


<b>Synthesis and Characterization of Cobalt apatite totally and half substituted by Microwave Irradiation. Antibacterial activity.</b>			<b>Chemistry</b>
		<b>Keywords:</b> Hydroxyapatite; Cobalt. FTIR spectroscopy, X-ray diffraction(XRD), Scanning electron microscopy (SEM).	
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<b>Abstract</b>			
<p>The present paper investigates the preparation of hydroxyapatite and Cobalt (Co) apatite totally and half substituted through a chemical synthesis process. The synthesized powders were characterized by the following analytical techniques: X-ray diffraction (XRD), FT-IR and Scanning electron microscopy (SEM). We have successfully prepared nano-Hap particles with various shapes by controlling pH and preparation temperature in the precipitation method. The HAP prepared in various conditions. It illustrates that the pH value, preparation temperature and component ratio play an important role in controlling the shape of the synthesized particles. It is interesting that those parameters have insignificant effect on size of the particles. The peak profiles of the XRD patterns may indicate that the crystallinity of the particles varies with the pH value and temperature. For examples, the particles prepared over the pH-9 solution at 60°C have high crystallinity than the particles prepared below pH- 9 solution. XRD analyses results showed that the crystallinity increase with increase the component ratio of cobalt. The antibacterial activity of these complexes against Gram positive and Gram negative has also been studied.</p>			

### 1. Introduction

Hydroxyapatite [HAp, chemical formula  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ] has attracted attention of researchers during the past 30 years as an implant material because of its excellent biocompatibility and bioactivity (Aoki, H., 1991; Suchanek, W. et al., 1998). It has also been extensively investigated for non-medical applications such as packing media for column chromatography, gas sensors, catalysts, host material for lasers, and plant growth substrates. All properties of HAp, including bioactivity, biocompatibility, solubility, and adsorption properties can be tailored over a wide range by modifying the composition through ionic substitutions (Aoki, H., 1991; Suchanek, W. et al., 1998; Elliott, J. C. 1994; LeGeros, R. Z. 1991).

Hydroxyapatite ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ; HAp) ceramic is a bioactive and biocompatible material which has been widely used in orthopedic surgery, tissue engineering and dental implant applications (Elliott, J. C. 1994; Hench, L. L., 1998; Jones, F. H., 2001; Karp, J. M. et al. 2003). Due to the chemical similarity between bone and mineralized bone of human tissue, synthetic HAp exhibits strong affinity to host hard tissues (Murugan R. and Ramakrishna S., 2005). HAp has excellent biocompatibility and bioactivity, thus it is used in the reconstruction of damaged bone or teeth (DeGroot, K., 1983; Narasaraaju, T. S. B. and Phebe, D. E., 1996).

HAp can be synthesized by many chemical processing routes such as solid state reaction [3, 8], coprecipitation and hydrothermal technique (Wang Y., et al. 2006; Byrappa K., 2001). Solid state reactions usually give stoichiometric and well-crystallized products but they require relatively high temperatures and long heat-treatment times. Moreover, the sintering capacity of such powders is usually low and ultimately results in inferior mechanical properties of the sintered matrices [12, 13]. In the case of coprecipitation, nanometer size powders can be prepared. However, their crystallinity and Ca/P ratio depend mainly upon the preparation conditions and are in many cases lower than for well-crystallized stoichiometric HAp. The hydrothermal technique usually results in HAp powders with a high degree of crystallinity and the Ca/P ratio close to the stoichiometric value (Byrappa K., 2001; Lin, K. et al. 2007). However, the obtained powders have typical agglomeration and their size distribution is relatively wide. Therefore, the size distribution of HAp powders cannot be well controlled using the normal hydrothermal method (Byrappa K., 2001).

The apatite (Hap) structure is so tolerant to ionic substitutions that  $\text{Ca}^{2+}$  in the crystals can be replaced by various divalent cations including  $\text{Mg}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Sr}^{2+}$ ,  $\text{Pb}^{2+}$  and  $\text{Ba}^{2+}$  (Brown, P. W. Constantz, B., 1994; Cheng, Z. H. 1998). Synthesized various Hap solid solutions containing  $\text{Ca}^{2+}$  and other cation, such as magnesium-calcium hydroxyapatite (MgCaHap) (Yasukawa, A. 1996). Cobalt is relatively rare element found in the earth's crust with a concentration of approximately 25 mg/g. (Hamilton, E. I., Sci. 1994). Although it occurs naturally in the environment, due to anthropogenic activity excessive amounts of cobalt are released from coal combustion and mining, processing of cobalt-containing ores and the production and use of cobalt chemicals. Cobalt has variety of uses: as a metal in electroplating, because of its hardness and resistance to oxidation, in alloys with iron, nickel and other metals, and also in magnet and stainless steels. Its salts have been used for centuries for the production of blue colors in porcelain, glass, pottery, and enamels, while compounds are used as paint pigments. Radioactive, artificial isotope cobalt-60 is an important gamma-ray source and is used extensively as a tracer and radio therapeutic agent (Schönfeld, E., 2002). In small amounts cobalt is essential for human health, because it is a part of vitamin B<sub>12</sub>. However, higher concentrations of cobalt may damage human health. Soils near mining and melting facilities may contain very high amounts of cobalt.

## 2. Materials and methods

### 2.1. Preparation of HAP

HAP powder were successfully prepared in a 2.45 GHz- 900 W multimode home model microwave oven. The HAP powder was prepared by microwave heating of a mixture that contain both phosphoric acid ( $H_3PO_4$ , adwic 85%) and calcium chloride dehydrate solutions ( $CaCl_2 \cdot 2H_2O$ , Riedel –dehaen, 99-103%) at pH 9.5 by the addition of ammonium hydroxide solution during the mixing process. The mixture was heated in the microwave oven for 6 minutes until complete dryness of the mixture then the precipitates were washed by distilled water and then filtered. The filtered precipitates dried by microwave for one minute.

### 2.2. Preparation of cobalt apatite half and totally substituted:-

Co apatite powders were successfully prepared in a 2.45 GHz - 900W multimode home model microwave oven. The Co apatite powder was prepared by microwave heating of a mixture that contain both phosphoric acid ( $H_3PO_4$ , adwic 85%) and cobalt chloride hexahydrate solutions ( $CoCl_2 \cdot 6H_2O$ , Riedel –dehaen, 99-103%) for totally substituted or microwave heating of a mixture that contain both phosphoric acid ( $H_3PO_4$ , adwic 85%), cobalt chloride hexahydrate solutions ( $CoCl_2 \cdot 6H_2O$ , Riedel –dehaen, 99-103%) and calcium chloride dihydrate solutions ( $CaCl_2 \cdot 2H_2O$ , Riedel –dehaen, 99-103%) for half substituted at pH 9.5 by the addition of ammonium hydroxide solution during the mixing process. The mixture was heated in the microwave oven for 8 minutes until complete dryness of the mixture then the precipitates were washed by distilled water and then filtered. The filtered precipitates dried by microwave for 2 minutes.

### 2.3. Samples Characterizations

The structural characteristics of the powders was investigated by FT-IR (Schimadzu FTIR; 8400S), the phase change and crystallinity were characterized by XRD (Schimadzu 7000), and the microstructure was observed by SEM (Jeol JSM-5400 LV). The particle sizes was also estimated by particle siz analyzer N5 Submicron particle Aize Analyzer Backman Coulter, Miami-Florida 13196, USA.

## 3. Results and Discussion

### 3.1. Factor which affecting on the preparation of HAP:

#### 3.1.1 Effect of pH

The variation of the solubilities of different phases present in these the phase precipitation medium as pH increases may changes the phases structure and the stoichiometry of the following phases.

The XRD patterns given in fig 1, indicate that the formation of HAP states over pH 9. Below this pH other compounds form which exist in the tricalcium phosphate.

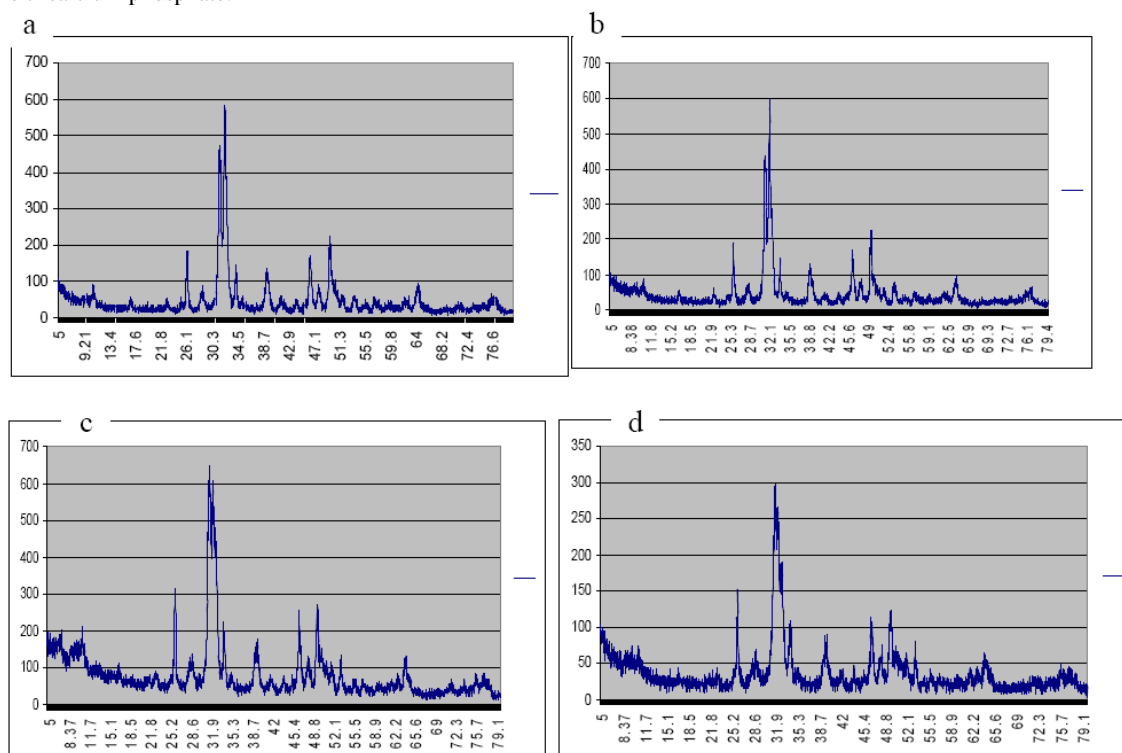


Figure. 1. XRD patterns of prepared HAp nanoparticles at (a) pH 7 (b) pH 8 (c) pH 9 (d) pH 10.

3.1.2 Effect of heating

The powder aged at 60 C displayed better crystallinity and larger crystallite size as show in Fig. 2b but with tricalcium phosphate impurities.

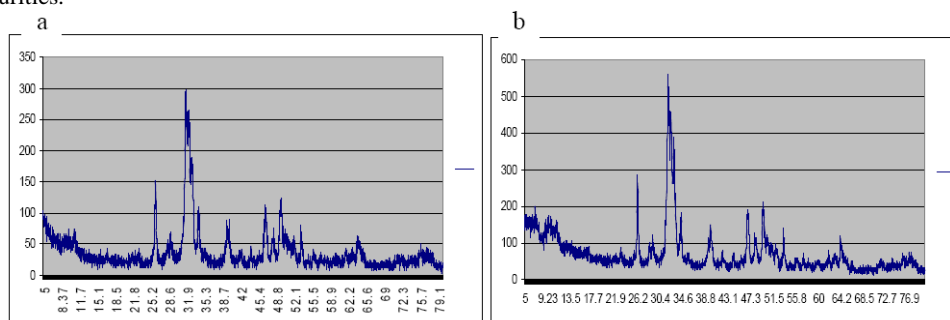


Figure. 2. XRD patterns of prepared HAP nanoparticles at pH 10 (a) without heating (b) with heating at 60 °C.

3.1.3. Effect of component ratio

Two precipitation were prepared at pH 10 one of them 1:1 CaCl<sub>2</sub>.2H<sub>2</sub>O : H<sub>3</sub>PO<sub>4</sub> and other 1.67: 1 CaCl<sub>2</sub>.2H<sub>2</sub>O : H<sub>3</sub>PO<sub>4</sub>. Both powders displayed characteristic HPA peaks as seen from Fig. 3. XRD analyses results showed that at 1:1.67 ratio give crystalline and the 1:1 ratio give amorphous.

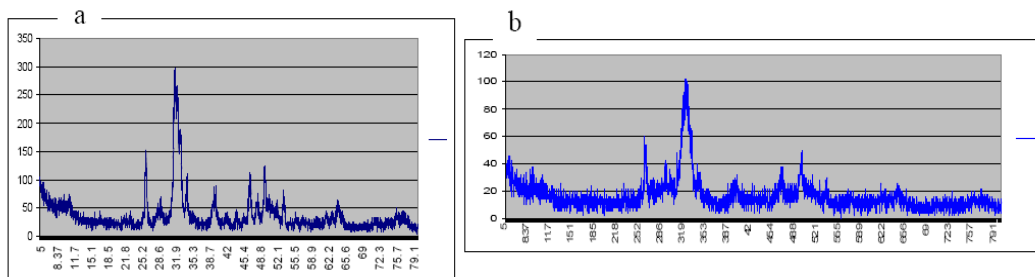


Figure. 3. XRD patterns of prepared HAP nanoparticles at pH 10 (a) component ratio 1.67:1 (b) component ratio 1:1.

3.2. FT-IR spectroscopy.

FTIR spectra of the purified Co-HAP powders are shown in Fig. 4b. these are typical spectra of Hap in Fig. 4a (Table 1). have indicated the vibrational modes of PO<sub>4</sub><sup>3-</sup> groups at 475, 574, 609, 966 and 1020–1120 cm<sup>-1</sup> and OH groups (630 and 3570 cm<sup>-1</sup>) of apatite phase for all the powders. FT-IR patterns also tend to coincide with the results from XRD by the way that the intensity of peak resolution of OH and PO<sub>4</sub><sup>3-</sup> bands are viewed with less intensity with the increase in Co concentration. The presence of adsorbed water could also be detected from FT-IR spectra in the region around 3300–3600 cm<sup>-1</sup>. In addition, some carbonate content also was seen (CO<sub>3</sub><sup>2-</sup> peak around 1600 cm<sup>-1</sup>), which are due to the adsorption of species remaining from the aqueous precipitation (NCCLS. 1993).

Table 1. Infrared assigned for the synthesized hydroxyapatite powder.

Infrared frequency (cm-1)	Assignment
475, 574, 609, 966	PO <sub>4</sub> <sup>3-</sup> bend ν <sub>4</sub>
630, 3570	OH structural
1020–1120	PO <sub>4</sub> <sup>3-</sup> bend ν <sub>3</sub>
3300–3600	OH structural
1660	CO <sub>3</sub> <sup>2-</sup>

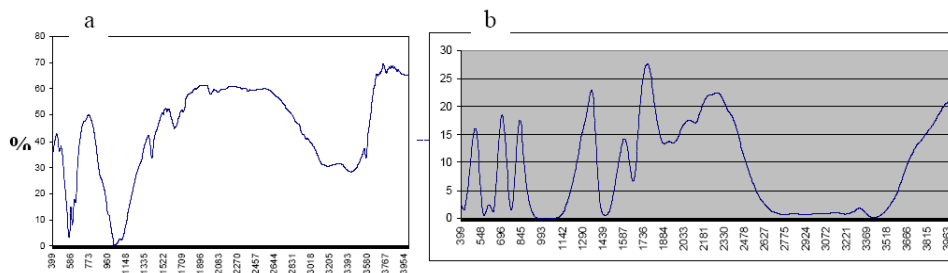


Figure. 4. FT-IR spectra of the (a) synthesized hydroxyapatite (b) Co-Hap complex.

3. 4. X-ray diffraction (XRD)

XRD patterns of the cobalt half and totally substituted by calcium are shown in figs. 5a and b respectively. Co strongly influences the crystallinity, morphology, crystal size and thermal stability of HAp. Pure HAp under the same conditions has well-crystallized perfect crystals. With the increase in Co content, the crystallites become smaller, more irregular and, owing to the size effect, they form agglomerates. In addition, The crystallinity degree progressively decreased with increasing the Co content. Cobalt has a lower ionic radius than calcium and a shorter bond length with oxygen ( Ca-O in HAP is in range 2.443 – 2.555 Å).

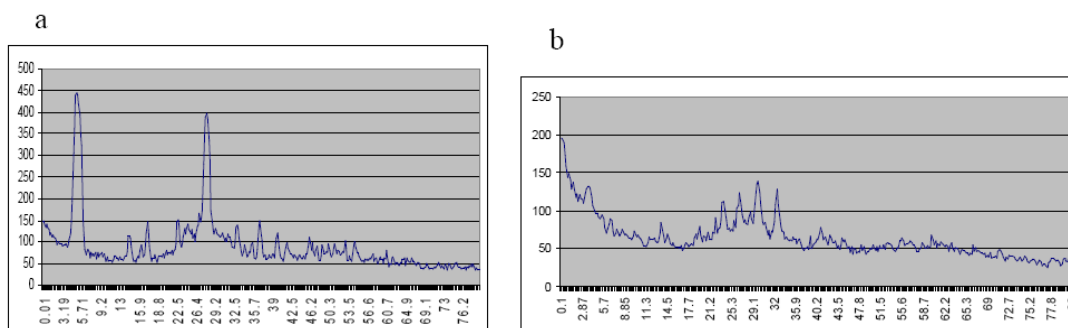


Figure 5. XRD patterns of the cobalt half and totally substituted by calcium (a) Co Ca HAp (b) Co HAp.

3. 5. Scanning Electron Microscopy (SEM).

With the increase in Co content, the crystallites become smaller, more irregular and, owing to the size effect, they form agglomerates. Co has a lower ionic radius than calcium and shorter bond length with oxygen, so Fig. 6a appear smooth and semi-crystalline while the fig. 6b have distortion.

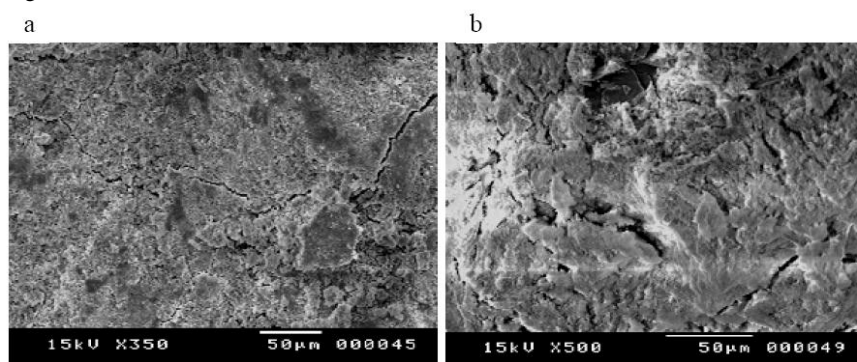


Figure 6. SEM patterns of the cobalt half and totally substituted by calcium (a) Co HAp (b) Co Ca HAp.

3. 6. Biological activity.

The experimental data (Table 2) indicate that the Hydroxyapatite and its Co complexes (Hap, Co apatite, Co Ca apatite) which tested, show a varying degree of activity against Gram-positive bacteria such as *Bacillus cereus*, *Staphylococcus aureus*, and Gram-negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*,. From the results, we show the cobalt apatite complex (totally substituted) has highly antibacterial ability more than others as shown in table.

Table 2. Antimicrobial activity of Hydroxyapatite and its Co complexes (half and totally substituted).

Compound	<i>Bacillus cereus</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>
Hap	-ve	-ve	-ve	-ve
Co Apatite	++	+	++	++
Co Ca Apatite	+	+	-ve	+

4. Conclusion

We have successfully prepared nano-Hap particles and Cobalt (Co) apatite totally and half substituted through a chemical synthesis process, by controlling pH and preparation temperature. The HAp prepared in various conditions. It illustrates that the pH value, preparation temperature and component ratio play an important role in controlling the shape of the synthesized particles. It is interesting that those parameters have insignificant effect on size of the particles. The peak profiles of the XRD patterns may indicate that the crystallinity of the particles varies with the pH value, temperature and component ratio. For examples, the particles prepared over the pH-9 solution at 60°C have high crystallinity than the particles prepared below pH- 9 solution. Also XRD analyses results showed that the crystallinity increase with increase the component ratio of cobalt.

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