAP both in presence of Muga Sericin

and its absence showed same phase plane but different sizes. The uniqueness of the synthesis that polymer nucleated HAP in its size which really mimic the natural HAP present in Osteoblast. (4). In the conventional precipitation process, the crystallite size increases with an increase in synthesis temperature in a regular fashion .The size, morphology and ordering of HAP precipitates have been shown significantly affected by the temperature and maturation condition. In microwave synthesis of nano HAP, the crystallite size shows an oscillating trend with an increase in microwave power. The HAP was directly obtained under the effect of microwave irradiation and not involving crystallographic transformation as in the case of precipitation process. (4).

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