

# Powder Characterization of Calcium Phosphate/Collagen for Bone Implant Application

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**Abstract.** Calcium phosphate (CaP) is a type of bioceramic material that is biocompatible and bioactive. It is usually used for bone implant application but it lacks mechanical strength. Therefore, sodium alginate (SA), a natural polymer, is combined with CaP to improve its properties of the CaP via precipitation method. The powder formed is then characterized by using FESEM, EDX, FTIR and DTA. Based on the FESEM result, it was confirmed that the SA particles were well embedded and homogeneously dispersed throughout the CaP matrix whereas the EDX result showed that the CaP and SA are pure and were not contaminated with other materials or substances. The FTIR result showed that the intensity of some peaks (3100-3600 cm<sup>-1</sup> and 1585-1625cm<sup>-1</sup>) increased due to the addition of SA but for some peaks the addition of SA leads to a decrease in intensity (1650-1300cm<sup>-1</sup> and 1190-976cm<sup>-1</sup>). This proves that the addition of SA in CaP influenced the intensity peaks and it was confirmed that chemical bonds were formed between these two substances. Meanwhile, the DTA result showed that CaP dehydroxylation occurred in the range of 650-1300C and the thermal degradation of SA happened at 245.80C but the SA thermal degradation temperature improved when it was added to the CaP matrix.

## 1 Introduction

Bones play an important part in the human body as they support body tissue and protect human organs. The human bone also acts as mineral storage (calcium and phosphorus) [1]. Bone defects like fractures, cancer, bone loss (osteoporotic) and other debilitating diseases can influence the function of bones in the human body. 60 to 70% of bone tissues consist of mineral calcium, phosphate and collagen, while the remaining percentage consists of inorganic elements and water [2]. Similarities between calcium phosphate (CaP) and the mineral content in bone tissue were the reason why calcium phosphate was chosen for this study [3]. Calcium phosphate (CaP) has been used for repairing bone defects, dental and bone tissue applications in the human body since 1920 [4]. Calcium phosphate (CaP) is

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a type of ceramic material that is biocompatible, non-toxic and resorbable. Furthermore, calcium phosphate (CaP) is a bioactive and osteoconductive material that can promote new bone growth and promote cellular function and expression [3,5]. The lack of the calcium phosphate (CaP) makes it limited in the application of artificial bone implant [3,5,6]. Due to that, the combination of different materials known as composite material have been introduced to improve the properties of calcium phosphate (CaP) [7].

Bio-composites for bone implant application usually consist of a combination of calcium phosphates with polymer materials (collagen, HDPE, PLGA, alginate etc.) or metal (stainless steel, Ti, Mg and etc.) to enhance the properties of CaP. In this study, polymer material is used due to the mechanical properties of the polymer that can be tailored for specific applications and easily fabricated into any desired shape. This gives polymers an edge over ceramics materials (CaP) [8]. However, synthetic polymers like PLGA, PLA, PGA among others can inhibit the deformation of cell growth due to their hydrophobic surface whereas some synthetic polymers are harmful to our body system because they go through acidic degradation and invoke a chronic immune response [9].

Sodium alginate (SA) was chosen as it is a natural polymer that is extracted from brown algae and it is hydrophilic, biocompatible and biodegradable. It is usually used in the food and chemical industry but it has recently been developed for living tissue applications in wound healing, drug and protein delivery [10-12]. Sodium alginate (SA) is soluble in water and that possesses rheological properties. Sodium alginate has been used as a thickening or gelling agent, colloidal stabilizer and as a blood expander in the pharmaceutical industry for a long time [13]. A combination of CaP and SA was prepared using the precipitation method. Precipitation method is one of the simple methods that can easily be done and used to control the morphology and mean size of the powder [14].

The addition of sodium alginate to hydroxyapatite (HA) showed an increase in intensity at 962 and 471  $\text{cm}^{-1}$  that leads to the growth rate of HA [10]. The alginate did not affect the subsequent modification of the characteristic band of calcium titanium phosphate (CTP) and HA. This showed that both ceramics were maintained in the microsphere [13]. A study by Kui Zhou showed that porous HA/SA 3D material was formed after weight loss occurred three times at 100°C, 300 to 400°C and 600-1300°C respectively. This was confirmed by the DSC-TG graph [15]. During the SEM analysis, it was found that the HA particles were homogeneously dispersed and well embedded throughout the SA particles [10,13].

Based on the literature, it was shown that HA has been used in the study of composite materials. However, in previous studies, calcium hydrogen phosphate was not widely used in combination with SA to produce composite material. The aim of this study is to explore the effect of calcium hydrogen phosphate in the SA filler as a CaP/SA composite with varying weight percentages of SA.

## 2 Sample Preparation

### 2.1 CaP/SA sample preparation

Calcium hydrogen phosphate ( $\text{CHPO}_4$ ) from Fisher Scientific was used as the matrix material and combined with sodium alginate (SA, R&M Chemicals) with different weight percentages at 0.5, 1.0, 3.0, 5.0, 10 and 20 wt% by using the precipitation method. The CaP (99.5wt%) was stirred with distilled water by using a magnetic stirrer at 80°C for 2 hours and then the SA (0.5wt%) powder was added while stirring vigorously. The mixture is continuously stirred for another 2 hours until a paste is formed and the paste will be stored at an ambient temperature for 24 hours. Thereafter, the solution will be dried in the oven at 80°C for 24 hours and then grounded using a mortar and pestle to form a fine powder.

The same procedure was used to prepare other composite materials, namely 1.0, 3.0, 5.0, 10 and 20wt%.

## **2.2 Sample morphology and characterization**

The microstructure of the powder was obtained by using field emission scanning electron microscope (FESEM) (JEOL JSM-7600F). The samples need to be prepared before running the FESEM analysis. The sample was coated with gold by using a sputter coater for 45seconds with 20-mA current and images of the microstructure were taken.

The Fourier transform infrared spectroscopy (FTIR) (Perkin Elmer Spectrum 100 Machine) was conducted to obtain the characteristic peaks of the chemical bond of the pure CaP and CaP/SA composites. The powder was analyzed by using the Perkin Elmer Spectrum 100 machine at a resolution of  $4\text{ cm}^{-1}$ .

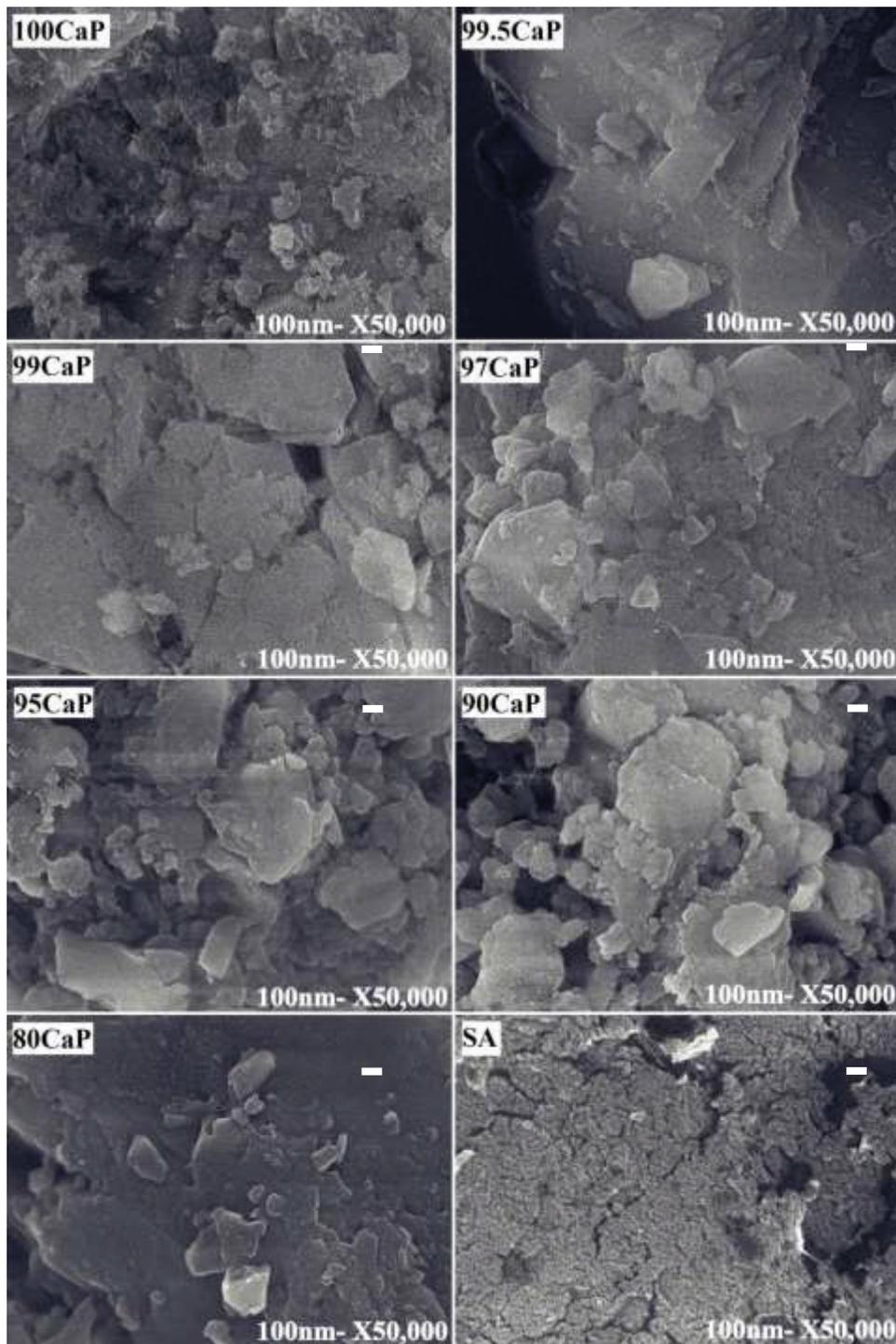
## **2.3 Thermal analysis**

Differential thermal analysis (DTA) (DTA L62) was conducted to analyze the phase reaction and phase diagram at different temperatures. The sample powder was filled into 1/3 of a crucible and weighed. The crucible was put into the furnace where the temperature and heating rate were set at  $1300\text{ }^{\circ}\text{C}$  and  $10\text{ min}/^{\circ}\text{C}$  respectively.

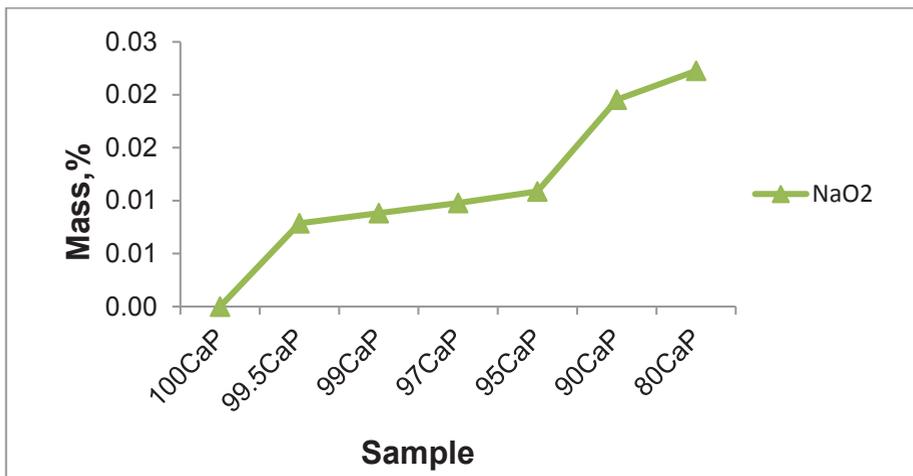
# **3 Result and Discussion**

## **3.1 Field emission scanning electron microscope**

Figure 1 shows the FESEM image for the pure CaP, SA and the CaP/SA composite. The structure of the CaP/SA particles showed that when there is an increase in the SA content, more flakey structures were formed in the particles caused by the structure of SA particles (Figure 2). All the combinations showed that the SA was identified with CaP because the SA fully covered the CaP surface [10,13]. The EDS results (Figure 2) showed that the sodium (Na) peak was identified in the composite and the extensive peak was formed when a higher wt% of SA added.



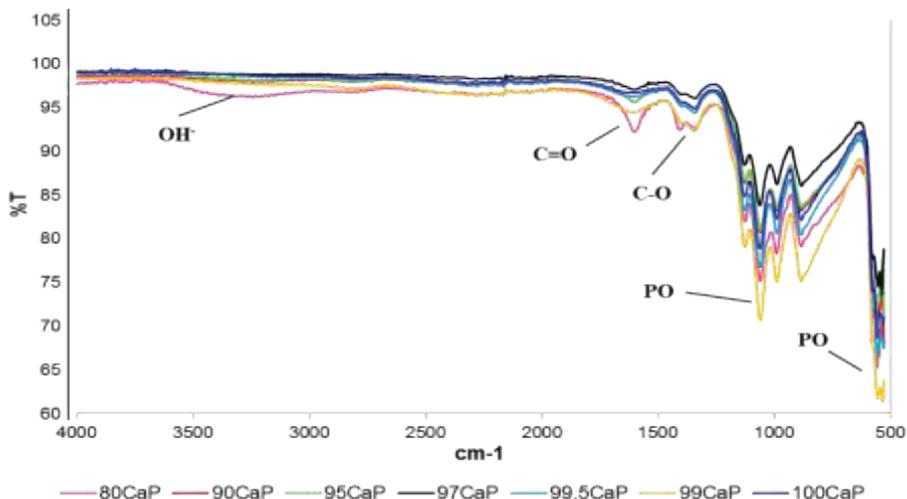
**Fig. 1.** FESEM image for all compositions of CaP/SA in X50,000



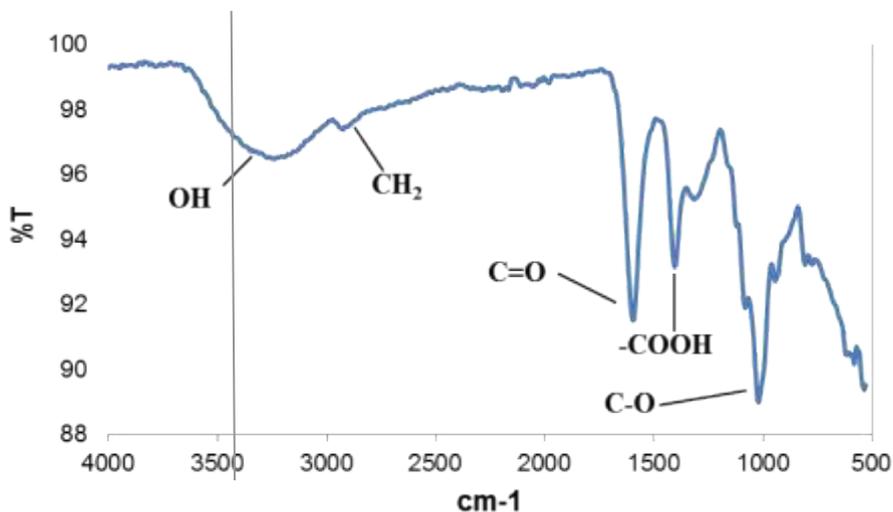
**Fig. 2.** Mass percentage of the Sodium (NaO<sub>2</sub>) for CaP/SA composite

### 3.2 Fourier Transform Infra-Red (FTIR)

FTIR spectra for the composite (CaP/SA), pure SA and CaP are shown in Figure 3 and Figure 4. The broad peak was detected in pure SA at range 3100-3600 cm<sup>-1</sup> due to water (H<sub>2</sub>O) absorption and stretching mode of surface hydroxyl groups [10,16]. Carboxyl (C=O) stretching vibration was detected at 1590-1750 cm<sup>-1</sup> [16] where the intensity of the peaks increased when there is an increase the SA content in the CaP/SA composite sample and the peak was more extensive for sample 80 CaP. The peak that was observed in the region between 1405 to 1456 cm<sup>-1</sup> and 1025.4 cm<sup>-1</sup> belongs to the carboxyl -COOH stretching band [16] and C-O stretching vibration [17] respectively where the peaks were detected in pure SA only. The peak V<sub>3</sub>, hydroxyl stretch carbonate and phosphate were observed between 1300 and 1650 cm<sup>-1</sup> [18] 976 and 1190 cm<sup>-1</sup> [18,19] respectively, where the highest peaks was observed for sample 99CaP which contains 1wt% of SA but the increasing wt% of SA led to a decrease in intensity. The peak for C1-H deformation manunuronic acid residue and hydroxyl stretch phosphate V<sub>4</sub> was observed between 878.9-882.4 cm<sup>-1</sup> [17] and 520-600 cm<sup>-1</sup> [18, 20, 21] respectively where both of these peaks were observed in sample 97CaP. Based on the results, the addition of the SA influenced the intensity peaks of the composite where the formation of chemical bonds occurred between both materials.



**Fig. 3.** FTIR result for pure CaP and CaP/SA for all compositions

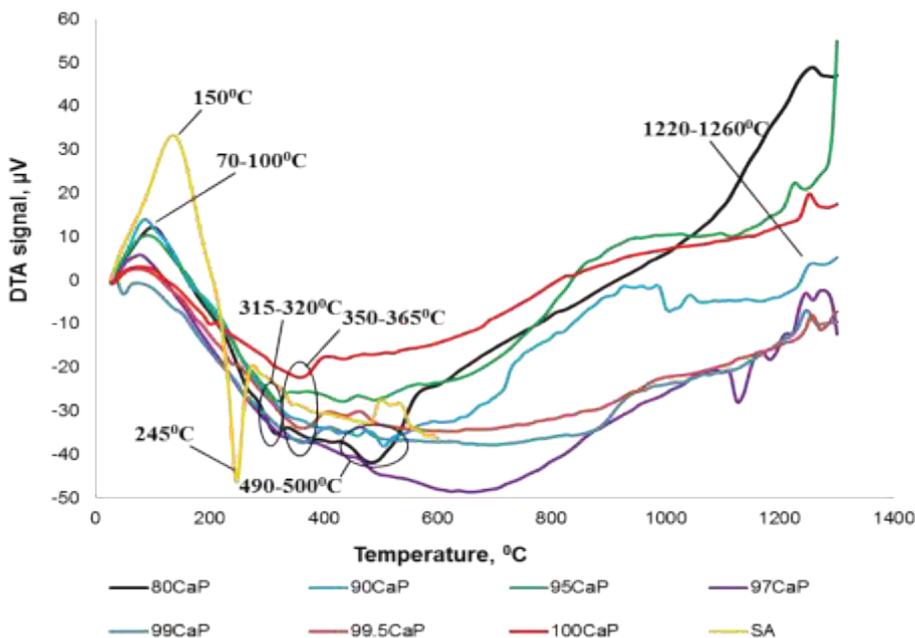


**Fig.4.** FTIR result for pure SA

### 3.3 Differential thermal analysis

DTA results for pure SA, CaP and CaP/SA composite are shown in Figure 5. Based on the figure, an exothermic peak was detected between 100°C and 70°C and the peak was also observed in pure SA at 150°C where oxidation occurred when the temperature started to increase and caused a change in the crystal structure of the SA as well as the loss of water in the samples [15, 21, 22]. The results showed the temperature approached 150°C (SA sample) when the wt% of SA was increased in the CaP/SA composite. A range between 240 until 360°C was observed as endothermic peaks occurred for all samples. A pure SA peak was detected at 245.8°C where thermal degradation of intermolecular side chains took place [21]. This also affected the CaP/SA composite where 5wt% of SA achieved a higher

temperature but the temperature decreased when a higher wt% of SA was added to the CaP. The crystal structure changed and chemical composition reduction was observed in pure CaP where the endothermic peak was detected at 357.8°C. The broad exothermic peak was induced at a range of 1100 to 1250°C where the highest peak was obtained at 100CaP and the peak gradually disappears when the wt% of SA increases. This peak occurred due to the dehydroxylation of CaP in the range of 650-1300°C and the break down reaction of SA [15].



**Fig. 5.** DTA pattern for the pure CaP and SA and CaP/SA composite.

## Conclusion

In conclusion, the addition of SA to CaP was successful as chemical bonds formed between the two substances as proven through the FTIR and FESEM results. Meanwhile, the thermal analysis results showed that CaP as the matrix can improve the properties of SA where the degradation temperature for SA increased when it was added to CaP.

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## References

1. C. VanPutte, J. Regan, A. Russo, Seeley's Essentials of Anatomy and Physiology, Chapter 6. Skeletal System: Bone and Joints (2013)
2. T. M. Keaveny, E. F. M., O. C. Y., Chapter 8: Bone Mechanics. Standard Handbook of Biomedical Engineering and Design, 1-28 (2004)
3. S. Ghosal, Densification and Mechanical Characterization of Pure and Doped Hydroxyapatite, National Institute of Technology, Rourkela, Degree Thesis (2012)
4. S. Sanchez-Salcedo, D. Arcos and M. Vallet-Regi, Upgrading Calcium Phosphate Scaffolds for Tissue Engineering Application, Key Engineering Materials. 377, 19-42 (2008)
5. C. Zhuo, X. Ye, Y. Fan, L. Ma, Y. Tan, F. Qing, X. Zhang, Biomimetic Fabrication of Three-Level Hierarchical Calcium Phosphate/Collagen/Hydroxyapatite Scaffold for Bone Tissue Engineering, Biofabrication. 6, 035013(2014)
6. L. Biolet, V. Lardot, A. Tricoteaux, A. Leriche, F. Cambier, M. Descamps. Processing and Properties of Calcium Phosphates Bioceramics by Hot Isostatic Pressing., MATEC Web of Conference. 7, 04020 (2013)
7. J. Hashim, SainsBahan. 1stedition. Johor Bahru:UniversitiTeknikal Malaysia (UTM) Press, pp. 212-258
8. G. Turco, E. Marsich, F. Bellomo, S. Semeraro, I. Donati, F. Brun, M. Grandolfo, A. Accardo, S. Paoletti, Alginate/Hydroxyapatite Biocomposite for Bone Ingrowth: A Trabecular Structure with High and Isotropic Connectivity. Biomacromolecules. 10, 1575-1583 (2009)
9. G. Chen, T. Ushida, T. Tateishi, A Biodegradable Hybrid Sponge Nested with Collagen Microsponges. John Wiley & Sons, Inc. (2000)
10. M. Rajkumar, N. Meenakshisundaram, V. Rajendran, Development of Nanocomposite based on Hydroxyapatite/Sodium Alginate: Synthesis and Characterization. Materials Characterization. 62, 469-479. (2011)
11. Y. L. Kuen, D. J. Mooney. Alginate: Properties and Biomedical Application. Progress in Polymer Science. 37, 106-126 (2012)
12. M. H. Sheridan L. D. Shea, M. C. Peters, D. J. Mooney, Bioabsorbable Polymer Scaffolds for Tissue Engineering Capable of Sustained Growth Factor Delivery. Journal of Controlled Release. 64, 91-102 (2000)
13. C.C. Ribeiro, C. C. Barrias, M. A. Barbosa, Calcium Phosphate-Alginate Microspheres as Enzyme Deliver Matrices. Biomaterial. 25, 4363-4373 (2004)
14. I. Mobasherpour, M. S. Heshajin, A. Kazemzadeh, M. Zakeri, Synthesis of Nanocrystalline Hydroxyapatite by using Precipitation Method. Journal of Alloys and Compounds. 430, 330-333 (2007)
15. K. Zhuo, X. Zhang, Z. Chen, L. Shi, W. Li, Preparation and Characterization of Hydroxyapatite-Sodium Alginate Scaffolds by Extrusion Freeforming., Ceramic International. 41, 14029-14034 (2015)
16. Z. Li, H. R. Ramay, K. D. Hauch, D. Xiao, M. Zhang, Chitosan-Alginate Hybrid Scaffold for Bone Tissue Engineering, Biomaterials. 26, 3919-3928 (2005)
17. S. K. Papageorgiou, E. P. Kouvelos, E. P. Favvas, A. A. Sapalidis, G. E. Romanos, F. K. Katsaros, Metal-Carboxylate Interactions in Metal-Alginate Complexes Studied with FTIR Spectroscopy. Carbohydrate Research. 345, 469-473 (2010)
18. I. Rehman, W. Bonfield, Characterization of Hydroxyapatite and Carbonate Apatite by Photo Acoustic FTIR Spectroscopy. Journal of Materials Science: Materials in Medicine. 8, 1-4 (1997)

19. A. Ślósarczyk, Z. Paszkiewicz, C. Paluszkiewicz, FTIR and XRD Evaluation of Carbonated Hydroxyapatite Powder Synthesized by Wet Methods. *Journal of Molecular Structure*. 744-747, 657-661 (2005)
20. L. Berzina-Cimdina, N. Borodajenko, Research of Calcium Phosphate using Fourier Transform Infrared Spectroscopy, *Infrared Spectroscopy-Material Science, Engineering and Technology*. Pp 123-148 (2012)
21. I. S. Neira, Y. V. Kolen'ko, O. I. Lebedev, G. V. Tendeloo, H. S. Gupta, F. Guitian, Y. Masahiro. An Effective Morphology Control of Hydroxyapatite Crystals via Hydrothermal Synthesis, *Crystal Growth and Design*. 9, 466-474 (2009)
22. X. Chaobo, L. Yongshang, L. Hongjuan, Z. Lina, Note: Preparation and Physical Properties of Blend Films from Sodium Alginate and Polyacrylamide Solutions. *Journal of Macromolecular Science: Pure and Applied Chemistry*. A37 (12), 1663-1675 (2000)
23. A. A. Said, Thermal Decomposition of Some Divalent Metal Alginate Gel Compound, *Polymer Degradation and Stability*. 39, 393-397 (1993)