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FABRICATION OF HYDROXYAPATITE NANORODS FROM LAND- SNAIL SHELL (*Helix pomatia*) ASSISTED BY MICROWAVE IRRADIATION

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Abstract

A highly inexpensive nano sized hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$ was synthesized from the mixture of cheaply available calcium precursor of land-snail shell (*Helix pomatia*) with di-ammonium hydrogen phosphate $(NH_4)_2HPO_4$ assisted by microwave irradiation. The grained powder of land–snail shell was dissolved in diluted hydrochloric acid and di-ammonium hydrogen phosphate solution was slowly added to the mixture while maintaining the pH at 9 - 10 using ammonium hydroxide (NH_4OH), followed by microwave irradiation for 30 min. The residue was collected and calcined at various temperatures from 500 - 1100 °C for 2 hrs, after the decomposition of the organic fragments and carbonate phases; the expected hydroxyapatite was afforded as nanorod of 10 – 20 nm scale with quantitative yield. Synthetically this protocol would have much precedence than other procedures since it is more facial and convenient method involving such a biological waste material of discarded land snail-shell. The, final products were characterized by X-ray diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), Field Emission Scanning Electron Microscope (FE-SEM), Field Emission Transmission Electron Microscope (FE-TEM) and Thermo gravimetric (TG-DTA) analysis. Further, the XRD pattern clearly confirms the formation of hydroxyapatite from the land-snail shell.

Key words: Land-snail shell, Hydroxyapatite, Microwave irradiation, Calcium phosphate and Bone substitute.

1. Introduction

In the recent years, considerable attention have been put forward towards the application of bio-ceramics in medicinal field is due to their potent rectification resistances, better compressive strength and comparatively lower density and weight. As a successful result of bio-physics exploration the damaged bones and tooth can be easily replaced by various bio-ceramics materials including hydroxyapatite and its analogues. In this view, hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) is one of the most important bio-material which has been extensively used for substituting the injured part of the human body, especially for bones, tooth and fractured or damaged form of various hard tissues etc. Very recently, it has been proved that the usages of calcium phosphates in biomedical field is an emerging practice which emphasis innumerable applications on biomineral phase as dentistry, bone substitute, bone repair materials and drug delivery systems (Knabe *et al*., 2000; Komath *et al*., 2000; Rabiee *et al*., 2008). Indeed, the main constituent of apatite calcium phosphate

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of bone mineral is made up of carbonate and small amount of magnesium and trace of other elements. Very interestingly, crystals studies of submicroscopic results reveal that the synthetic hydroxyapatite bears a resemblance with the structure of natural calcium phosphate core present in the bones and teeth of (Rabiee *et al*., 2013; Sinha *et al*., 2001) living vertebrate. Due to this similarity, the artificially prepared HAP found to have enhanced bio-activity and bio-compatibility to the respective naturally occurring calcium phosphate. In addition, the widespread applicability of synthesized HAP is paid to their amicable chemical interaction through hard and host tissues. Because of these type of interaction, the scientist are highly motivated to investigate the influence of HAP involving various field, for example, in medicines as implants and as coating on prostheses or as bone filling material. Though, the preparation of HAP encountered with some difficulties, there are several literatures which describes the various synthetic methods including precipitation, sol-gel, combustion synthesis and plasma etc (Brown *et al*., 1991; Young *et al*., 1982; Arita *et al*., 1995; Brendel *et al*., 1992; Bartenfelder *et al*., 1991; Roy *et al*., 1974). However, most of the conventional methods are employing through hydrothermal, micro emulsion and mechanochemical process (Bezzi *et al*., 2003; Anee Kuriakose *et al*., 2004; Hattori *et al*., 1990; Remant Bahadur *et al*., 2008; Muray *et al*., 1995; Lim *et al*., 1996 Kim *et al*., 2000; Yeong *et al*., 2001) respectively.

Subsequently, the fabrication of nanosized HAP with definite size, shape and purity is also a challenging task for the scientist since its makes a significant role in medicinal and materials sciences (Sadat-Shojai *et al*., 2010). Comparatively, the nanosized HAP is considered to be a preeminent material for bone substitutes than the microsized HAP due to their strong resorbability and prominent bioactivity (Cai *et al*., 2007; Wang *et al*., 2010; Dong *et al*., 2009; Dorozhkin *et al*., 2010). Further, the enhancement in the densification and sintering is also apparently conveying the keen expedient over the application of HAP powder (Eriksson *et al*., 2011; Bose *et al*., 2009; Bose *et al*., 2010; Bianco *et al*., 2009). At the outset, the overall advantageous behind the nano sized HAP offers a great deal of interest to search a novel synthetic protocol for accomplishing engineered nano-HAp.

The protocol associated with microwave-irradiation is one of the finest methods to synthesis nanosized HAP, which covers shorter reaction time and lesser-energy consumption and etc. However, most of the protocols designate these methods by combining both conventional and microwave irradiation technique under heating or room temperature for a period of particular time (Zyman *et al*., 2011; Parhi *et al*., 2004; Park *et al*., 2008; Yoon *et al*., 2005; Jalota *et al*., 2004; Jalota *et al*., 2006). In the past few years, there have been found many reports which deal the fabrication of nano sized HAP through microwave-irradiation using the surfactants (CTAB) and chelating (EDTA) agent to furnish the particles size of 5 – 50 nm scale (Liu *et al*., 2004; Liu *et al*., 2004; Kalita *et al*., 2010; Sarig *et al*., 2002; Lak *et al*., 2008; Pon-On *et al*., 2008; Meejoo *et al*., 2006; Yang *et al*., 2004; Elkady *et al*., 2011; Sampathkumar *et al*., 2000; Sidharthan *et al*., 2006). Recently, Gopi *et al*. reported the microwave coupled hydrothermal protocol influenced by the concentration of surfactant (CTAB) for accessing nano-HAp with different morphologies (Gopi *et al*., 2013).

In addition, the usefulness of biogenic material for the preparations of HAP is also a privileged process because of its valuable impact on environmental and economical benefits. However, this methods need to be more encouraged in order to utilize the biological waste and control over the pollution. Moreover, to the best our knowledge, the microwave – assisted synthesis of nanosized HAP from land-snail shell has been less explored. Connecting this view, we are interested to focus on the microwave – assisted synthesis of Nano sized HAP from land- snail shell (Helix Pomatia) since it is highly enriched with $CaCO_3$ and also it is easily available from natural sources.

Thus, the land-snail shell, a waste material after use of flesh, has been used as calcium originator to synthesize highly pure and inexpensive hydroxyapatite using di-ammonium hydrogen phosphate under microwave irradiation condition. The synthesized powder was characterized by XRD, FTIR, FE- SEM, FE-TEM and TG-DTA analytical techniques.

2. Materials and methods

2.1. Synthesis of HAP

The skeletons of land-snails shells were collected and washed with water, followed by distilled water to get rid of unwanted deposits and muds. They were air and vacuum dried for 24 hr and crushed by pistol mortar to obtain powdered mass of 200 mesh size particles. The stoichiometric amount of land snail-shell powder was dissolved in dilute hydrochloric acid and the mixture was added to a solution of di-ammonium hydrogen phosphate at 9 – 10 pH using aqueous ammonia. The resulting mixture was stirred for 20 min and immediately transferred to a domestic microwave oven and irradiated at 800 W energy of frequency employing 2.45 GHZ for 30 min continuously. After the irradiation, the residue was washed thrice with de-ionized water and then dried in a vacuum oven at 60 °C for 2 hrs.

2.2. Evaluation and measurement

The phase purity of the as-synthesized nano sized hydroxyapatite was analyzed by X-ray powder diffraction using Cu-kα radiation and the surface morphology was determined by FE- SEM (JEOL JSM 6701-F USA) and FE-TEM (JEOL 2100 F JAPAN) techniques. The FTIR spectroscopy (RXI Perkin Elmer) was used to identify the functional groups of as-synthesized hydroxyapatite. The TG-DTA analysis was carried out using (NETZSCH STA 449F3) instrument for examining their thermal stability.

3. Results and Discussion

3.1. X –Ray diffraction study

In order to check the crystallinity, the as-synthesized HAP powder and the calcined powders at various temperatures were subjected to XRD analysis using Cu-kα radiation. In the plot (a), the broadening of the peak indicates that the particles are small in size and less crsytallinity of the as-synthesized HAP powder dried at 60° C after microwave irradiation as shown in the Fig 1**.**

To check the effect of calcination, the powder was heated at different temperatures ranges from 500 °C to 1100 °C and analyzed the phases. This result shows that all the observed major peaks designate the presence of nanosized HAP in all the samples. As the temperature increases, the sharpening of the peak at 31.78°, 32.92° and 34.06° increases corresponding to the planes (211), (300) and (202), which represents the strong improvement in the crystallinity of the calcined nanosized HAP. Evidently, these results were good in agreement with standard JCPDS Card No-09-0432 and JCPDS Card No-89-6437.

$D=k\lambda/\beta\cos\theta$. Where
$\beta=FWHM\times\pi/180$
$K=0.94$
$\lambda=1.5406$ Å

Further, the size of the crystallite was calculated for as-synthesized and calcined powders by Scherer formula from the XRD pattern, which discloses that the cryastallite size was apparently 10.3 nm for as-synthesized nanosized HAP and 14 – 15 nm for the calcined HAPs, consequently, the increased crystallite sizes of the calicined HAPs are attributed to the set temperatures between 500 – 1100 ^{o}C (Simpson *et al*., 1968) as depicted in the Table - 1.

3.2. FTIR Studies

The FTIR spectrum of the HAP, as-synthesised nano-HAP at various temperatures are shown in the Fig 2. The characteristic peaks corresponding to stretching vibration of PO_4^{3-}

Table - 1: Calculation of crystallite size of Nanosized HAP powders

S. No.	Samples	Crystallite Size (nm)
1.	As-Synthesized HAP dried at 60° C	10.3
2.	Calcined at 500° C	14.7
3.	Calcined at 900° C	14.9
4.	Calcined at 1100° C	15.4

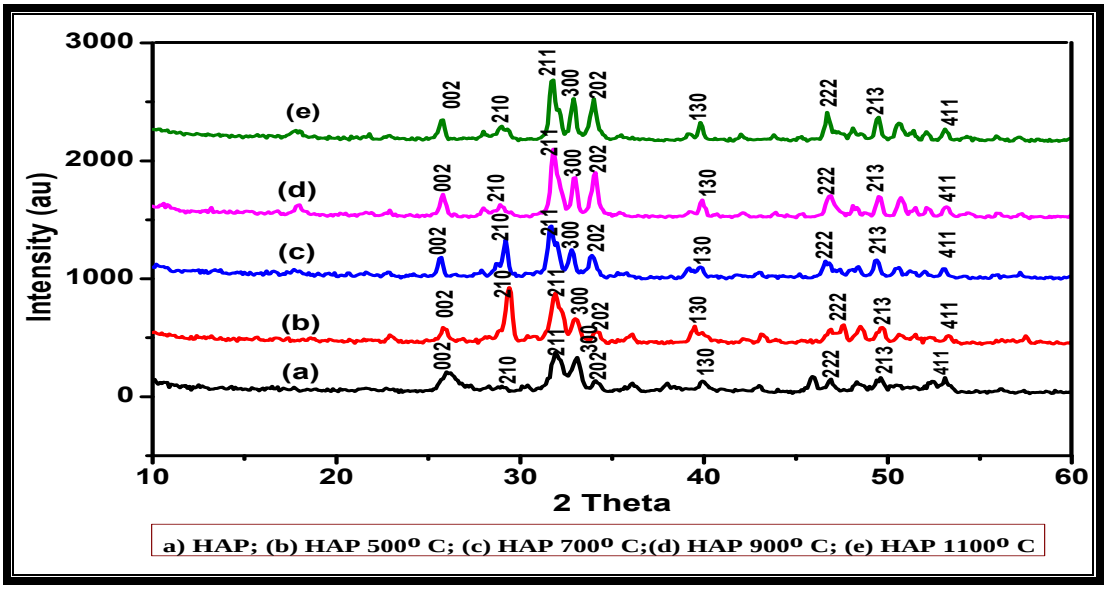

Fig – 1: XRD partern of as-synthesiszed and calcined nanosized HAP powders.

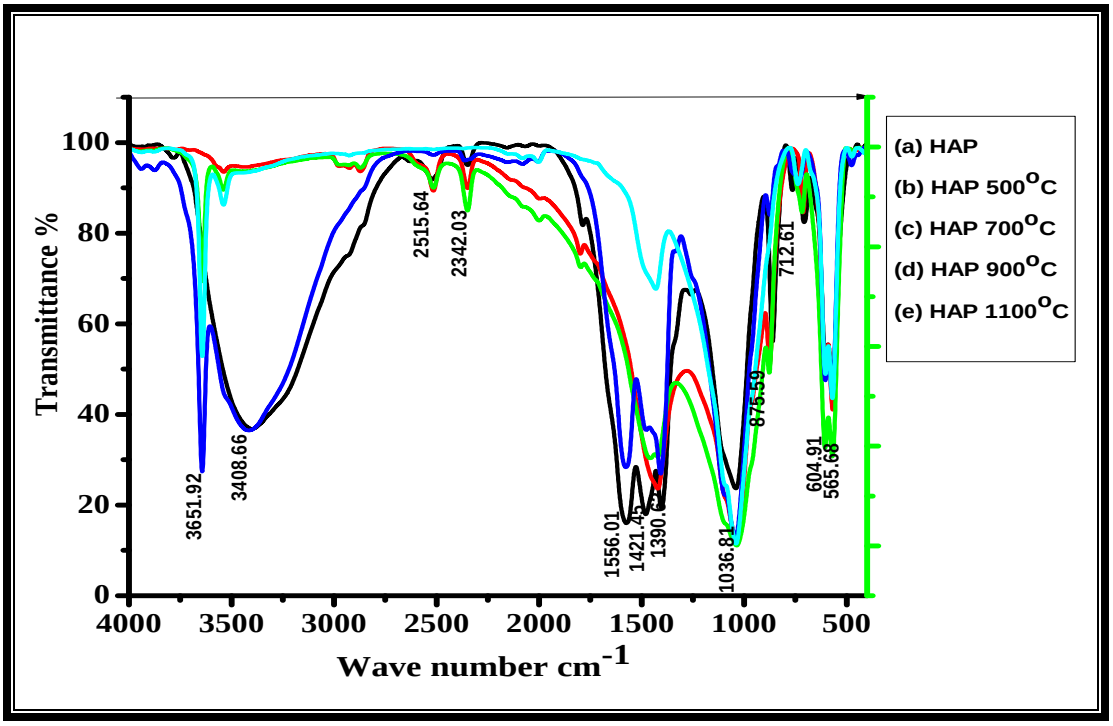

Fig – 2: FTIR Spectrum of as-synthesiszed and calcined nanosized HAP powders.

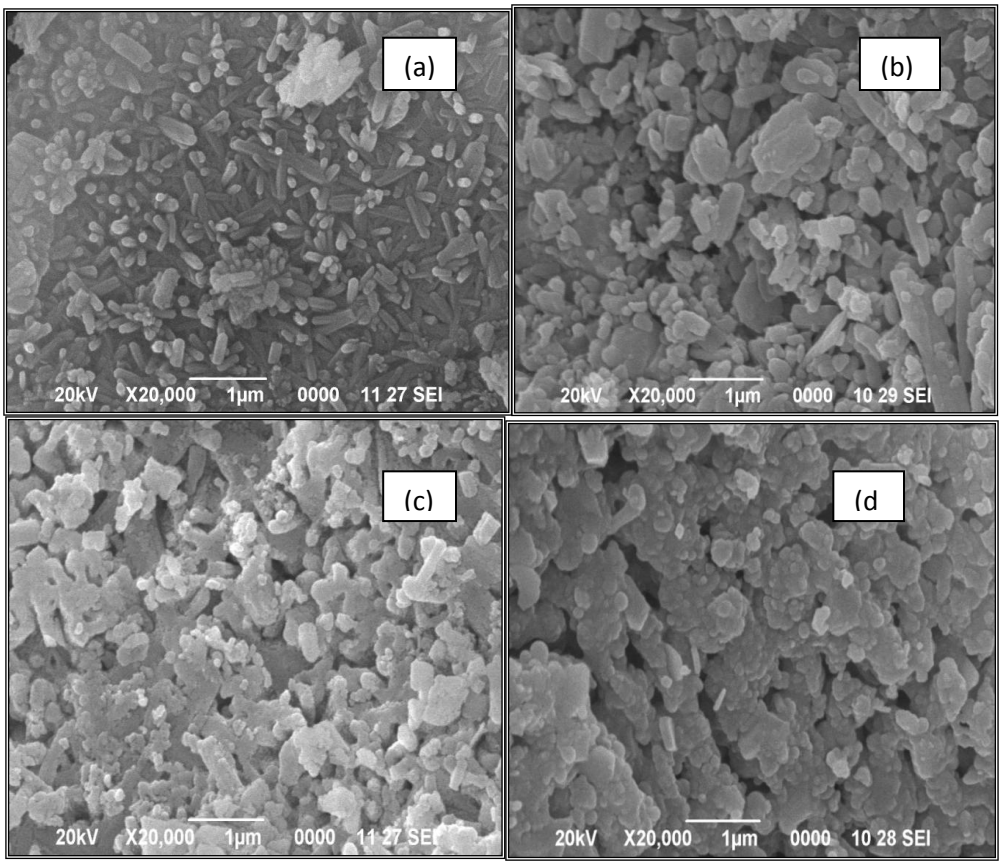

Fig - 3: FE-SEM images of nanosized HAP powders
(a) As-synthesiszed HAP (b) HAP at 500˚C (c) HAP at 700˚C (d) HAP at 900˚C

ions at 1035.81cm^{-1}, and the peaks at 568.02 cm^{-1}, are assigned to the deformation of PO_4^{3-} ions (Murugan *et al*., 2003). The broad OH stretching band around 2515.64 cm^{-1} - 3651.92 cm^{-1}, proves the adsorption for H_2O molecules.

A region of peak at 1390.62 cm^{-1}, designate the existence of carbonate in trace level. The formation of apatite was confirmed by the appeared doublet at around 604.32 cm^{-1} -568.02 cm^{-1}, which denotes the bending mode of P-O bonds in phosphate ions. Further, the peaks at 1421.45 cm^{-1}, 1556.01 cm^{-1} are responsible for stretching mode of CO_3^{2-} (Murugan *et al*., 2005), this may be associated with weak bonding interaction between carbon and oxygen in the present study.

3.3. SEM Analysis

Figure.3 shows the morphology of as-synthesized (a) calcined HAP (b-e) at different temperature ranges from 500 – 1100 °C. The SEM results reveals that the as-synthesized HAP has rod like structure with less crystallite of highly agglomerised powders. The gradual changes in the morphology of the calcined nanosized HAPs are due to the increase in the crystallinity of the powder incorporated with the sintering temperatures. The initial temperature at 500 °C, the calcined HAP possess rod-like homogenous microstructure, as the temperature increases, aggregation increases which results distorted spherical and rod-like shape with average size of 1 µm (500 – 900 °C). This is due to the decreasing of the probability of respective growths in grains

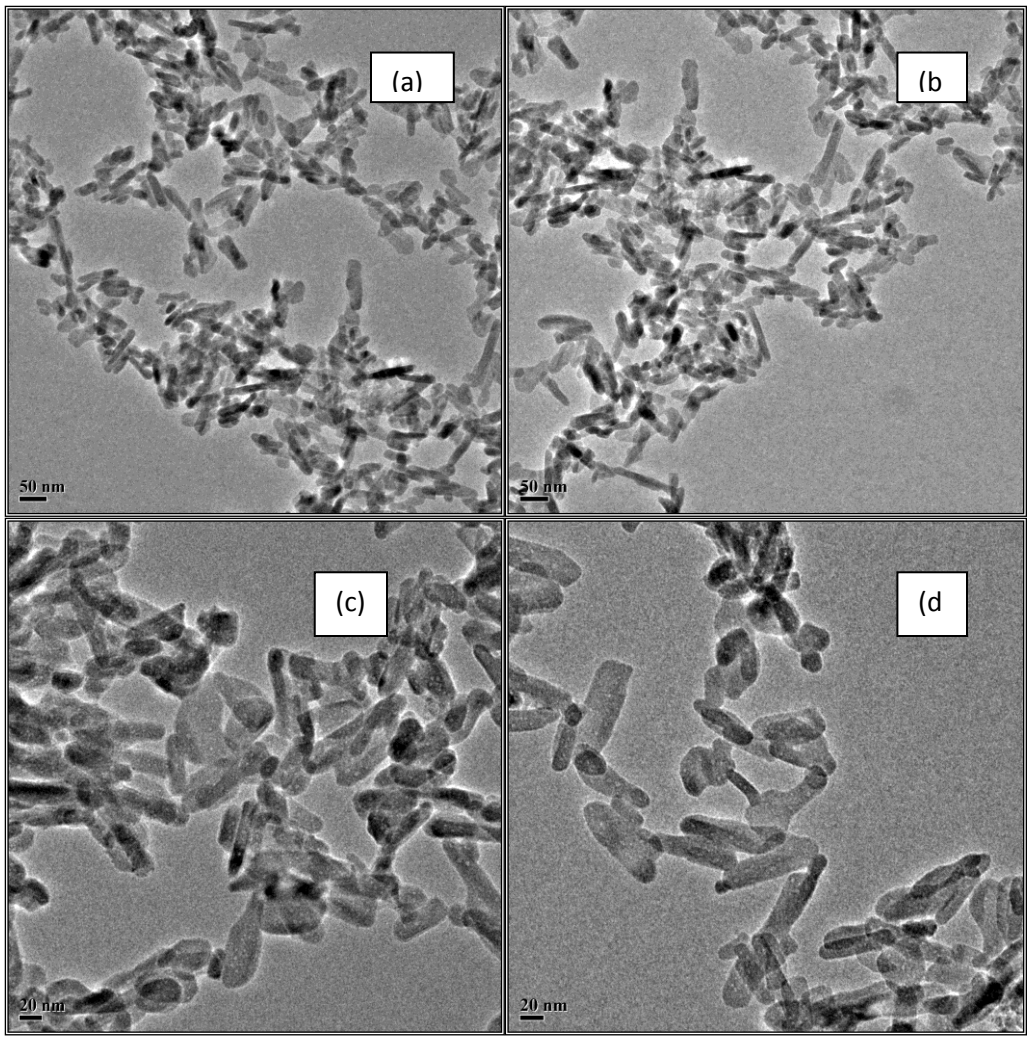

Fig - 4: FE- TEM images of as-synthesiszed nanosized HAP powders
(a) and (b) shows highly agglomerated nano-HAP powder with average size of 50nm; (c) and (d) displays poor agglomeration of the individual particles with 20nm size of nano-HAP powder

and the improvement of densification of the particles (Dasgupta Adak *et al*., 2011).

3.4. TEM Analysis

To further confirm the shape and size of the nano-HAP crystallite, they were investigated by TEM analysis. Fig 4 demonstrates the micrograph of the as-synthesized HAP powder obtained after microwave irradiation without calcination.

From the micrograph, it has been observed that the particles were highly agglomerated as rod-like shape and the size of particles were found to be 20 nm. This result was more promising with the grain size calculated by Scherer formula.

3.5. TG-DTA Analysis

In order to check the thermal solidity of land-snail shell, they have exposed to TG-DTA analysis as shown in the Fig (5). The major weight loss observed for land-snail shell at the temperature between 700 °C – 850 °C was attributed to the decomposition $CaCO_3$ to drive calcinated species which is depicted in the following equation.

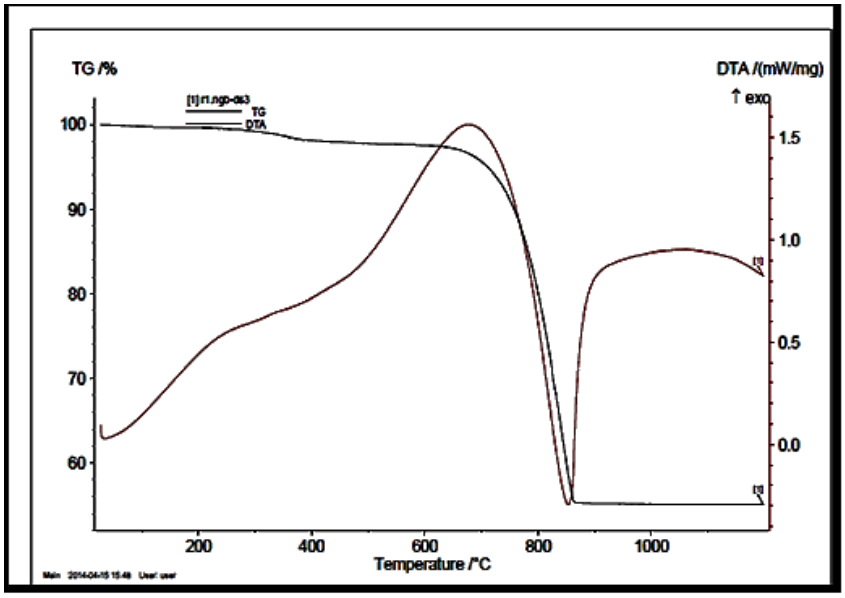

Fig - 5: TG-DTA Thermo gram of land-snail shell

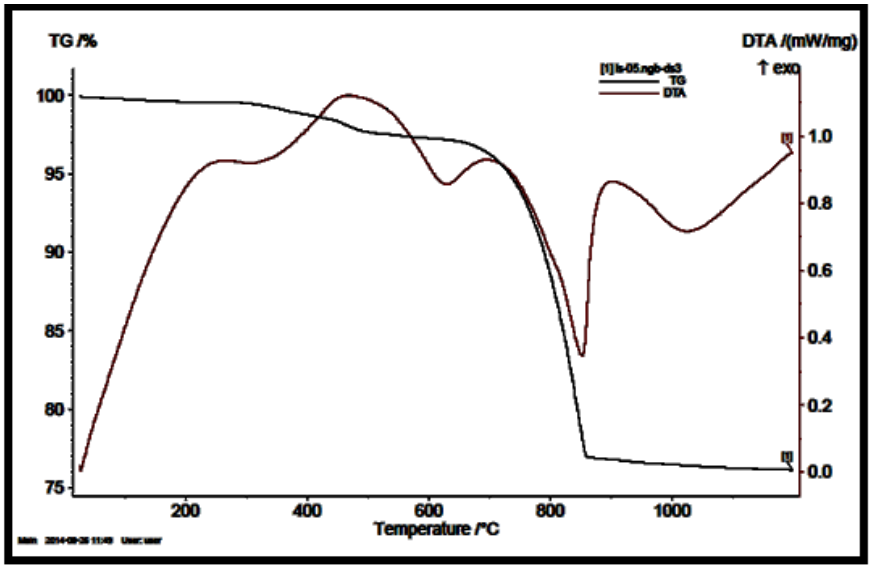

Fig - 6: TG-DTA Thermo gram of nanosized HAP

$$CaCO_3 \rightarrow CaO + CO_2 \uparrow$$

Further, no change in the weight of the snail shell was observed albeit up to 1200 °C respectively. This result strongly proves the excellent thermal stability of land - snail shells at higher temperatures.

Also, the experimental report confirms the occurrence of $CaCO_3$ and small amount of $MgCO_3$ and other organic materials present in the land-snail shells (Singh *et al*., 2011).

Similarly, TG-DTA analysis was also carried out to investigate the thermal solidity of as-synthesized nano-HAP as shown in Fig 6. The major weight losses of the nanosized HAP are ascribed to the dehydration of physically adsorbed water molecules from HAP powder to form

precipitation complex. Besides, no weight loss was found when it was heated up to 1200 °C is due to its thermally stable at higher temperature.

4. Conclusion

In conclusion, by a more facial and convenient method employing highly inexpensive, stoichiometric pure and thermally stable HAP nano-rods was synthesized using cheaply available land-snail shell (*Helix pomatia*) with di-ammonium hydrogen phosphate [$(NH_4)_2HPO_4$)] under microwave irradiation. The purity of the prepared HAP powder was verified by various analytical techniques having an admirable physico-chemical and *in vitro* physiological properties. The HAP- nanorods can be used as a bone substitute for filling bone deficiencies and as a coating material for orthopedic. And also the land-snail shell initiated HAP to be probable bio-ceramics which could be used for bio-medical applications.

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