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# Synthesis of hydroxyapatite by using calcium carbonate and phosphoric acid in various water-ethanol solvent systems

**Research Article**

Arzu Engin[a]*, İsmail Girgin[b]

[a]*Afyon Kocatepe University, Faculty of Engineering,*
*Chemical Engineering Department,*
*03200 Afyonkarahisar, Turkey*

[b]*Hacettepe University, Faculty of Engineering,*
*Mining Engineering Department,*
*06800 Beytepe, Ankara, Turkey*

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**Abstract:** The purpose of the present study is to synthesize hydroxyapatite by using $CaCO_3$ and $H_3PO_4$ in various water-ethanol solvent systems. It was observed from experiments that formation of ammonium phosphate compounds hindered the formation of calcium phosphates in ethanol medium. Although the reactivity was better in aqueous medium, the carbonate contents of the products obtained were above 8.5%. Best results with a carbonate content as low as 3.82% was obtained in 50% ethanol containing mixed-aqueous medium at 80°C and the FTIR analysis showed that the product was a carbonated apatite with a calculated composition of $14CaO \bullet 4.2P_2O_5 \bullet CO_3 \bullet 7.2H_2O$. The amorphous and porous phosphate compound obtained with a BET surface area of 106.6 $m^2\ g^{-1}$ seems to be useful as adsorbent in wastewater treatment. Upon sintering of the amorphous product at 750°C, crystalline hydroxyapatite with a BET surface area of 25.9 $m^2\ g^{-1}$ is obtained that may be used in biomedical applications.

**Keywords:** *Synthesis • Hydroxyapatite • XRD • FTIR*

## 1. Introduction

Synthetic hydroxyapatites, $Ca_5(PO_4)_3OH$, known for their high chemical and thermal stabilities, unique sorption properties, biocompatibility and bioactivity, have been widely studied. Various techniques were developed for the synthesis of hydroxyapatites, based on solid state reactions [1,2], chemical precipitation reactions [3-11], hydrothermal reactions [12-18], sol-gel methods [19-23] and mechanochemical methods [24-26] using different calcium and phosphorus containing starting materials.

Few investigations have been done on the synthesis of hydroxyapatite using calcium carbonate and phosphoric acid. Some work have been done on the conversion of natural calcium carbonates with aragonite structure (coral, nacre) into hydroxyapatite [27-32] and Yoshimura *et al.* [13] studied on the conversion mechanism of synthetic calcite into hydroxyapatite by hydrothermal reaction with phosphoric acid.

The effect of alcohols on the formation of calcium phosphates have been reported previously [32,33] and it was shown that addition of ethanol and fluoride to the initial solutions of $CaCl_2$-$NaH_2PO_4$ and $Ca(NO_3)_2 \bullet 4H_2O$-$KH_2PO_4$ facilitated the transformation of amorphous calcium phosphate to crystalline form. The presence of ethanol in $Ca(NO_3)_2 \bullet 4H_2O$ and $(NH_4)_2HPO_4$ solutions [34] was found to favor the changes during the gradual formation of calcium phosphates, from the cluster to the crystallite. Ethanol solutions of $Ca(NO_3)_2 \bullet 4H_2O$ and $(NH_4)_2HPO_4$ were used [35] to synthesize nanocrystalline hydroxyapatite by sol-gel technique at 85°C and the presence of ethanol was said to provide a thermally stable hydroxyapatite formation at a lower temperature than the other existing methods.

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* *E-mail: aengin@aku.edu.tr*

As no work was found in the literature, this study was aimed at synthesizing by using calcium carbonate and phosphoric acid in ethanol presence.

# 2. Experimental Procedures

## 2.1 Materials and Methods

Synthesis experiments were conducted in a 250 mL thermostatically controlled (±1°C) three-necked Pyrex reactor with a reflux condenser, a thermometer and a pH electrode. Merck grade reagents ($CaCO_3$, $H_3PO_4$, $NH_4OH$), absolute ethanol (Delta Teknik) and distilled water were used in the experiments.

Ethanol containing aqueous solvents (25, 50, 75% (v/v) ethanol) were prepared. No buffer solution was used to control the pH of the reaction medium but 30% (w/w) $NH_4OH$ solution was used to adjust the pH. For this purpose, a series of titration tests were conducted on the initial reaction composition excluding $CaCO_3$ to determine the amount of the $NH_4OH$ for keeping the pH value of the $H_3PO_4$-solvent system at values of 10 (basic), 7 (neutral) and 4 (acidic).

5 grams of calcium carbonate was added into the reaction vessel containing 50 mL of solvent and then the pre-determined quantity of ammonia solution was added to adjust pH. Afterwards, $H_3PO_4$ was added dropwise from approximately 2 mL burette to start the reaction in alkaline medium to obtain a final Ca/P molar ratio at 1.66 while the system was stirred magnetically. Reactions at room temperature were continued for one hour after mixing all reagents, yet, experiments conducted at 80°C were continued for one hour after the medium temperature reached 80°C. After completion of the reaction, the solids were separated by filtration under vacuum using Whatman 42 filter paper, the products were dried at 105°C for two hours and analyzed. The experimental steps were shown in the flow sheet given in Fig. 1.

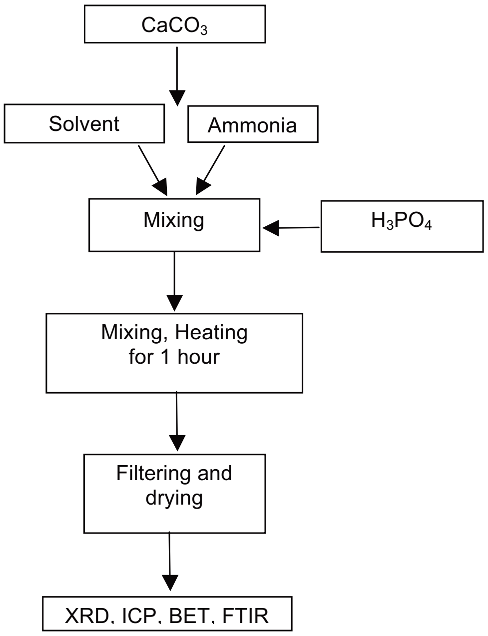

**Figure 1.** Flow sheet of the experiments.

The samples synthesized were characterized by XRD analyses (Rigaku D-MAX 2000) using Cu-Kα radiation, FT-IR analyses (Perkin Elmer), particle size analyses (Sympatec Laser Sizer), B.E.T. (Quantachrome Monosorb), Ca and P analyses (Iris Intrepid ICP-OES). The carbonate contents of the samples were determined by $CO_2$ analysis [27].

**Table 1.** Results of the experiments at room temperature.

| Solvent | Solvent+$H_3PO_4$ Predetermined pH | Final pH | Ca (%) | P (%) | $CO_3$ (%) | Ca/P Mole Ratio (Calculated) |
|---|---|---|---|---|---|---|
| Water | 10 | 10.1 | 36.97 | 3.49 | 44.44 | 1.63 |
| Water–EtOH (%25) | 10 | 10.0 | 39.06 | 6.81 | 33.42 | 1.91 |
| Water–EtOH (%50) | 10 | 9.9 | 36.66 | 9.83 | 23.38 | 1.66 |
| Water–EtOH (%75) | 10 | 9.6 | 34.16 | 12.95 | 15.82 | 1.41 |
| Et-OH | 10 | 6.9 | 27.76 | 11.05 | 41.25 | 0.02 |
| Water | 7 | 8.2 | 37.23 | 9.43 | 26.56 | 1.60 |
| Water–EtOH (%25) | 7 | 8.1 | 39.80 | 6.91 | 36.15 | 1.77 |
| Water–EtOH (%50) | 7 | 8.4 | 37.73 | 15.29 | 8.51 | 1.62 |
| Water–EtOH (%75) | 7 | 7.5 | 35.82 | 15.55 | 9.70 | 1.46 |
| EtOH | 7 | 7.0 | 28.30 | 12.49 | 31.87 | 0.44 |
| Water | 4 | 7.8 | 34.21 | 11.85 | 18.34 | 1.44 |
| Water–EtOH (%25) | 4 | 7.9 | 33.72 | 12.65 | 15.27 | 1.44 |
| Water–EtOH (%50) | 4 | 8.0 | 31.98 | 15.00 | 9.05 | 1.34 |
| Water–EtOH (%75) | 4 | 7.3 | 31.00 | 14.69 | 16.40 | 1.06 |
| EtOH | 4 | 7.1 | 26.51 | 11.57 | 27.11 | 0.56 |

# 3. Results and Discussion

## 3.1 Synthesis at room temperature

A series of experiments were done at room temperature using water, water-ethanol and ethanol as solvents. In these experiments, $H_3PO_4$ was added dropwise onto a slurry containing $CaCO_3$, solvent and ammonia in the reaction vessel and the reaction was continued for one hour after mixing all the reagents. Ca, P and carbonate contents of the products together with the final pH value of the reaction medium are given in Table 1. Fig. 2 gives the XRD patterns of the products.

Chemical analysis results show that the products contain carbonate and XRD patterns verify the presence of calcium carbonate. Considering the carbonate equivalents as unreacted calcium carbonate, the remaining calcium and phosphorus contents were used to calculate the Ca/P mole ratios for the products formed in Table 1. Fig. 3 shows the effect of the solvent systems on the calcium carbonate contents, final pH and the Ca/P mole ratios of the products formed.

As is evidenced from the experimental results (Table 1), the solvent systems behave very differently in the reaction at room temperature. Maximum carbonate contents are observed in the products obtained in pure water and ethanol solvents. Carbonate contents decrease gradually from 44.4% to 18.3% in aqueous medium with decrease of final pH values from 10.1 to 7.8. Although the carbonate contents are lowered from 41.3% to 27.1% in pure ethanol, final pH values almost stay constant between 6.9 to 7.1, no calcium phosphate peaks are observed in the XRD patterns but some new unidentified peaks, typical of ammonium phosphates, are formed (Fig. 2). Among the mixed-aqueous solvents, the best result was obtained in the solvent containing 50% ethanol at final pH value of 8.4 with formation of a product (Ca/P mole ratio of 1.62) containing 8.5% carbonate.

## 3.2 Synthesis at 80°C

Experiments in Section 3.1. were repeated at 80°C. Experimental results and the XRD patterns are presented in Table 2 and Fig. 4, respectively. Fig. 5 gives the effect of the solvent systems on the unreacted calcium carbonate contents, final pH and the Ca/P mole ratios of the products formed.

Upon increase of the reaction temperature to 80°C, the reactivity was observed to increase tremendously for all the solvent systems, the final pH values reached to almost constant values between 9 to 10 and no crystalline calcium phosphate phase was observed in the XRD patterns. The lowest final pH values and the highest carbonate contents were attained with pure ethanol solvent. Similar to room temperature, mixed water-EtOH (50% ethanol) solvent gave the best results at the predetermined solvent+$H_3PO_4$ pH values of 10 and 7 with carbonate contents lowering to 3.83 and 3.82%, respectively. The final pH values for both cases were around 10 and the Ca/P mole ratios were determined as 1.57 and 1.52, respectively.

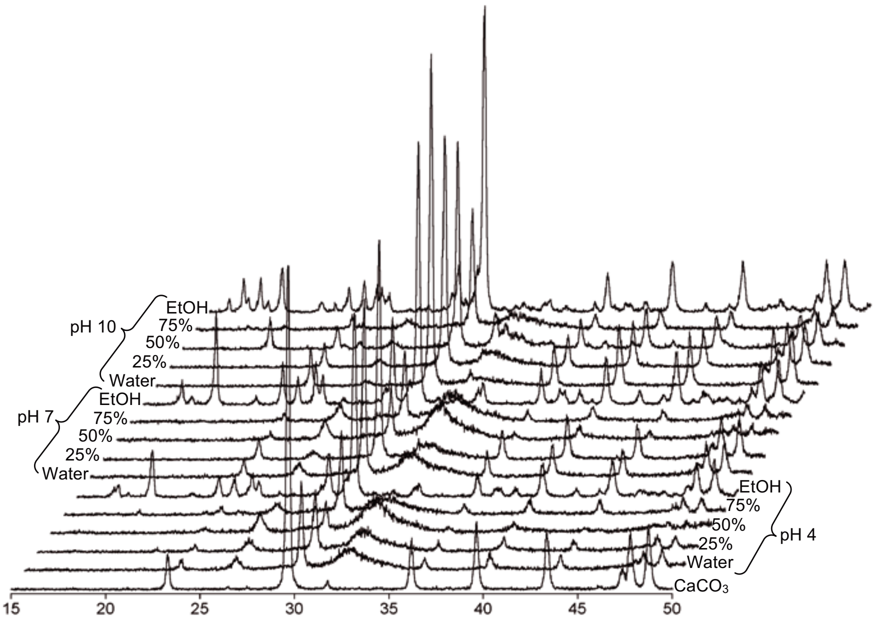

**Figure 2.** XRD patterns of the products obtained at room temperature.

**Table 2.** Results of the experiments at 80°C.

| Solvent | Solvent+$H_3PO_4$ Predetermined pH | Final pH | Ca (%) | P (%) | $CO_3$ (%) | Ca/P Mole Ratio (Calculated) |
|---|---|---|---|---|---|---|
| Water | 10 | 9.1 | 35.14 | 13.95 | 7.81 | 1.66 |
| Water–EtOH (%25) | 10 | 10.0 | 34.48 | 14.52 | 5.12 | 1.66 |
| Water–EtOH (%50) | 10 | 10.1 | 33.76 | 15.41 | 3.83 | 1.57 |
| Water–EtOH (%75) | 10 | 10.1 | 35.37 | 15.26 | 5.78 | 1.60 |
| Et-OH | 10 | 9.3 | 35.09 | 14.97 | 8.51 | 1.52 |
| Water | 7 | 9.5 | 34.86 | 14.05 | 7.09 | 1.66 |
| Water–EtOH (%25) | 7 | 9.9 | 34.17 | 14.65 | 5.45 | 1.61 |
| Water–EtOH (%50) | 7 | 10.0 | 35.45 | 16.72 | 3.82 | 1.52 |
| Water–EtOH (%75) | 7 | 9.4 | 35.03 | 15.43 | 5.60 | 1.56 |
| EtOH | 7 | 9.3 | 33.07 | 13.97 | 9.27 | 1.49 |
| Water | 4 | 9.4 | 36.92 | 16.92 | 5.78 | 1.51 |
| Water–EtOH (%25) | 4 | 9.8 | 32.41 | 14.94 | 7.00 | 1.44 |
| Water–EtOH (%50) | 4 | 10.1 | 34.70 | 15.80 | 4.27 | 1.56 |
| Water–EtOH (%75) | 4 | 9.6 | 33.10 | 15.13 | 7.30 | 1.44 |
| EtOH | 4 | 8.9 | 29.88 | 13.91 | 15.21 | 1.10 |

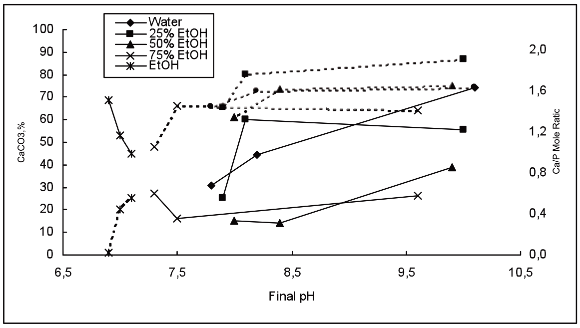

**Figure 3.** Effect of solvent on the reaction at room temperature.

## 3.3 Characterization of the products prepared in water-EtOH (50%) medium at 80°C

The increase of reaction time from 1 hour to 7 hours to observe the effect of reaction time on calcium carbonate content in the product showed no difference in the chemical composition and the XRD patterns but slight changes in particle size distribution (Fig. 6) and a decrease in the BET surface area from 106.6 m$^2$ g$^{-1}$ to 77.3 m$^2$ g$^{-1}$ were observed.

Heating tests were done on the sythesized amorphous calcium phosphate product between 700-900°C to obtain crystalline product and the optimum temperature was determined to be 750°C for the formation of crystalline calcium phosphates. Fig. 7 gives the XRD pattern of the product heated at 750°C for 3 hours showing the formation of hydroxyapatite (HAP) with well defined characteristic peaks (JCPDS No: 9-432). The BET surface area of the heated product was determined as 25.9 m$^2$ g$^{-1}$.

The FTIR spectra of the dried and the heated samples are shown in Fig. 8. The unresolved IR absorption peaks belonging to phosphate groups near 565 and 600 cm$^{-1}$ for the dried sample indicate that this sample has poor crystallinity with respect to the heated sample [33]. The broad adsorption peak between 1000-1150 cm$^{-1}$, the peak at 962 cm$^{-1}$ and the peak at 473 cm$^{-1}$ are assigned to $PO_4^{3-}$ group vibrations. The bands at 632/3572 cm$^{-1}$ are assigned to liberational/str hing modes of hydroxyl groups and the bands near 3420 and 1635 cm$^{-1}$ arise from the adsorbed water molecules [36]. Finally, the peaks at 874, 1421 and 1467 cm$^{-1}$ are assigned to $CO_3^{2-}$ group vibrations in the apatite structure that are characteristic for carbonated apatites. The carbonate bands for the 750°C heated sample near 1421 and 1467 cm$^{-1}$ correspond to the incorporation of $CO_3^{2-}$ groups for the $PO_4^{3-}$ sites in the structure [37].

## 3.4 Discussion

When the reaction is conducted in pure ethanol at room temperature, there is evidence that ammonium phosphate type compounds are formed. As these compounds are mostly insoluble in ethanol, consumption of phosphorus hinders the formation of calcium phosphates. On the other hand, presence of water coming from ammonia solution used for pH adjustment causes slight dissociation of ammonium phosphates. This dissociation formed a buffer action at around pH 7. Upon increase of the reaction temperature to 80°C in ethanol medium, the increase in solubilities of both the calcium carbonate and the ammonium phosphates render the formation of calcium phosphate. Even then,

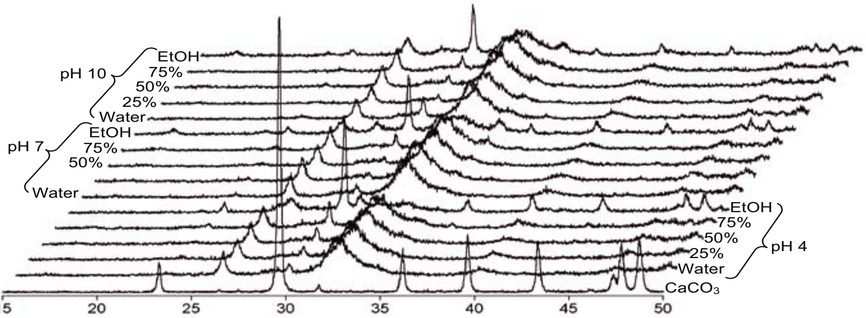

**Figure 4.** XRD patterns of the products obtained at 80°C.

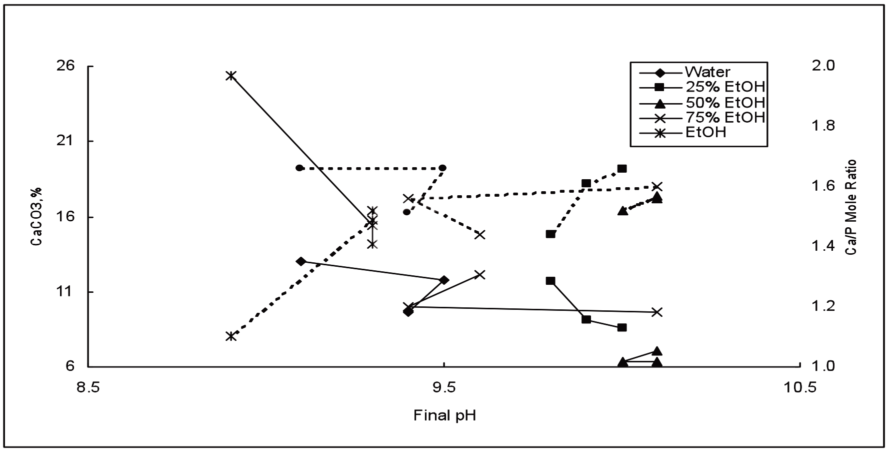

**Figure 5.** Effect of solvent on the reaction at 80°C.

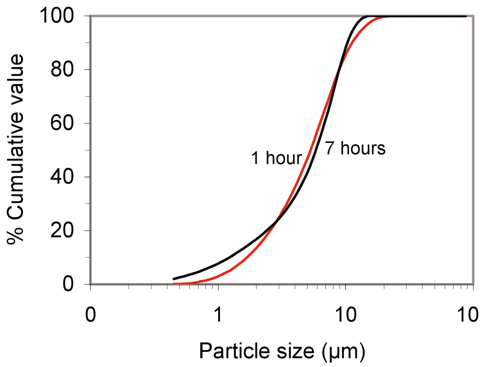

**Figure 6.** Effect of reaction time on the particle size of the products.

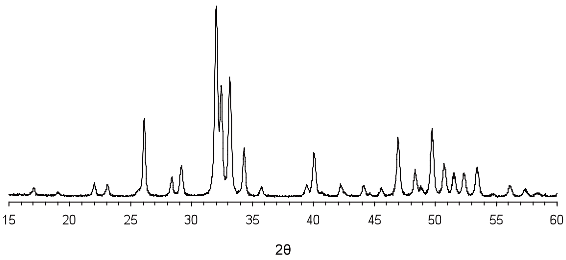

**Figure 7.** XRD pattern of the product heated at 750°C.

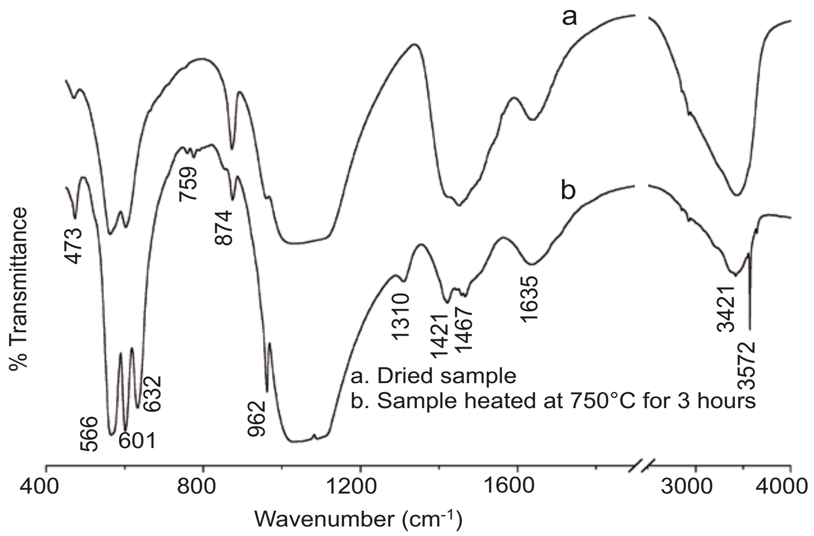

**Figure 8.** FT-IR spectra of the dried and the heated samples.

the carbonate contents of the products obtained are above 8.5% and all are amorphous in nature.

In aqueous medium at room temperature, the reactivity increases with decrease of final pH value from 10.1 to 7.8 with a corresponding decrease in carbonate content from 44.4 to 18.34%. As the temperature is increased to 80°C, the carbonate content is observed to decrease to 5.78% at the lowest solvent+$H_3PO_4$ predetermined value of 4 and the products obtained are amorphous in character.

Best results with carbonate contents as low as 3.82 and 3.83% are obtained in 50% ethanol containing mixed-aqueous medium at final pH values around 10. The FTIR analysis shows that the product is an amorphous carbonated calcium phosphate with a calculated chemical composition of $14CaO{\bullet}4.2P_2O_5{\bullet}CO_3{\bullet}7.2H_2O$ and a Ca/P mole ratio of 1.67. When this sample is heated at 750°C for 3 hours crystalline hydroxyapatite is formed but FTIR analysis gives evidence of the presence of carbonate groups incorporated for phosphate sites in the structure.

## 4. Conclusion

Experimental results show that ethanol is effective in the synthesis of calcium phosphates from calcium carbonate and phosphoric acid in ammoniacal solutions. Although pure ethanol medium seems to be unsuitable for the synthesis of calcium phosphates due to formation of insoluble ammonium phosphates, the least carbonate containing amorphous compound is obtained in 50% ethanol containing mixed-aqueous medium. This amorphous and porous carbonated calcium phosphate with a BET surface area of 106.6 $m^2$ $g^{-1}$ seems to be useful as an adsorbent in wastewater treatment, while the crystalline hydroxyapatite obtained by sintering of the amorphous compound for three hours at 750°C with BET surface area of 25.9 $m^2$ $g^{-1}$ may be used in biomedical applications.

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