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Hydroxyapatite Synthesis and Comparison with Commercial Hydroxyapatite

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Abstract – Hydroxyapatite (HA) is the most commonly used synthetic, inert, and biocompatible material having no organic substance. HAs having calcium phosphate in their structures are biomaterials used in injured organs or tissues in human body. These make quick and strong recovery when used in body. Besides, due to the reproductive characteristics of bone cells like HA structure and the stability of HA material in body fluid they are commonly used in biomedical applications. HA is very similar to the mineral structure of the bones and teeth, and is often used for synthetic bone grafts production. Because of these properties, the synthesis, isolation and commercialization of this kind of materials gain great importance. In this study, HA was synthesized with a precipitation and microwave radiation technique, and then the properties of synthesized HA are compared with the commercial HA. The characterization and comparison of the materials was made with various methods, general internal structure development and pore size relations were determined by FTIR, XRD and SEM.

Keywords -
Hydroxyapatite, Alloplast Graft, Biocompatibility, bioceramic,

1. Introduction

Biomaterials are recently developing topic having great importance. These materials are used to restore the function of injured living organism tissues and are produced from natural materials or synthetically to be compatible with body organs. Recent studies in this topic especially focused on medicine and engineering applications [1]. Biomaterials are used especially in orthopedics and dentistry when the tissues in human body are damaged or when organs lose their function.

In this context, Hydroxyapatite is a biomaterial used in reconstructions of the damaged parts of the body and has hexagonal crystal structure [1]. With $Ca_{10}(PO_4)_6(OH)_2$ (Ca/P

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mole ratio is 1.67) chemical formula and its physical properties, HA is similar to the mineral structure of the bones and the teeth, it also makes the essential inorganic structure of the hard tissues in a living organism. This molecule, due to its biocompatibility and bioactivity, is commonly used in many areas including the production of various prosthesis as artificial bone or bone filler material, repairing the fractured and broken bones, coating of the metallic biomaterials, orthopedic applications, bone filler material (which does not require long stability) applications, tooth implants and controlled drug delivery systems [2]. HA is also used as filling material in lacuna (the cavity in bone) during tumor surgery and as a bridge to cover the defect in the bone during the broken bone repair, in feeding the tooth root in dentistry and in implant coatings. Hydroxyapatite as being used as coating in many medical applications also increases the adhesion of the polymer and the bone formation [3-6]. In addition, since it can perfectly absorb many elements, it has been used as an absorbent during the chromatography for purification and separation. Hydroxyapatite is also used in protein purification, DNA isolation, separation of sub-classes of enzymes and nucleic acids. Beside these, the use of porous HA blocks and HA ceramics as various treatment agents in continuous-release drug delivery systems is being investigated. It has been reported that the use of HA in the preparation of the antibiotic and anticancer drugs causes slow release [7].

Hydroxyapatite is present in body fluid and it has a quite stable structure. This compound can stay without decomposing at high temperatures and it shows high bioactivity due to their solubility characteristic [8]. Especially, glass-ceramics consisting of hydroxyapatite bioglasses and hydroxyapatite crystals do not cause any unwanted reaction in body and generally cause a minimal tissue reaction as bonding to the bone chemically, make an hard and reliable bone/implant interface, and do not cause any cell lost in a tissue covering them. Most of them are in hydrophilic structure. Nowadays, bioceramics are successfully used in most parts of the body, and as a result of continuing investigations, the given applications increase each passing day [9-13].

The bone is a living material consisting of cells and a blood support. Not leaving any scar from the beginning to the end of the life is an indicator of a unique talent of self-perpetuation and reshape, and an example of a tissue as being dynamic. Besides providing the mechanical stability in the body, it is a mineral storage as a matter of calcium and phosphate. The bones constitutive the skeletal system of the living creatures, are in natural composite structure supported by HA crystals in collagen anaphase. Even if depending on the species, age and the gender, by weight, the bone is approximately 25% water, 45% inorganic mineral salts (calcium phosphate, calcium carbonate, magnesium phosphate, and a little amount of sodium and iron) which make the hard structure of the bone, and 30% organic substances on the other hand make the flexibility. The main structure of the bone's mineral phase consists of calcium, phosphate and oxygen atoms. The mineral in the bone is a sort of calcium and phosphate apatite which is essentially similar to the crystal structure of HA. The organic part of the bone consisting of collagen as a main component has a soft nature and the component providing the hardness of the bone is hydroxyapatite [2].

There are different methods which can be used to synthesize hydroxyapatite such as precipitation, solid-state, hydrothermal, sol-gel, and reverse micelle in literature. These methods are preferred depending on the characteristics of the easiness of the process, low cost, obtaining the pure product in an intended stoichiometry, not needing any complex equipment. Out of these, while solid-state reactions provide stoichiometric synthesis of well-crystallized HA powder, long time heat process at high temperature increases the

process cost. It was seen in many investigations that the methods to synthesize this compound are expensive and performed at multiple steps. Investigations performed showed that the most advantageous of the methods is the chemical precipitation. The main advantageous of this method; easy synthesis without needing any complex devices or high temperatures, making the mass production, easy obtaining the synthesis conditions, and easy synthesis of products with intended properties [7-11].

In this study, the methods for hydroxyapatite synthesis in literature were modified and the cheaper and the more suitable method were investigated. HA was synthesized in the Organic Synthesis and Catalysis Laboratory, and the characterization of the synthesized substance was made by using FTIR, XRD and SEM instruments (Figure 1,2,3 and 4). Then, it was compared with commercial HA. In this study, the advantages of the applied synthetic method compared to other methods; are being applicable in room temperatures, the easiness and the practical of the process, the production of the homogeneous and high-purity products.

2. Materials and Methods

In this study, obtaining of the closest product to the commercial hydroxyapatite via optimization under different laboratory conditions was aimed. Normal and microwave-supported precipitation methods were used to obtain the closest product to the commercial HA. As Ca and P source for HA synthesis, calcium nitrate tetra hydrate and diammonium hydrogen phosphate were used. On the other hand, pH adjustment was performed by using ammonium hydroxide. Solution ratios were adjusted to have Ca/P mole ratio of hydroxyapatite as 1.67. Thus, optimum conditions in hydroxyapatite synthesis were determined for low cost, purity, fast and easy method and ideal surface morphology were determined. The characterizations of the synthesized compounds were made by SEM, XRD and FTIR instruments (Figure 1 and Figure 2).

2.1 Experimental Studies

2.2. Synthetic Body Fluid

Using sodium sulphate, sodium hydrocarbonate, sodium chloride, potassium chloride, calcium chloride, hydrogen chloride, disodiumhydrogenphosphate, magnesium chloride, and hydroxymethyl aminomethane, was prepared according to the related literature [7, 11].

2.3. Hydroxyapatite Synthesis

9.86 g of $Ca(NO_3).4H_2O$, and 3.31 g of $(NH_4)_2HPO_4$ were weighed and dissolved in distilled water in two separate 250 mL volumetric flasks. The pH values of the solutions were adjusted to pH≥9 by NH_4OH (0.1 M). Then, the solution was stirred for 15 minutes, and $(NH_4)_2HPO_4$ was slowly added on it. The reaction mixture was stirred for another 4 hours providing pH and temperature control. After the stirring was completed, formation of a white precipitate was observed. For the completion of this precipitation, it was waited for 10 hours. By this, the reactants were completely reacted and the formation of Ca/P mole ratio was provided. Later on, the precipitate was filtered and washed for 3 times with distilled water. Obtained pure precipitate was dried in 100 ^oC oven for 24 hours. Prepared pure HA was powderized and FTIR, XRD and SEM analysis were performed.

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3. Results and Discussion

In order to understand that the produced powder is hydroxyapatite or not, we firstly investigated it by XRD. With analysis performed, it was determined that the compound is HA having carbonate in it. It was determined that all peaks in analysis of HA crystal are completely compatible when compared with the ones of commercial HA. The results of FTIR performed are also compatible with XRD results (Figure 1 and Figure 2). It was determined in our study that the effect of the experiment time on the precipitation and the purity degree is important. By increasing or decreasing of the optimum time, deviation was observed from commercial HA structure. The grain sizes of the synthesized compound were calculated by using microscopic methods. Synthesized HA having nano-sized grains is more practical for biological applications when it is compared with the normal sized one. The precipitation method we applied is the easiest way to obtain the nano structured compounds similar to the commercial HA. The SEM images of the HA we synthesized shows that the grain structures are in nano size

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Hydroxyapatite Sample

Synthesized Hydroxyapatite

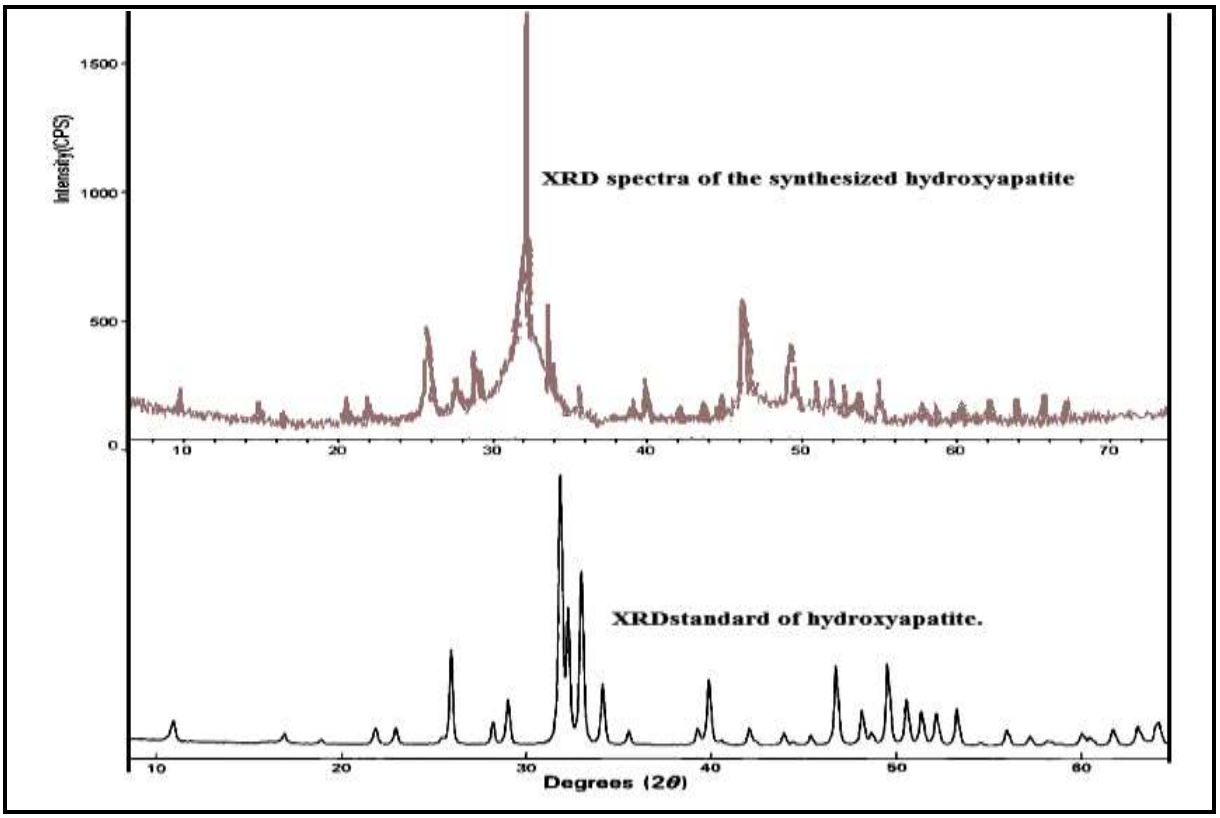

Figure 1. XRD pattern of synthesized and standard hydroxyapatite

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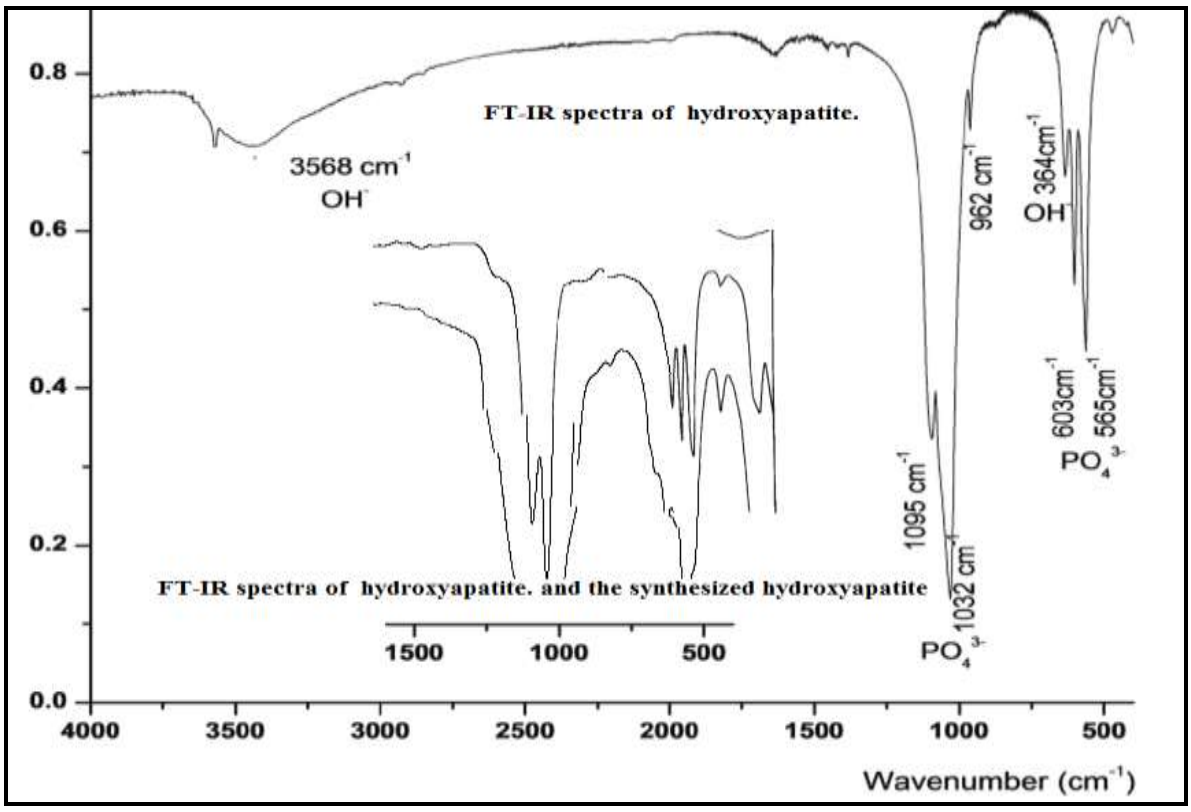
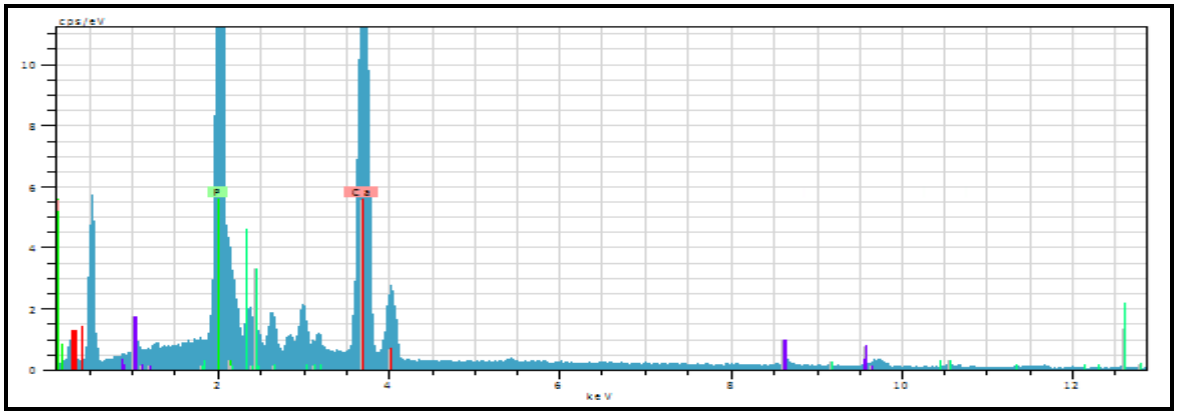


Figure 2. FTIR spectra of standard HA-synthesized HA composite

The SEM images of the synthesized and standard HA crystals structure are shown in Figure 3 and 4. Having a porosity and light roughness in the structure of HA is an important factor in the effectiveness of the bone production. As seen in SEM analysis, some parts are not homogeneous. And this shows that the mixing was not well done. The connections among the pores in the mixture are clearly seen in SEM images.



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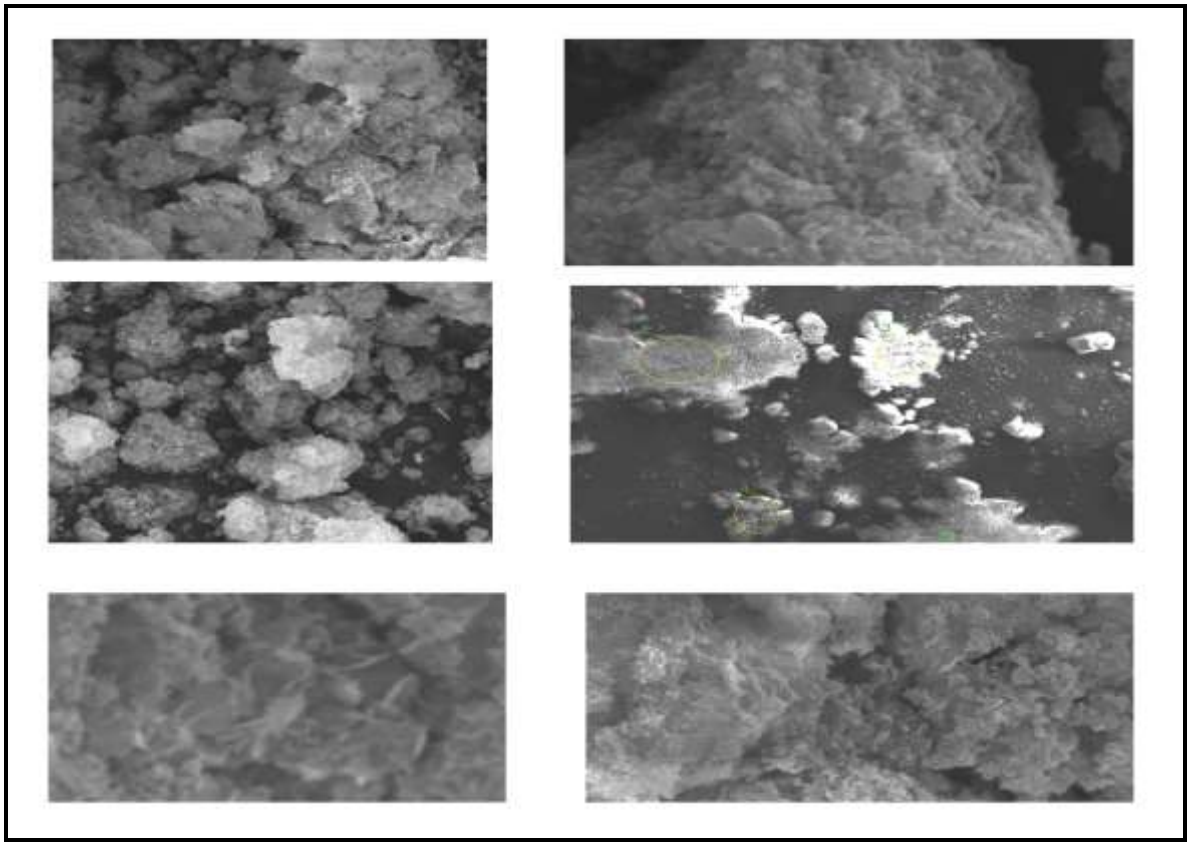

Figure 3. Scanning Electron Micrograph of the synthesized and HA crystals.

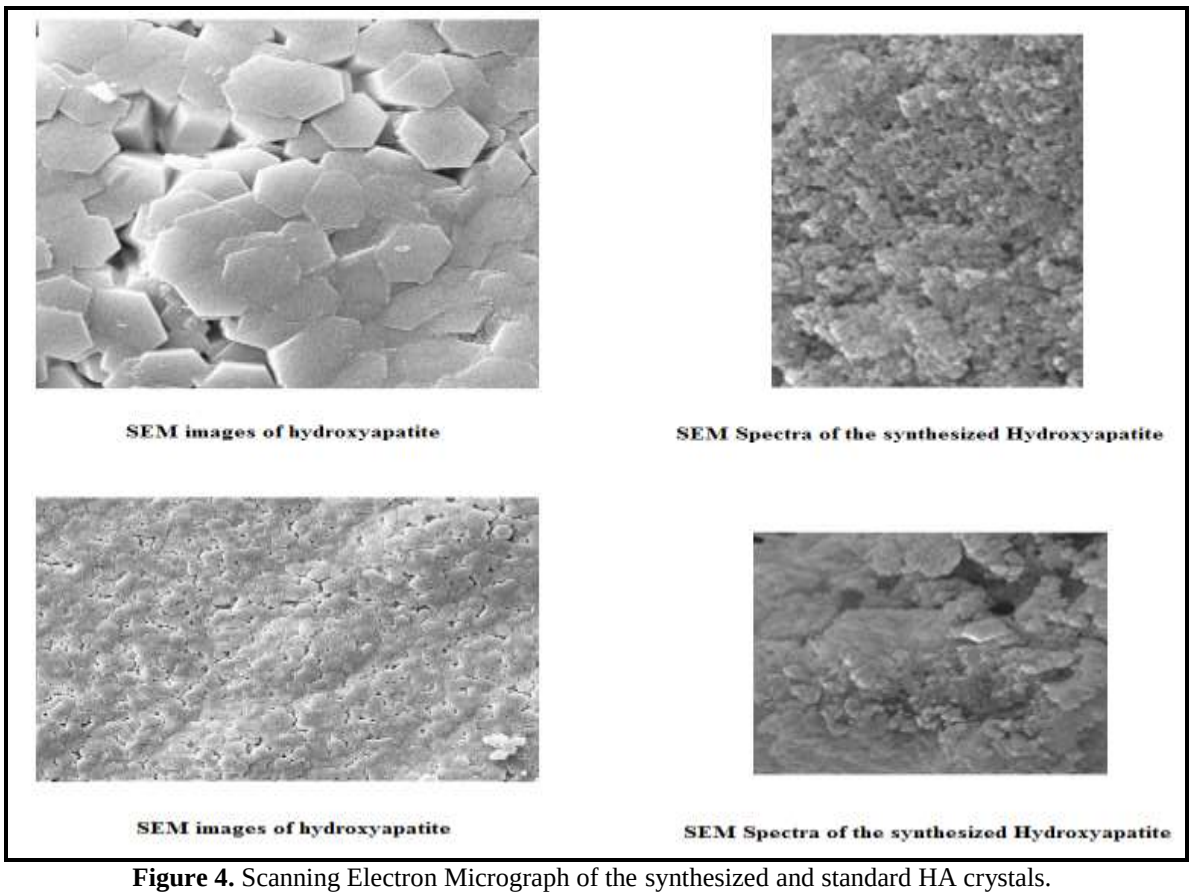

Figure 4. Scanning Electron Micrograph of the synthesized and standard HA crystals.

4. Conclusions

In this study, HA synthesis was made at room temperature by using ammonium dihydrogenphosphate and calcium nitrate. The obtained HA was recrystallized and transformed into granular structure. Later on, it was compared with the commercial HA and the similarities and differences were analyzed. Tests performed showed that HA synthesized in the laboratory are compatible with the commercial HA.

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References

[1] A. Pasinli, R. Aksoy, *Yapay Kemik Uygulamaları için Hidroksiapatit,* BiyoTeknoloji Elektronik Dergisi, 1 (2010), 41-51.

[2] V.P. Orlovskii, V.S. Komlev, S.M. Barinov, *Hydroxyapatite and Hydroxyapatite-Based Ceramics*, Inorganic Materials, 38 (2002), 973–984.

[3] M. Gümüşderelioğlu, *Biyomalzemeler*, Bilim ve Teknik Dergisi, TÜBİTAK, Temmuz özel sayısı, 2002.

[4] O. Güllü, M. Onay, *Metalurji ve malzeme mühendisliğinin bilinmeyen yönü biyomalzemeler*, Hurdacı Dergisi, 3, 2007.

[5] J.B. Park, J.D. Bronzino, *Biomaterials: Principlesandapplications*, CRC press, 2002.

[6] A. Evcin, D.B. Kepekçi, İ. Barut, *Hidroksiapatit Tozlarının Plazma Sprey Yöntemiyle Paslanmaz Çelik Üzerine Kaplanması*, 5. Uluslararası ileri Teknolojiler Sempozyumu (IATS'09), Karabük, 2009.

[7] T. Başargan, *Püskürtmeli kurutucuda hidroksiapatid polimer malzemelerin Hazırlanması*, İTÜ Fen Bilimleri Enstitüsü Yüksek Lisans Tezi, Kimya Mühendisliği Programı, 2010.

[8] T.V. Thamaraiselvi, S. Rajeswari, *Biological Evaluation of Bioceramic Materials*, , Trends Biomaterials of Artifical Organs, 18, 1, (2004), 9–17.

[9] L. Larry, H. and J. Wilson, *An introduction to bioceramics*, World Scientific Publishing Co., 1-25 (1993), 139-189.

[10] P. W. Brown, B.C. *Hydroxyapatite and related materials*, CRC Press, (1994), 3-45.

[11] E. Hasret, *Hidroksiapatit sentezi, karakterizasyonu ve adsorban özelliğinin incelenmesi*, İstanbul Teknik Üniversitesi, Fen bilimleri Enstitüsü, Yüksek lisans tezi, Kimya Mühendisliği, 2010.

[12] A. Afshar, M. Ghorbani, N. Ehsani, M.R. Saeri, C.C. Sorrell, Someimportant Factors in TheWetPrecipitationProcess Of Hydroxyapatite, *Materialsand Design*, 24 (2002), 197–202.

[13] I. Bogdanoviciene, A. Beganskiene, K. Tonsuaadu, J. Glaser, H.J. Meyer, A. Kareiva, *Calcium hydroxyapatite, $Ca10(PO4)_6(OH)_2$ ceramics prepared by*

aqueous sol–gel processing, Materials Research Bulletin, 41 (2006), 1754–1762.

[14] N.Ö. Engin, A.C. Taş, *Preparation of Porous $Ca_{10}(PO_4)_6(OH)_2$ and b-$Ca_3(PO4)_2$ Bioceramics*, J. Am. Ceram. Soc., 83 7 (2000), 1581–1584.

[15] Ş. Yılmaz, G.K. Delikanlı, *Metalik biyomalzemelerde son gelişmeler*, *TİMAK-Tasarım İmalat Analiz Kongresi,* İzmir, 2006.

[16] T.V. Safronova, M.A. Shekhirev, V.I. Putlyaev, D., Tret'yakovYu. *Hydroxyapatite-Based Ceramic Materials Prepared Using Solutions of Different Concentrations*, Inorganic Materials, 43 8 (2007), 901–909.