



Synthesis of Hydroxyapatite / Zeolite Nanocomposite for Medical and Dentistry Applications

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Abstract: Bone is a real nanocomposite which is formed of two organic (collagen) and inorganic (nano-hydroxyapatite) phases. The construction of new composite as bone and tissue substitute is one of the notable topics in bone and tissue engineering. In this study, nano-hydroxyapatite and its composites, including 2.5, 5 and 7.5% (w/v) zeolite were synthesized. XRD and FTIR tests were utilized to investigate the crystal structure and determine the functional groups, respectively. Considering the XRD test, nano-composite of hydroxyapatite-zeolite has more crystallinity compared to the pure hydroxyapatite. Finally, according to the results, the use of 7.5% zeolite for the synthesis of nano-composite of hydroxyapatite-zeolite is recommended as a bone substitute.

Keywords: Hydroxyapatite, Zeolite, Nano-Composite, Tissue Engineering, Bone Substitute

INTRODUCTION

Nanotechnology is the invention and utilization of materials, apparatus, and systems by controlling the materials in the nanometer scale in the atoms, molecules and supermolecular structures. Medical nanotechnology means the utilization of nanotechnology in medical science and healthcare that has the ability to act in the prevention, diagnose, control and reliable and early curing of the diseases. One of the objectives of the medical nanotechnology is understanding of biological processes for diagnosis and diseases curing improvement (Freitas, 2002).

The bone is a real nanocomposite which is a specialized form of the connective tissue for forming the body skeleton system and originates from the mesenchymal stem cells (Gurevich et al., 2002; Krause et al., 2001; Bianchi et al., 2001; Gurevitch et al., 2003). The bone matrix is formed of two important organic and inorganic phases in the nanoscale. The inorganic phase contains the hydroxyapatite, carbonate, citrate, sodium, magnesium, chlorine, fluorine, strontium, lead, zinc, copper and iron and the organic phase contains collagen, water, non-collagen proteins (bone morphogenic proteins, osteocalcin, osteonectin, etc.), cytokine, lipids, polysaccharides (Meyer et al., 2005; Murugan and Ramakrishna, 2004; Kim, Knowles and K. HE., 2005), various types of related cells to the bone tissue (osteoblast, osteoclast, and osteocyte) (Costa-Pinto et al., 2008; Ducy, Schinke and Karsenty, 2000). Losing the tissue and inefficiency of the organs due to the injuries and diseases, in spite of the vast development in medical science, have created serious sanitary problems. In general, about 2.8% of the bone fracture happens in men and 2% of the bone fracture happens in women (Krause, 2001).

Hydroxyapatite is a calcium phosphate that has the properties regarding proper biocompatibility and bioactivity that is very similar to the tooth and natural bone in terms of natural structure and crystalline phase. It has been proved that the nanostructure compound of it has very higher functional specifications compared to its micro-scaled compound. It has been suggested to use the biomaterials that beside the properties regarding proper biocompatibility and bioactivity, compensate the weakness of the low strength and adhesion of the hydroxyapatite. Hydroxyapatite has various applications in medicine and dentistry, such as replacement of bone tissue and coating the body implants. The research has shown that the nanostructured hydroxyapatite shows higher mechanical properties and more proper biocompatibility compared to the micro-scaled samples in the body environment. These properties are optimized when the nanoparticles of hydroxyapatite have more uniform size and shape and minimum agglomeration (Shafiei Zaadeh, 2010).

Despite the bone conduction and good compatibility of nano-hydroxyapatite, it could not be used for the load-bearing scaffolds due to lower mechanical properties and for its reinforcement, it has been suggested to use the biomaterials that in addition to the proper biocompatibility and bioactivity properties, can compensate the low strength and adhesion of the hydroxyapatite. The hydroxyapatite combination with organic and inorganic polymers is one of the desirable materials that has attracted great interest in materials and orthopedic sciences (Marino, Sitinger and Risbud, 2005).

Zeolites are hydrated aluminosilicate that have the cations of alkali metals and alkaline earth metals and have unlimited structure. Among the specifications of these compounds, the cationic exchange, having reversibility capability and water attraction and repulsion without formation of significant change in its molecular structure could be mentioned. Natural zeolites, regularly have negative charge in their surface and so they only have the cationic exchange capability (Bowman, 2003).

There are various techniques for determining the structure, properties and physicochemical specifications of zeolites. The most important methods are: the methods including X-ray like X-ray diffraction (XRD), X-Ray Fluorescence (XRF), Scanning Electron Microscope (SEM), Infrared Radiation (IR), Ion Exchange Chromatography (IEC), etc. In the XRF method, the excitement process is performed by the radiation of the x-ray beam. Under these conditions, the existing elements in the sample are excited by the primary x-ray and emit secondary x-rays that are specific for each element (Kowalczyk et al., 2006).

One of the most important sources of getting structural fundamental information of various compounds is the XRD method. The condition for the happening of the x-ray diffraction could be described by studying monochrome beam diffraction due to the incidence to a plane of atoms or ions. The crystalline compounds that their atomic distances are in the range of the x-ray wavelength can reflect the incident x-ray that this phenomenon is the basis for the structural analysis using the x-ray diffraction method (Ming et al., 1993).

A research was carried out by Nikpour et al. (2013), entitled "chemical synthesis of hydroxyapatite/chitosan nanocomposite for medical and dentistry applications". In order to study the crystal structure and determining the functional groups, the XRD and Fourier-Transform Infrared Spectroscopy (FTIR) tests were utilized. The results of the XRD test showed that the grain size of the pure hydroxyapatite and its composites were all lower than 50 nm (Nikpour, Rabiee and Jahanshahi, 2012). A research was carried out by Zeshki et al. (2012), entitled "Synthesis and investigation of the phase transformation of the hydroxyapatite-silver nanocomposite powder". In order to study the formed phases, thermal stability, microstructure, grain size distribution, and elements distribution in the synthesized nanocomposites the XRD, IR, SEM, Dynamic light scattering (DLS) and elements map analysis were utilized. The results showed that the presence of silver as the reinforcing phase has resulted in the thermal stability decrease of the hydroxyapatite phase and increases its decomposition to the Tri-calcium phosphate phase. Also, the microstructural analyses show that the silver distribution in the matrix was uniform up to 4% of the silver and the grain sizes are in the nano range (Zeshki, 2012). Palanivelu et al. (2013) performed a study about the hydroxyapatite synthesis and spectroscopic specifications by the sol-gel method. The XRD pattern of the hydroxyapatite proves that the

crystal size is lower than 45 nm and the FTIR patterns of the phosphate and hydroxyl groups and SEM images of the hydroxyapatite show the platelets shape with the size in the range of 30 – 75 nm (Palanivelu and Rubankumar, 2013).

There has been high consideration on Nanocomposites due to their excellent bending strength compared to the composites with high grain sizes. The hydroxyapatite-based nanocomposites have great potential for replacement of bones due to the improvement of mechanical and biological properties. The aim of this research is to synthesize the hydroxyapatite/zeolite nanocomposite. The characterization of the surficial properties of the products was performed using the XRD, FTIR and AFM apparatuses.

Materials and Methods

All the chemicals used in this research are of high grade of purity and were used without purification. Also, deionized water has been used for washing and preparing some of the solutions. All the reactions were taken place at room temperature. In this project, the raw zeolite was used that was provided by the Afrand Tooska Company and was the commercial grade clinoptilolite that was obtained from the Semnan district. The di-ammonium phosphate $(\text{NH}_4)_2\text{HPO}_4$ with the molecular mass of 132.05 g/mol, tetrahydrate calcium nitrate $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ with the molecular mass of 236.15 g/mol and also 25% purity ammonia (NH_4OH) with the molecular mass of 17.031 g/mol were used. The performed tests in this research were categorized into two main groups of products synthesis and structural features investigation.

In order to synthesize the hydroxyapatite, at first, the solutions containing calcium and phosphorus were prepared separately by the tetrahydrate calcium nitrate with the chemical formula of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and di-ammonium phosphate with the chemical formula of $(\text{NH}_4)_2\text{HPO}_4$, respectively. The synthesis of the powder was performed in the pH near to 11 and at room temperature. The used reactor contained Pyrex three-spin balloons that were used according to the different volumes. These balloons were positioned on the mixer with the connection to the refrigerant equipped with the cold water input and output in the paraffin bath and in case of requirement of the homogeneous solution preparation, the magnetic stirrers were used.

The required amount of tetrahydrate calcium nitrate and di-ammonium phosphate were weighted with the precision of 0.0001 g by the one-piece digital scale made by A&D Japanese Company. Then, the solutions containing Ca and P were prepared by the demineralized water. In all the synthesis steps, the pH of the solutions was adjusted to the desired value by ammonia. For simultaneous measurement of the pH and temperature, the Swiss-made Metrohm 691 pH meter was used.

The calcium-containing solution transferred to the reactor and the phosphorus-containing solution was gradually added to it using a burette in 60 minutes and drop by drop. All the time that the reaction took place, the solution in the reactor was stirred so the product would form uniformly. At the end of the two solutions combination, the obtained solution was put at room temperature for one night. After the passage of the mentioned time, the obtained solution was filtered, dried and was put in the oven with the temperature of 100°C for 24 hours and was dried at the intended temperature and related process that finally a white powder was obtained (Zhang and Xia, 2009).

In order to synthesize the hydroxyapatite/zeolite nanocomposite, various compounds of zeolite solution were obtained by dissolving 2.5, 5 and 7.5 percent zeolite powder. Then, the tetrahydrate calcium nitrate was added to it in the stirring state and after four hours of vigorous stirring, the di-ammonium phosphate was gradually and in 60 minutes added to it using a dropper and the stirring continued for two more hours so a good and uniform suspension could be obtained. Throughout the process, the pH of the solution was kept constant around 11 using the ammonia solution. Finally, the obtained concentrated suspension was put at room temperature for one night. Then the obtained solution was filtered, dried and was put in the oven with the temperature of 100°C for 24 hours and finally, a white powder was obtained. The synthesized solutions were named as HA/Z 2.5, HA/Z 5 and HA/Z 7.5.

In this research, two methods for analysis and investigation of the obtained materials and composites were used that were: 1- X-Ray Diffraction (XRD) for determining the structure of the nanograins and characterizing the existing phases. 2- Fourier-Transform Infrared Spectroscopy (FTIR) for determining the functional groups of the products.

In order to study the crystal structure, the x-ray diffraction patterns were used that were obtained by the XRD apparatus that works under the voltage of 30 kV and current of 25 mA. The x-ray diffraction method is a method for studying the structure of the crystalline materials and is among the methods that its excitation agent is the electromagnetic waves (with various wavelengths). The x-ray region in the electromagnetic spectrum is between the gamma and ultraviolet rays. Using this range of spectrums, some information about the structure, material and the number of elements could be obtained. Therefore, these x-ray methods have vast applications in analytical chemistry.

In this research, the XRD apparatus of the faculty of metallurgy and materials science of the Semnan University was used.

In order to determine the chemical composition, the FTIR method in the $400 - 4400 \text{ cm}^{-1}$ wavenumber and in the transmission state was used. The FTIR method functions based on the radiation adsorption and investigating the molecules vibration directions and multi-atoms ions. This method is used as a powerful and developed method for determining the structure and calculating the chemical types. The FTIR apparatus has many advantages compared to ordinary IR method due to the utilization of mathematical Fourier conversion that from its examples the high data collection rate and better signal to noise ratio could be stated. The absorbed energy from the infrared light, by the chemical bonds or specific functional groups in specified wavelengths, lead to the reduction of the passing light intensity and is usually considered as a function of the wave number. It's important to attend to this point that all the molecule bonds are not capable of absorbing the infrared light energy, even if the beam frequency matches the movement frequency, only the bonds with bipolar moments can adsorb the infrared beam. In this method, the FTIR apparatus of Islamic Azad University, Ayatollah Amoli branch was used (Perkin Elmer U.S.A model).

Results

The XRD pattern of the raw zeolite is shown in Figure 1. As could be seen in the Figure, the main peak in the 2θ angle for the raw zeolite is in the 22° that clearly shows the crystal structure of the sample. The main crystal structure is related to the SiO_2 and the Kalium (CaO), Hematite (Fe_2O_3) and alumina (Al_2O_3) phases could also be observed. Also, the obtained XRD patterns show that the zeolite in the clinoptilolite type.

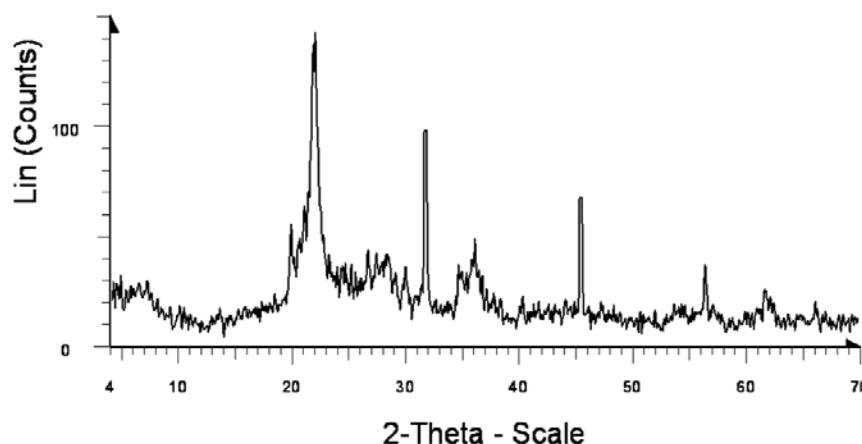


Figure 1: XRD pattern of raw zeolite

The chemical composition of zeolite is shown in Table 1. As could be seen in Table 1, the main elements include Si, O, Al, Ca, Fe, Na, Mg and a small amount of Mn, S, P, and Ti. According to the XRF analysis results shown in Table 1, the used zeolite in this research has 78% of SiO_2 , Al_2O_3 , and Fe_2O_3

Table 1: The determination of the chemical composition of zeolite using the XRF

Composition	SiO_2	AL_2O_3	Fe_2O_3	Cao	Na_2O	Mgo	Mno	P_2O_5	SO_3	L.O.I
Weight percentage (%)	65.5	10.68	1.82	0.51	3.91	1.69	0.011	0.041	0.029	13.29

Figure 2 shows the EDX diagram of the raw zeolite. The main constituent elements of the zeolite are specified in this Figure and the silicon is its main constituent.

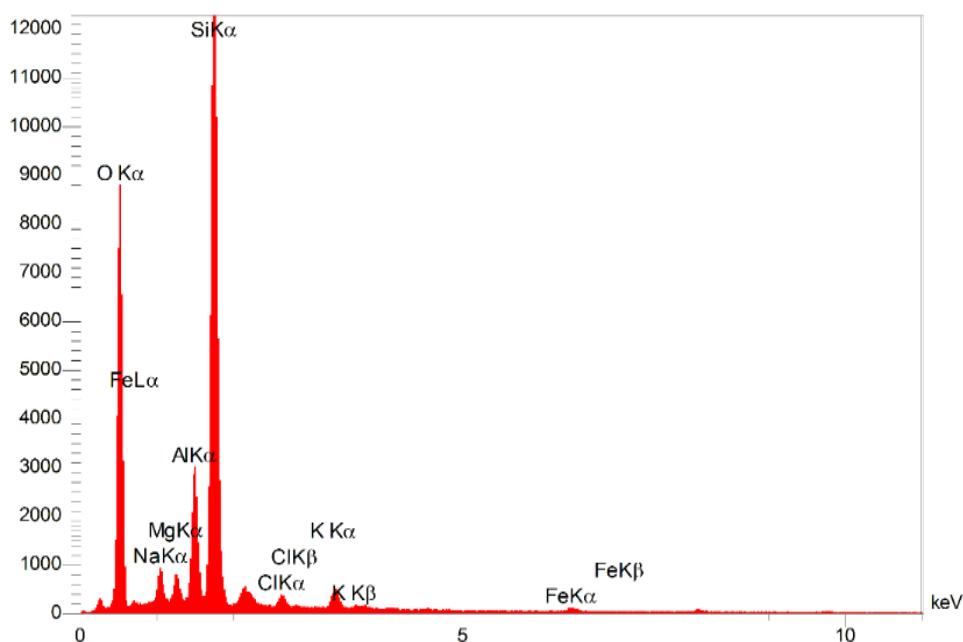
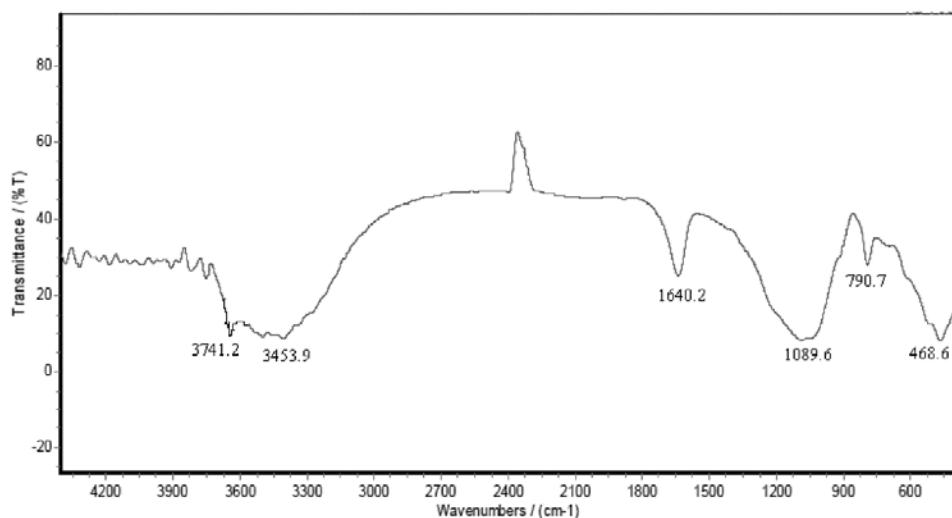


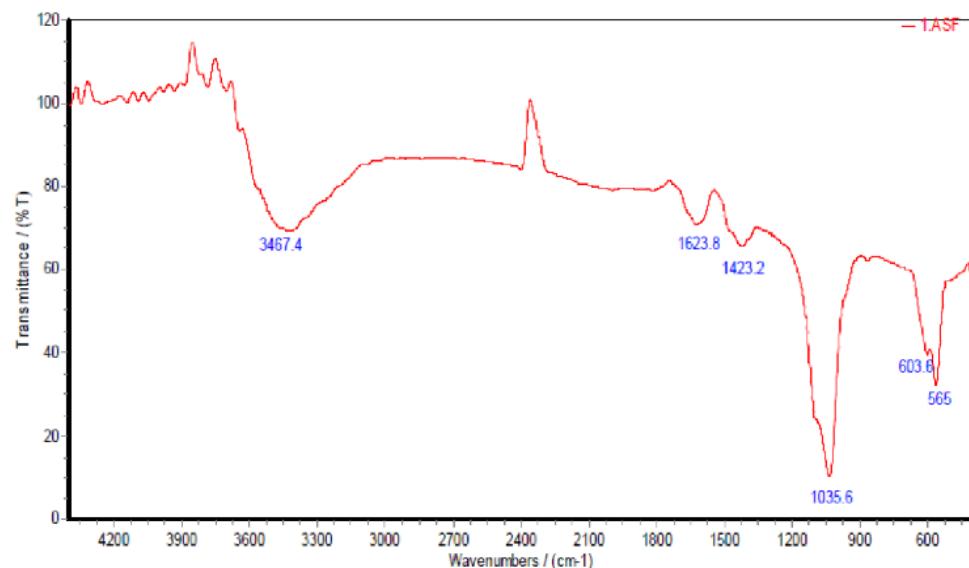
Figure 2: EDX graph of the zeolite

The FTIR spectrum of the nano zeolite is shown in Figure 3. The nano zeolite has the hydrogen bonds in its watery structure in (3741.2 cm^{-1}) and symmetrical and asymmetrical stretching vibration of the OH groups (1640.2 cm^{-1}), a wide weak band that is created by the asymmetrical stretching vibration of the Si – O and Al – O bonds (1089.67 cm^{-1}) and weak bonds from the bending vibration of the O – Si – O and O – Al – O (472.5 cm^{-1}) bonds could be observed in the Figure.

**Figure 3:** FTIR pattern of zeolite

The properties of the synthesized nanohydroxyapatite and the composites produced from it in this research, hydroxyapatite-zeolite nanocomposites containing 2.5%, 5% and 7.5% of zeolite, were studied using the FTIR and XRD tests.

In order to determine the chemical composition, the FTIR method in the transmission state was used. Generally, the results confirm that the synthesized powder has the functional group peculiar to the hydroxyapatite. Figure 4 shows the FTIR spectrum related to the synthesized hydroxyapatite and Figure 5 to Figure 7 show the FTIR spectrum related to the hydroxyapatite-zeolite nanocomposites containing 2.5%, 5% and 7.5% of zeolite. In the produced composites the Si – O – Si bonds and PO_4^{3-} have overlap in the 1100 cm and this bond is related to the asymmetrical stretching vibration. The bond in the 1035 cm is due to the asymmetrical stretching vibration Si – Al – O group. The absorptions in the 790 cm and 601 cm are related to the symmetrical stretching vibration of Si – Al – O and PO_4^{3-} . The peak near to the 3400 cm is related to the adsorbed water or the O – H stretching bond. The peak in 16 is in the bending O – H state.

**Figure 4:** The FTIR spectrum related to the synthesized hydroxyapatite

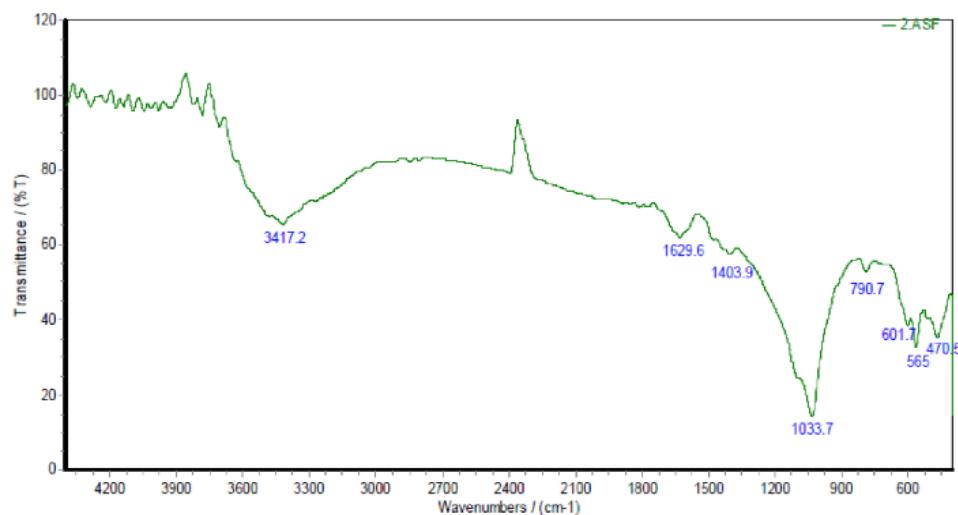


Figure 5: The FTIR spectrum related to the hydroxyapatite-zeolite nanocomposite containing 2.5% of zeolite

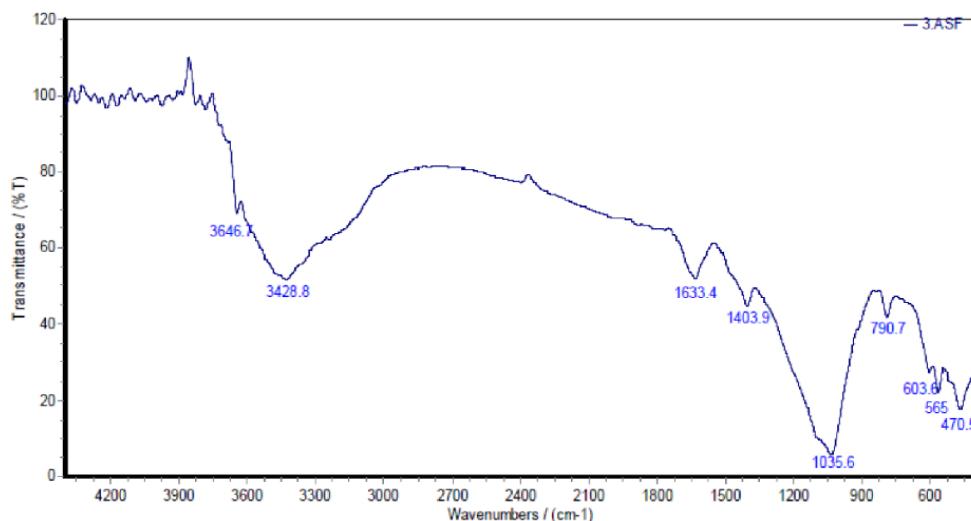


Figure 6: The FTIR spectrum related to the hydroxyapatite-zeolite nanocomposite containing 5% of zeolite

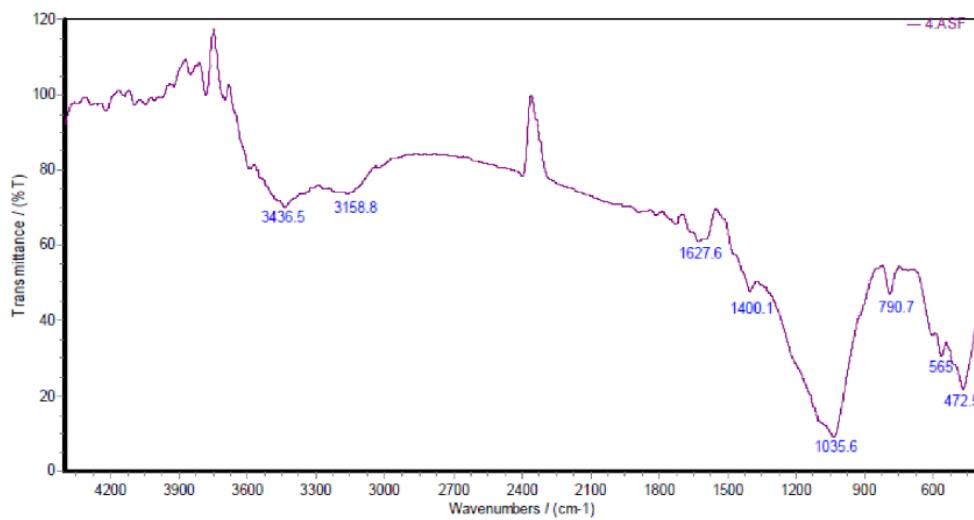


Figure 7: The FTIR spectrum related to the hydroxyapatite-zeolite nanocomposite containing 7.5% of zeolite

Figures 8 to 11 show the XRD test results of the samples. As could be seen, for the HA particles there are peaks in 26, 29 and 32.2 that are related to the (0 0 2), (2 1 0) and (3 0 0) planes of the HA crystal, respectively, that specify the formation and presence of the hydroxyapatite particles and are representative of its high crystalline structure. It could be seen in the Figures that the peaks related to the hydroxyapatite still exist after the formation of the composites but their intensity has decreased and it seems that there is no meaningful difference between their size differences that could probably be related to the good interaction between the nanohydroxyapatite and zeolite matrix and it could be seen that with the increase of the zeolite content, the crystallinity of the compounds increases. Also, it could be observed that another peak is formed in the 22.2 in the composites that is related to the (2 1 1) plane in the hydroxyapatite crystals. With the increase of the zeolite content, the 22.2 peak gets a bigger size that is a representative of the crystallinity increase that could be due to the effective interaction of the nanohydroxyapatite in the zeolite matrix.

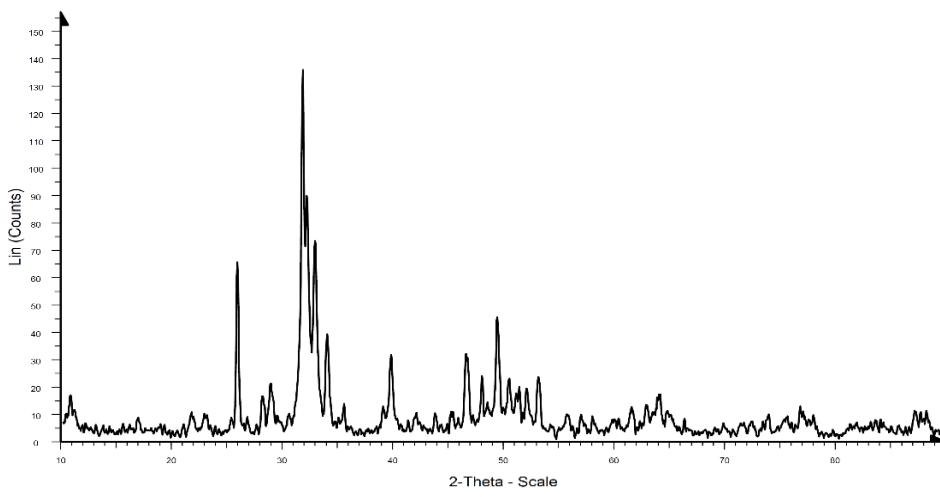


Figure 8: The XRD test results related to pure hydroxyapatite

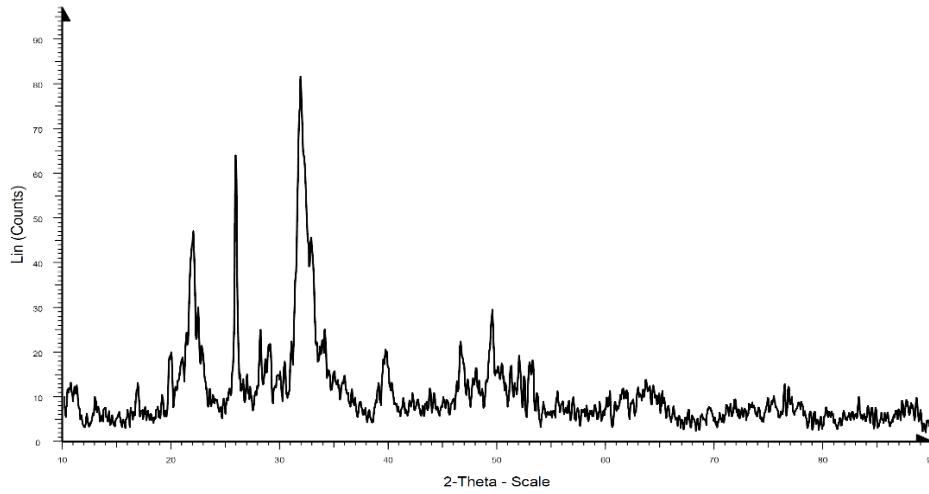


Figure 9: The XRD test results related to the hydroxyapatite-zeolite nanocomposite containing 2.5% of zeolite

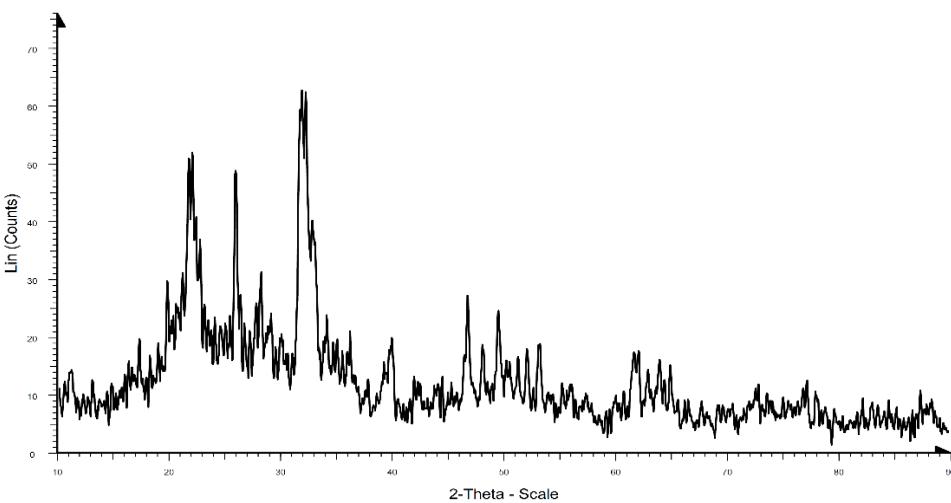


Figure 10: The XRD test results related to the hydroxyapatite-zeolite nanocomposite containing 5% of zeolite

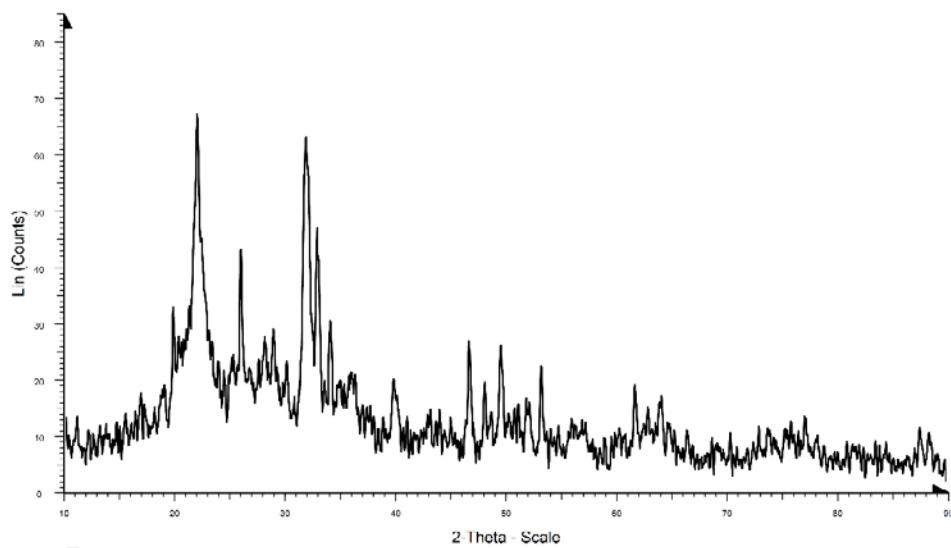


Figure 11: The XRD test results related to the hydroxyapatite-zeolite nanocomposite containing 7.5% of zeolite

Discussion and Conclusion

Various materials and compounds have been used as a replacement for the bones so far and the trend of utilization of new compounds with the aim of construction of superior alternatives and elimination of previous materials deficiencies continues. The research about the bone and tissue replacements show that the nanocomposites could be proper replacements. Overall, the nanocomposites, due to the properties like higher surface to volume ratio and also because of proper properties that were mentioned about the pure hydroxyapatite, zeolite and the composites composed of these two compounds, the research about this area could be helpful. But, there has been no specific research about the construction and study of the hydroxyapatite/zeolite nanocomposite properties as a replacement for the bone and tissue.

Due to the point that bone has the nanocomposite structure containing of about 60% apatite, 90% water and 20% collagen and also since apatite is positioned in it as the very fine blocks with the dimensions of about 5-60 nm and also due to the good and proper properties of zeolite as a scaffold and bone matrix, this research

has investigated the synthesize of the hydroxyapatite/zeolite nanocomposite with various properties of zeolite and the has dealt with the properties of obtained nanocomposites.

The results of the FTIR test shows that the pure hydroxyapatite contains all the related functional groups and is correctly synthesized. Also, the results of the XRD show that the hydroxyapatite/zeolite composite has increased the crystallinity of the pure hydroxyapatite and effective interactions have been created between the pure hydroxyapatite and zeolite.

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