

MARINE SCIENCE AND TECHNOLOGY BULLETIN

Surface modification with P-aminohippuric acid on biogenic apatite (fish bones) particles.

Evren Tan¹, Bayram Kizilkaya^{1*}, Nail Ucyol¹, Hasan Basri Ormanci¹, Ayhan Oral²

¹Faculty of Marine Science and Technology, Canakkale Onsekiz Mart University, Canakkale, Turkey

²Faculty of Science and Art, Çanakkale Onsekiz Mart University, Canakkale, Turkey

ARTICLE INFO

Article history:

Received: 14.11.2014
Received in revised form: 19.12.2014
Accepted : 22.12.2014
Available online : 31.12.2014

Keywords:

Fish bone
P-aminohippuric acid
Surface modification

ABSTRACT

The aim of this study with fish bones as a waste in fish processing is to investigate whether they can provide more efficient and multi-functional materials by chemical modification. As modification chemical, p-aminohippuric acid was selected and modified on the bone particle surface by esterification method. The results showed that surface modification was performed successfully. P-aminohippuric acid bonded on the surface of bone was calculated as 190.64 µmol/g. The point of zero charge (PZC) of bone apatite (H) and modified bone (HA₅) was investigated and determined as 7.25 and 6.80, respectively. SEM-EDS spectrums showed that nitrogen element of p-aminohippuric acid on the surface of HA₅ could be observed in EDS spectrum clearly. Additionally, nitrogen amount of H and HA₅ was detected as 8.037% and 8.565%, respectively.

Introduction

Bones are generally composed of fibered protein-collagen which is an organic composition (30%) and inorganic hydroxy apatite Ca₁₀(PO₄)₆(OH)₂ (HAP) (about 70 %) (Kizilkaya et al. 2010; Dimovic et al. 2009). As an alternative, bones are natural materials with low costs when compared to synthetic HAP as the source of biogenic apatite. A lot of researches have been carried out in order to use waste products in various fields recently.

The removal of HAP cations is enabled with the replacement of calcium ions with ions on the surface (Banat et al. 2000; Ozawa et al. 2003; Smiciklas et al. 2006; Dimovic et al. 2009). Some researchers have been carried out upon the usage of bones which are one of waste products. The usage of animal bones and removal and adsorptions of cobalt (Dimovic et al. 2009), zinc (Banat et

al. 2000), chrome (Chojnacka, 2005), copper and nickel (Alasbeb et.al. 1999) were researched accordingly. It is seen that due to its low solubility in water, high stability in degradation and oxidize, high surface space and the capacity of buffering; HAP is applicable in respect of heavy metal sorption and removal. Synthesizing the synthetic HAP, the removal of cobalt (Smiciklas et al. 2006), lead (Janga et al. 2008), copper (Corami et al. 2008) and cadmium (Zhu et al. 2008) was also investigated in this respect. Besides; using fish bones, adsorption and removal of ions of copper, cobalt and nickel in aqueous solutions were researched. In order to remove metal ions; anchovy, sardine, bogue, bluefish, and sea bream were used. It was treated to the bones with nitric acid, sodium hydroxide, hexane, alcohol, hydrogen peroxide and water so as to remove the fatty acids and contamination. It was understood that the powders of bones purified with NaOH, Hexane, and Ethanol were white and those purified with H₂O, H₂O₂ and NHO₃ were yellow. It was observed that during the adsorption the white colour of fish bone powder transformed into blue, red and yellow, respectively, in solutions of Cu⁺², Co⁺² and Ni⁺² (Kizilkaya et al. 2010; Kizilkaya and Tekinay, 2011).

*Corresponding author

E-mail address: bayram342001@yahoo.com (B. Kizilkaya)

Tel: +90 286 218 00 18 fax: +90 286 218 05 43

For chemical modifications and applications; HAP, synthesized in laboratory, was used. Hydroxy apatites ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, HAP) are materials that have lots of applications and characteristics as adsorbent, catalyzed and biomaterial. High biologic reactivity of apatites can easily lead in change of ion with organic groups such as CO_3^{2-} or RPO_4^{2-} , RSO_4^{2-} and RCOO^- . Besides, P-OH groups, exist on the surface of hydroxyapatite, are very suitable for the adsorption and reaction of mineral and organic types. Organophosphates can have high stabilization within the range of large pH and temperature. Some organophosphate products (PP-HA), occurred by the reaction of phosphanate ($\text{C}_6\text{H}_5\text{-PO}_3^{2-}$) and phosphinate ($\text{C}_6\text{H}_5\text{-HPO}^{2-}$) with $\text{Ca}(\text{OH})_2$ and $\text{NH}_4\text{H}_2\text{PO}_4$ were synthesized on the surface of hydroxyapatite. It was seen that modified products, acquired, had more surface space than hydroxyapatite. While the surface of pure hydroxyapatite is $140\text{m}^2/\text{g}$, surface spaces of the products modified with organophosphate are $267\text{m}^2/\text{g}$ (Hammari et al. 2006).

It is known that synthetic polymers such as polymethylmethacrylate are used as antibiotic transmitter in bones. Afterwards medicine application, surgery is required in order to remove polymethylmethacrylate because it causes some clinical problems within body. Therefore, it is used for the release of natural polymers such as collagen or polylactic acid. As such kind of materials are both biocompatible and biodegradable; they can be easily released from the body innocuously. Hence, remarkable researches have been carried out in order to develop new strategies based on HA/composite materials for a controlled medicine distribution. Tetracycline is a therapeutic molecule and it is frequently used in the treatment of bacterial infections and bone diseases. In this respect using glycidylmethacrylate redox starter, polymerization and modification were applied on the surface of HAP. Glycidylmethacrylate can easily react with amino groups. Tetracycline medicine was added on HAP modified with Poli-glycidylmethacrylate (PGMA). The results showed that HA/PGMA composite material can be used for medicine release as a biomaterial (Murugan and Ramakrishna, 2004).

Also; using stearic acid (Sa), which is another organic acid, synthetic HAP surface was modified. During the modification process, the changes on the surface of HAP were examined using Sa with different percentages between 1% and 11%. The reaction is composed of the esterification reaction between OH groups on the surface of HAP and carboxyl (COOH). It is observed that surface of HAP was modified successfully and after modification the range of particle size changed. It is clearly seen that Sa modifications in different proportions come from aliphatic CH_3 and CH_2 groups at 2954 cm^{-1} according to FT-IR spectrum. It is determined that due to the increase in the proportion of Sa aldehyde, the peak occurred at 1548 cm^{-1} is the asymmetric COOCa vibration bands (Li and Weng, 2008).

In the recent years a lot of studies have been carried out in order to bond polymers on the surfaces of material and upgrade the biocompatibility mechanic characteristics. Using composite poly (lactid-glycolide) (PLGA, LA/GA=80/20), polymerization (g-HAP) and modification were operated on the surface of HAP. The reaction was

performed with the copolymerization of ring expansion in the catalytic tin octoat (SnOct_2). It is observed that the sizes of HAP were between 30 and 40 nm and their mechanic properties were pretty developed (Hong et al. 2006). In another study upon HAP modification, the surface of HAP was firstly bonded with 3-aminopropil-trietoksisilan (APS) by silanization. Afterwards ring expansion polymerization was applied to amine groups in margin that belong to APS molecule benzil-glutamate N-carboxyanhidrite (BLG-NCA) on the surface of HAP. It is understood that composite materials acquired have different dispersion characteristics in dichloromethane (Wei et al. 2009).

Material and methods

Supply of fish bones and pre-treatments

Tuna fish bones provided from Çanakkale Dardanel Factory were used as waste fish bones. Firstly purification and milling and then homogenization were applied to the waste fish bones provided. According to our previous experiences, the most efficient way for cleansing method is using alkali solutions. Accordingly, unprocessed fish bones were treated with mechanic mixer in 60°C with NaOH solution until they were purified from contamination. The cleansing process is completed after the control whether they still had organic or any other residual by the analysis with FT-IR. Finally, the waste fish bones were dried in the incubator and pulverized with the aid of homogenizator and grinder.

Materials and chemicals

In the laboratory experiments, magnetic stirrer-heater with contact thermometer (WiseStir MSH-20D), centrifuge (Nüve NF400), ultrasonic banyo, Ultra pure water (SG, Ultra Clear 2001-B), pH meter (InoLab pH 7110), mechanic mixer (WiseStir HS-120A) were used. P-Aminohippuric acid (SIAL.A1422) was used in the experiments.

In the analysis of the products acquired, elemental analysis (LECO, CHNS 628), Scanning Electron Microscope (SEM-EDS, Jeol), FT-IR (Perkin Elmer, Spectrum One-ATR), DTA-TG (Perkin Elmer-Diomand) were used.

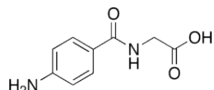
Functionalization of bone surfaces with organic acids

Esterification reaction occurs in acidic environment by extracting a molecule of H_2O via hydroxyapatite-OH group and carboxyl group (-COOH)-OH. Preparing a solution of ester compound 25 mL acetonitrile 0.1 M (2.5×10^{-3} mole) (A_5), a 50 mL flask was taken for the reaction at first. Afterwards 2.5 gr H was added to the solution and refluxed in boiling point for 8 hours in inert atmosphere. The mixture was set aside in room temperature for 12 hours. The mixture was then centrifuged with acetonitrile and ethanol at 2000 rpm, washed five times and finally the modified solid phase was dried at 45°C in incubator. Technical information of p-aminohippuric acid used in esterification reaction is given in Table 1.

Determination of point of zero charge (PZC)

For determination of PZC, 0.01 M KNO_3 solutions were firstly prepared in a 100 ml flask. The initial pH of this

Table 1. The technical information of p-aminohippuric acid used in the esterification reaction.

Chemical	Molecules	M _A	Molecular Formula	Code
p-aminohippuric acid		194.19	C ₉ H ₁₀ N ₂ O ₃	A ₅

solution (PHB) between pH 4 and 10 was adjusted with 0.1 M HCl and NaOH. Then, it was added H and HA₅ products to prepared solution. The solution was stirred at constant temperature with a magnetic stirrer for 48 hours. The pH of the final solution (PH_f) was measured and recorded after 48 hours. PZC was determined with obtained curve against the initial pH between the difference of the initial pH (pH_i) and the final pH (pH_f) ($pH = pH_i - pH_f$).

Results and discussion

Photos of p-aminohippuric acid, bonded on the surface of the bone, are given in Figure 1a. It is clearly seen that unprocessed particles (H) are white and the product HA₅, occurred thanks to organic acid and surface modification, is light yellow and white. These colour differences show that acid modification was successfully applied. Modification scheme of p-aminohippuric acid is shown in Figure 1b.

The point of Zero Charge of H and HA₅ is given in Figures 2 and Table 2. It is obviously seen that H and HA₅ have different breakpoints in x axis according to Figure 2. It is also seen that HA₅ have a better linear curve than H. Besides, the points of Zero Charge of H and HA₅ are 7.25 and 6.80, respectively.

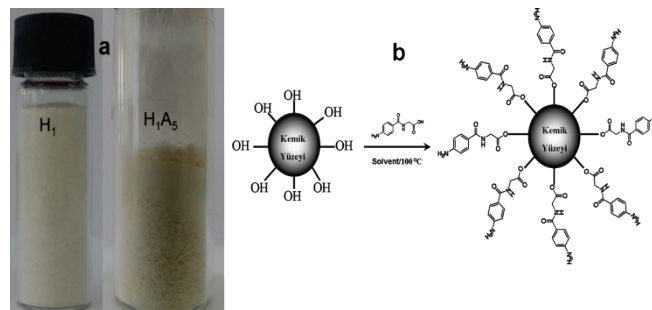


Figure 1. Photos of bone (H) and HA₅ products (a), chemical modification of p-aminohippuric acid over bone surface (b).

Table 2. PZC curves of H and HA₅ products.

	PZC _D	PZC _T	R ²
H	7.25	6.69	0.993
HA ₅	6.80	6.62	0.999

PZC_D: Experimental PZC.

PZC_T: PZC and R² values of experimental data calculated with linear regression.

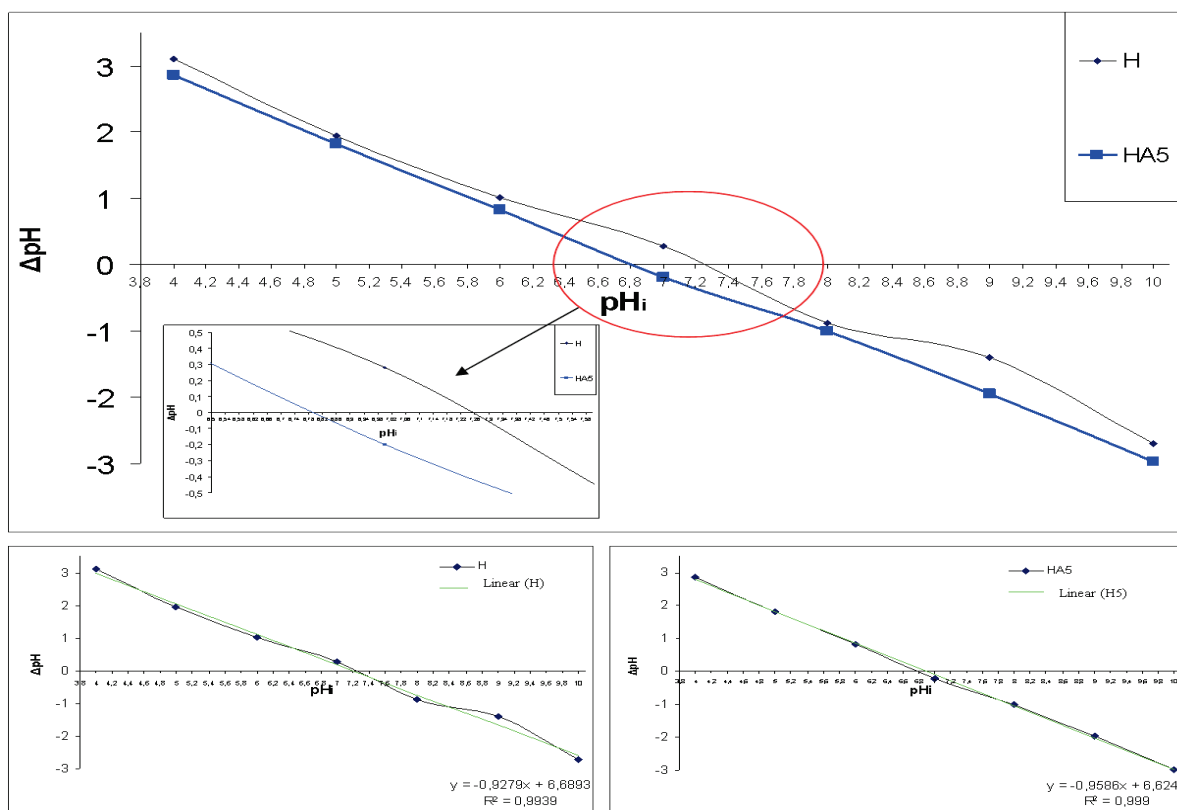


Figure 2. PZC curves of H and HA₅ products.

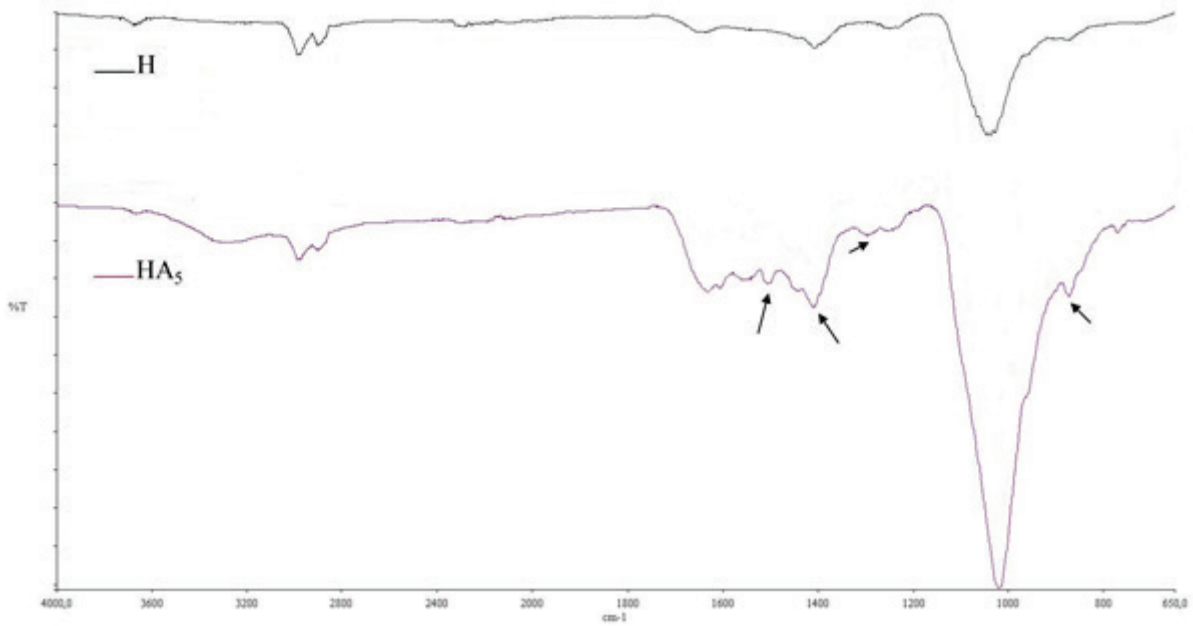


Figure 3. FT-IR spectrums of H and HA₅ products.

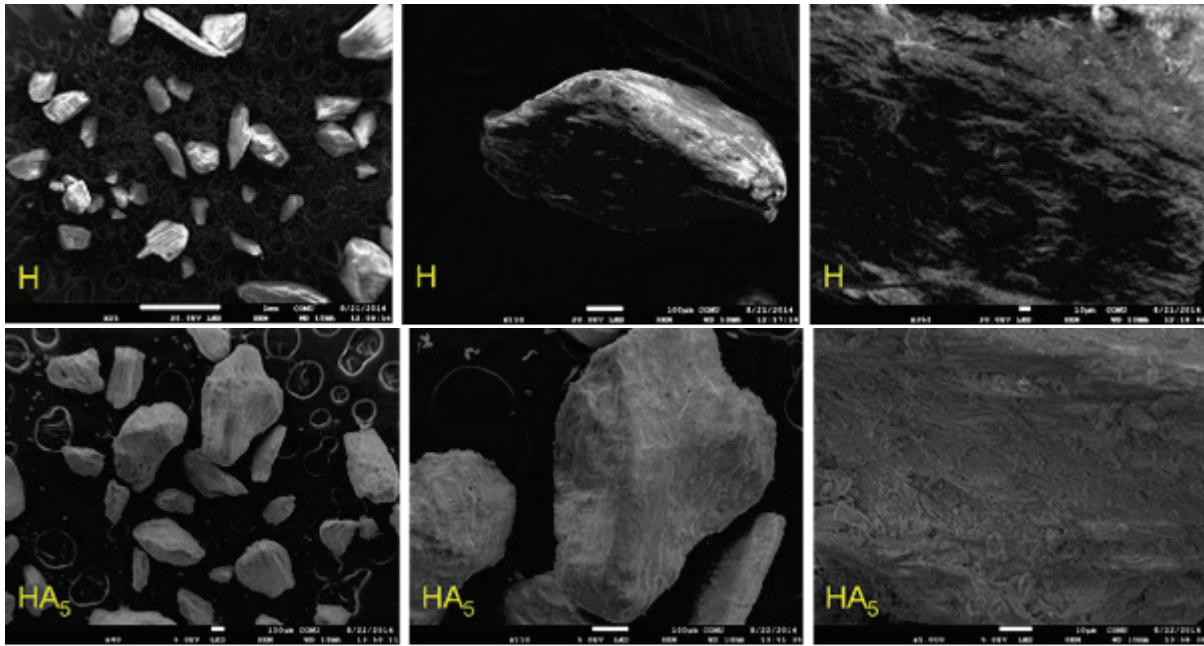


Figure 4. SEM images of H and HA₅ products.

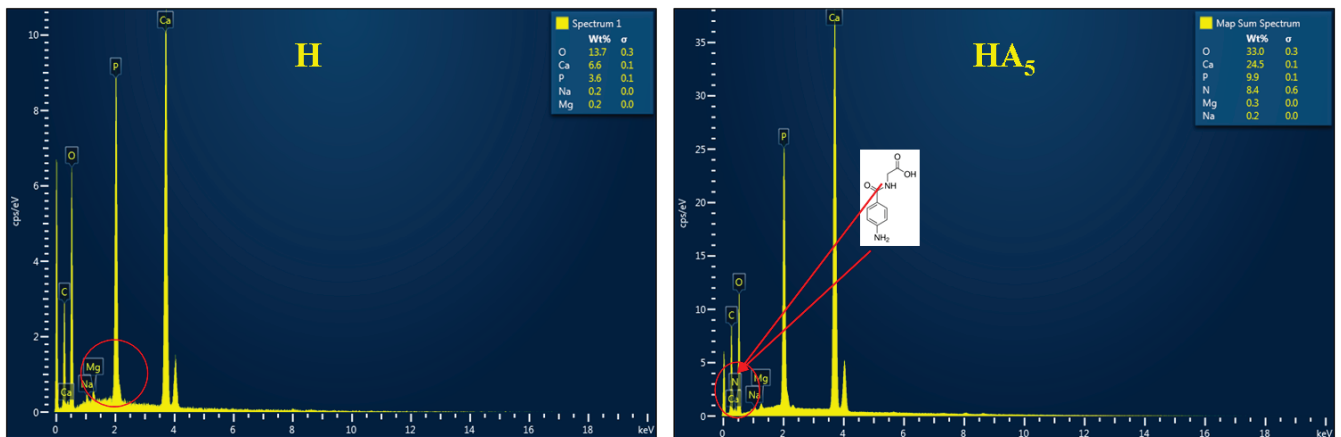


Figure 5. EDX spectrums of H and HA₅ products.

FT-IR spectrums of H and HA₅ compounds are given in Figure 3. When FT-IR data of compounds are examined; it is observed that in HA₅ compound the amplitude ascends due to the convergence of stretching vibrations of -OH bond in 3285 cm⁻¹ molecule and -OH bond that exists in the structure of hydroxyapatite (H), and the intensity also ascends due to the convergence of N-H bending vibrations in 1633 cm⁻¹ and stretching vibrations of carbonyl (-C=O), and finally in 1504 cm⁻¹ the intensity ascends due to stretching of unsaturated C-C bond because of its aromatic structure. It can be said that HA₅ compounds are modified according to the data received from FT-IR spectrum.

SEM images and EDX analysis of H and HA₅ compounds (100 and 10 μm) are given in Figure 4 and 5. When these images are examined in different options of zoom, it is seen that it has fairly smooth and rigid surface. In the images it can be seen that the surface roughens due to the corrosive effect of acidic groups and a fibered structure occurs on the surface of HA₅.

As it can be seen in EDX analysis of H; Ca (6.6%), P (3.6%) and O (13.7%) percentages of the scanned surface supports the structure prospected in respect of weight. In EDX analysis of HA₅ compounds, the compound weighs N in the percentage of 8.4% on the surface.

Table 3. Thermal analysis data of H and HA₅ materials.

	TGA				
	TGS (°C)	MDT (°C)	20% DT ¹ (°C)	30% DT ² (°C)	RA (%) (800°C)
H	211	316	354	503	66.22
HA ₅	204	343	372	562	67.24

TGS: The temperature degradation starts; MDT: Maximum degradation temperature; DT¹ and DT²: Degradation temperature of loss of mass 20 % and 30 %; RA: Residue amount.

When thermal data of H compound (Table 3) is examined, it is determined that thermal degradation starts at 211 °C, maximum degradation temperature is 316 °C, and the temperatures that loss of mass (20% and 30%) is observed are 354 and 503 °C respectively. It is observed that total loss of mass at 800 °C is 33.88%; namely 66.22% of it is residue. According to thermal data of HA₅ compound, thermal degradation starts at 204 °C, maximum degradation temperature is 343 °C, and the temperatures that loss of mass (20% and 30%) is observed are 377 and 735 °C respectively. It is observed that total loss of mass at 800 °C is 32.76%; namely 67.24% of it is residue.

Table 4. Elemental Analysis Data of H and HA₅ Materials

	C			N		
	C ¹ (%)	C ² (mmol/g)	CAB _C (μmol/g)	N ¹ (%)	N ² (mmol/g)	CAB _N (μmol/g)
H	14.174	-	-	8.031	-	-
HA ₅	15.962	1.490	165.55	8.565	0.381	190.64

C¹(%) ve N¹(%): Percentage rate of carbon and nitrogen amount in material totally.

C²(mmol/g) ve N²(mmol/g): Millimole amount of total carbon and nitrogen bonded on the surface per each bone (gram).

CAB_C and CAB_N (μmol/g): Micromole amount calculated over element C and N of compound amount bonded on the surface of HAP per each bone (gram).

Elemental Analysis of Modified Materials

In Table 4 the percentage amount of total C and N in the material, millimole amount of total C and N bonded on the surface per bone (gram), and micromole amounts calculated over C and N element bonded on the surface per each bone (gram) are given.

According to the data in the table, H compound includes 14.174% (C) and 8.031% (N) before the modification. In HA₅ compound it is seen that total percentage of C is (C¹) 15.962%, millimole amount of C bonded is (C²) 1.490 mmol/g per each bone (gram), the millimole amount calculated over element C of compound amount bonded per each bone (gram) is (CAB_C) 165.55 μmol/g, the proportion of total percentage of N is (N¹) 8.565%, millimole amount of total N bonded per each bone (gram) is 0.381 mmol/g, and the micromole amount calculated over element N of compound amount bonded per each bone (gram) increases to (CAB_N) 190.64 μmol/g. Since the compound used in the modification had not included S; S¹, S² and CAB_S values did not change dramatically.

Conclusions

It is known that environmental pollution has recently reached a hazardous level for human beings and environment with increasing world population, technological development and uncontrolled industrialization. Lots of natural and waste materials have some characteristics such as being transformable and usable. Therefore, a lot of researchers carry out studies upon waste materials in order to transform them into usable ones.

Acknowledgements

The authors acknowledge TUBİTAK and this study was supported by TUBİTAK Project No: 213M200. The authors wish to thank Merve Nihal SÖZEN for her help in English proofreading.

References

- Alasbeb, S., Banat, F. and F. Mobai. 1999. Sorption of copper and nickel by spent animal bones. *Chemosphere*, **39**(12):2087-2096.
- Banat, F., Asheh, S.A. and F. Mohai. 2000. Batch zinc removal from aqueous solution using dried animal bones. *Separation and Purification Technology*, **21**:155-164.

- Corami, A., D'Acapito, F., Mignardi, S. and V. Ferini. 2008. Removal of Cu from aqueous solutions by synthetic hydroxyapatite: EXAFS investigation. *Materials Science and Engineering B*, **149**:209-213.
- Chojnacka, K. 2005. Equilibrium and kinetic modelling of chromium(III) sorption by animal bones. *Chemosphere*, **59**:315-320.
- Dimovic, S., Smiciklas, I., Plecas, I., Antonovic, D. and M. Mitric. 2009. Comparative study of differently treated animal bones for Co²⁺ removal. *Journal of Hazardous Materials*, **164**:279-287.
- El Hammari, L., Laghzizil, A., Saoiabi, A., Barboux, P. and M. Meyer. 2006. Chemical modification of porous calcium hydroxyapatite surfaces by grafting phenylphosphonic and phenylphosphite acids. *Physicochemical and Engineering Aspects*, **289**:84-88.
- Kizilkaya, B. and A.A. Tekinay. 2011. Comparative study and removal of Co and Ni (II) ions from aqueous solutions using fish bones. *Science of Advanced Materials*, **3**:949-961.
- Kizilkaya, B., Tekinay A.A. and Y. Dilgin. 2010. Adsorption and removal of Cu (II) ions from aqueous solution using pretreated fish bones. *Desalination*, **264**:37-47.
- Li, Y. and W. Weng. 2008. Surface modification of hydroxyapatite by stearic acid: characterization and in vitro behaviors. *Journal of Materials Science: Materials in Medicine*, **19**:19-25.
- Murugan, R. and S. Ramakrishna. 2004. Coupling of therapeutic molecules onto surface modified coralline hydroxyapatite. *Biomaterials*, **25**:3073-3080.
- Ozawa, M., Satake, K. and R. Suzuki. 2003. Removal of aqueous chromium by fish bone waste originated hydroxyapatite. *Journal of Materials Science Letters*, **22**:513-514.
- Smiciklas, I., Dimovic, S., Plecas, I. and M. Mitric. 2006. Removal of Co²⁺ from aqueous solutions by hydroxyapatite. *Water Research*, **40**:2267-2274.
- Jang, S.H., Jeong, Y.G., Min, B.G., Lyoo, W.S. and S.C. Lee. 2008. Preparation and lead ion removal property of hydroxyapatite/polyacrylamide composite hydrogels. *Journal of Hazardous Materials*, **159**:294-299.
- Zhu, R., Yu, R., Yao, J., Mao, D., Xing, C. and D. Wanga. 2008. Removal of Cd²⁺ from aqueous solutions by hydroxyapatite. *Catalysis Today*, **139**: 94-99.
- Hong, Z., Zhang, P., Liu, A., Chen, L., Chen, X. and X. Jing. 2006. Composites of poly(lactide-co-glycolide) and the surface modified carbonated hydroxyapatite nanoparticles. *Journal of Biomedical Materials Research Part A*, **81**(3):515-522.
- Wei, J., Liu, A., Chen, L., Zhang, P., Chen, X. and X. Jing. 2009. The surface modification of hydroxyapatite nanoparticles by the ring opening polymerization of *g*-benzyl-L-glutamate *N*-carboxyanhydride. *Macromolecular Bioscience*, **9**:631-638.