

RESEARCH ARTICLE

## How can Hydroxyapatite Nano Crystal be prepared from Egg-Shell?

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### ARTICLE INFO

#### Article History:

Received 2022-01-03

Accepted 2022-03-04

Published 2022-09-30

#### Keywords:

Nanoparticle,  
Hydroxyapatite (HA),  
TEM,  
XRD,  
FT-IR.

### ABSTRACT

Hydroxyapatite (HA) with chemical formula of  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$  is prepared from eggshell and di-potassium-phosphate with chemical formula of  $\text{K}_2\text{HPO}_4$  in the present work. Adequate amount of egg-shell containing Calcium Oxide (CaO) was soaked in di-potassium phosphate solution (ratio: Ca/P=1.67) at 37 °C at different soaking times to obtain nano-crystalline HA which was characterized by X-Ray Diffraction (XRD), Transition Electron Microscopy (TEM) and FT-IR studies. The crystal grain size is very little dependent on the soaking time in di-potassium-phosphate solution, and the grain sizes are almost uniform and regular in shape. The egg-shell derived HA nanoparticles could be used to make scaffolds of different shapes and pore sizes for clinical uses. This process of low temperature HA synthesis is simple, and environmentally friendly for the mass production of pure hydroxyapatite nano-crystalline.

### How to cite this article

Gheisari H., How can Hydroxyapatite Nano Crystal be prepared from Egg-Shell?, J. Nanoanalysis., 2022; 9(3): 240-243.  
DOI: 10.22034/jna.2022.1948935.1287

## INTRODUCTION

Nanometer scale of HA has been proved to be an osteo-conductive material that also binds chemically to enamel [1]. HA with the chemical formula of  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$  has the capability to form chemical bonds with surrounding hard tissues [2]. Also, HA was used for the removal of heavy metals from wastewater [3]. So far, different methods have been developed to prepare HA, such as sol-gel method [4] and chemical precipitation [5]. Several attempts have also been made earlier to prepare HA from egg-shell [6]. As the physical properties of HA are process dependent as well as particle size dependent, every method has its own demerits. Most of these methods are a little bit complicated and require high temperature processing. It is still a challenge to prepare high quality powder of HA for direct clinical use. In this study, a simple method to synthesize high quality uniform size HA- nanoparticles from egg-shell and di-potassium phosphate solution is reported. Di-potassium phosphates used instead of sodium as we noticed that the formation of HA is more

spontaneous in the case of potassium salt. Finally, the evaluation and characterization of HA were made by electronic microscopy, XRD, and FT-IR studies.

## EXPERIMENTAL

To prepare the HA, egg-shells were properly cleaned and washed with water first, then heated to dry with the heating rate of 5 °C/min and finally kept at 150 °C for 2 h. After 2h organic substances came out and shell became black. The furnace temperature was adjusted with increasing rate of 20 °C/min and fixed between 750 and 850 °C and sintered for 4h. The black shell converted slowly to snow white powder. The snow white powder grab from egg-shells ( $\text{CaCO}_3$ ). Obtained pure CaO was immediately used for the preparation of HA using appropriate amount of di-potassium phosphate in deionized water solution. The obtained CaO was slowly added to the solution in a beaker and mixed well for 25 min. Moreover, the pH value of the solution was kept about 11 by adding  $\text{NH}_4\text{OH}$  solution. Subsequently, the beaker was placed in the heating chamber that was kept at 37 °C. The

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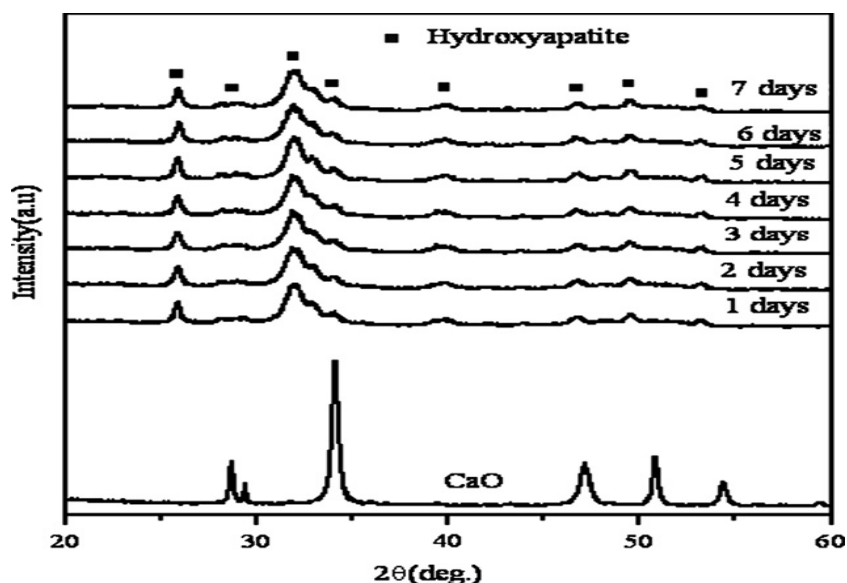


Fig. 1. XRD patterns of the CaO obtained from the egg-shell and those of the HA nanoparticles prepared from this CaO powder soaked in di-potassium phosphate solution for different days.

self-reaction was allowed to complete in different beakers for different periods of soaking time viz. 24h.

In continue, HA particles were separated from the solution and washed several times with water to remove any external ions. Then the powder was dried in the oven at 37°C for a week. Nano crystalline obtained powder is specified to confirm HA. It shall be noted that when CaO is mixed with water, a small amount of calcium hydroxide might be produced. The values of pH were recorded before and after the soaking time.

## DISCUSSION

Table. 1 showed the changes in pH of the solution with soaking time kept at 37°C. It is noticed that the variation of pH with soaking time is very small and the corresponding changes of grain sizes of HA product are also very small. Such a small change of pH might be due to the loss of water by evaporation during longer soaking process at 37°C which increases acidity. To evaluate the prepared HA powder, the XRD and FT-IR have been carried as depicted in Figs. 1 and 2. The crystalline phases identified mostly correspond to HA (HSTR, 8-576). It is apparent from the XRD pattern (Bruker, Model S9 Zaiess, Switzerland) that all the peaks corresponding to HA are identified and agree with the reported. There is no change is observed in the XRD peak positions for different samples prepared with different soaking time during the

days. Thus, a little broadening of the concerned peaks along with slight reduction in intensity indicates little reduction of grain size with soaking time compared with that of smaller soaking time sample. This is due to the fact that the first growing particles are little larger compared to those of slow growing particles. Peak broadening analysis of the pattern obtained after 7 days allowed estimating the grain size reduction of 20 nm by appropriate disconsolation of the concerned  $2\gamma$ ?. Also, the values of  $2\gamma$  of the XRD pattern of HA powder are matched well? with the reported work. In addition, the average grain size of the samples was evaluated from the broadening of the most intense peak of the chosen phases using single line profile analysis based on equivalent Voight representation [7-9].

Fig. 1 also shows the XRD patterns of the CaO obtained from the egg-shell and HA nanoparticles prepared from this CaO powder using various soaking times. FT-IR spectra were studied for different HA powder samples as shown in Fig. 2. Infra-red transmittance spectra of the prepared HA powders prepared with different soaking times are almost identical and show the presence of two bands around 742 and 2610  $\text{cm}^{-1}$  corresponding to the vibration of hydroxyl. Those bands at 2048 and 2089  $\text{cm}^{-1}$  are the characteristic bands of phosphate stretching vibration, whereas the bands at 614 and 572  $\text{cm}^{-1}$  are due to phosphate bending vibration. The FT-IR spectral behavior of all prepared HA samples also agreed well with that of the reported

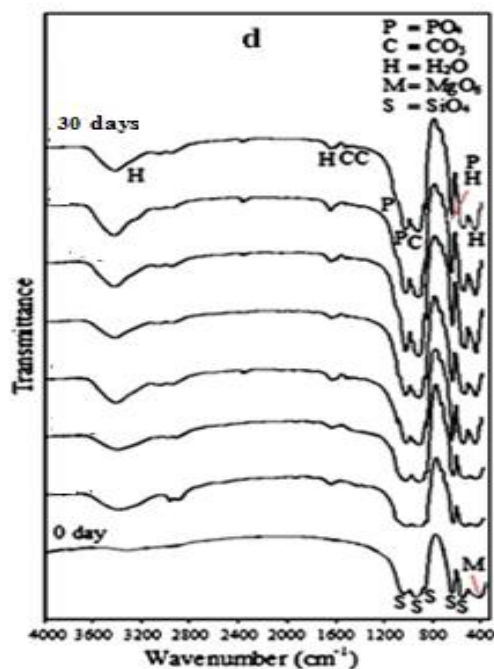


Fig. 2. FT-IR transmittance spectra of hydroxyapatite for samples soaked 7 days.

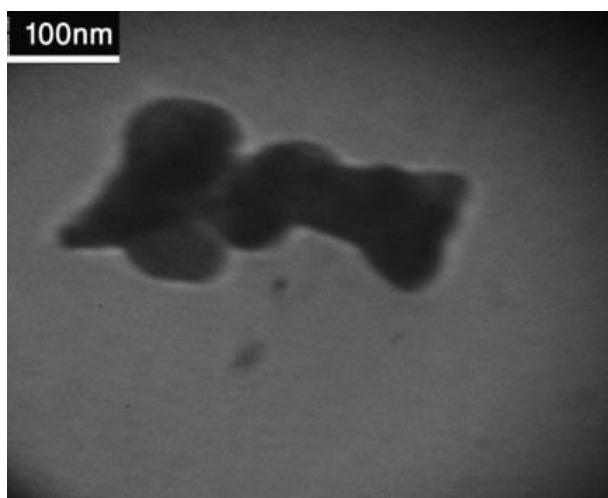


Fig. 3. The TEM micrographs of crystalline HA powders for soaking time after 7 days.

sample. TEM micrographs, Fig. 3 clearly indicated the nano-structure 100 nm particle size). Based on the XRD and FT-IR analyses, the white powder prepared from the egg-shell, CaO is confirmed to be pure nano crystalline HA with original chemical formula of  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ . Pure nanoparticles are very important for biomedical material [10-12] to make scaffolds of different sizes, shapes and pore sizes by mixing with appropriate biocompatible polymer binders which are being attempted in our laboratory.

### CONCLUSION

In conclusion, the source of CaO is egg shell, the prepared HA is very pure. The simple self-assisted chemical reaction method was used to prepare pure fine particles of HA from egg-shell. Besides, this method can be used for the mass production of HA from waste materials that might be used for water purification. The crystal grain size is very little dependent on the soaking time in di-potassium phosphate solution, and the grain sizes are almost uniform and regular in shape. The



egg-shell derived HA nanoparticles could be used to make scaffolds of different shapes and pore sizes for clinical uses.

#### ACKNOWLEDGMENTS

The authors are grateful for the kind supports by the Najafabad Branch, Islamic Azad University, Najafabad, Iran and University of Toronto, ON, Canada.

#### CONFLICT OF INTEREST

The author declare no conflict of interest.

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