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An application on fish bones by chemical modification of histidine as amino acid.

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ABSTRACT

In this study, fish bones as a waste in fish processing was investigated whether they could be used as multi-functional materials by chemical modification. Histidine was used and modified on the surface of bone particle by esterification method. The reaction was carried out in water. The results showed that the surface modification of bone particles was performed successfully. Histidine bonded on the surface of bone was calculated as 110.83 $\mu\text{mol/g}$. The point of zero charge (PZC) of bone apatite (H) and modified bone (HA_4) was investigated and determined as 7.25 and 6.98, respectively. SEM-EDX spectrums showed that nitrogen element of histidine on the surface of HA_4 could be observed in EDS spectrum clearly. According to EDS spectrum analysis, nitrogen amount of HA_4 which was formed to the one of histidine composition was detected as 16.2 %, nitrogen amount of H and HA_5 was detected as 8.037% and 8.565 %, respectively.

Introduction

Nowadays, it is known that environmental pollution has reached hazardous levels for human being and environment due to increasing population, technological developments and uncontrolled industrialization. It is also observed that natural resources are rapidly spent in order to meet up the needs of world population. Different kinds of methods are used for eliminating wastes but most of these methods are both expensive and insufficient. In the recent years, lots of studies focus on recycling of wastes in order to transform them into sufficient materials. A lot of natural and waste materials have some features such as being transformable and applicable thanks to their chemical properties. For this

reason, many researchers study upon waste products so as to transform them into applicable materials. One of these natural waste products is fish bone. Bones generally consist of protein-collagen as organic composition (30%) and hydroxyapatite $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ (HAP) (about 70%) as inorganic composition (Dimovic et al. 2009; Kizilkaya et. al. 2010; Kizilkaya and Tekinay, 2011; Tan et al. 2014). Especially, bones are natural materials as the source of biogenic apatite with low cost when compared to other synthetic HAP. Recently, many researches have been carried out in order to use waste products in various fields. Hydroxyapatites ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, HAP) are materials that have lots of applications and characteristics as adsorbent, catalyser and biomaterial. P-OH groups that exist on the surface of hydroxyapatite are very suitable for the adsorption and reaction of mineral and organic types (Hammari et al. 2006; Tan et al. 2014). Tetracycline is a therapeutic molecule and it is frequently used in the treatment of bacterial infections and bone diseases. In this

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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 Polyglycidylmethacrylate (PGMA). The results showed that HA/PGMA composite material can be used for medicine release as a biomaterial (Murugan and Ramakrishna, 2004; Tan et al. 2014).

It is observed that in respect of heavy metal sorption and removal, HA is applicable due to its low solubility in water, high stability in oxidation and degradation, high surface space and high capacity of buffering. In this area, synthetic HA was synthesized and its removal of cobalt (Smiciklas et al. 2006), lead (Janga et al. 2008), copper (Corami et al. 2008), cadmium (Zhu et al. 2008) was studied too. Moreover; using the fish bones that were prepared copper, cobalt and nickel removal and adsorption in aqueous solutions were investigated. Anchovy, sardine, bogue, blue fish and gilded sea bream were used for removal of metal ions. In order to remove fatty acids and other contaminations, the bones were treated with nitric acid, sodium hydroxide, hexane, alcohol, hydrogen peroxide, and water. It is determined that the bone powders cleaned with NaOH, hexane and ethanol are white and the bone powders cleaned with H₂O, H₂O₂ and NHO₃ are yellow. After adsorption, it is observed that in Cu⁺², Co⁺² and Ni⁺² solutions white fish bones turned into blue, red, and yellow, respectively (Kizilkaya et al. 2010; Kizilkaya and Tekinay, 2011).

Material and methods

Materials and Chemicals

In the laboratory experiments magnetic stirrer-heater with contact thermometer (WiseStir MSH-20D), centrifuge (Nüve NF400), ultrasonic bath, Ultra-pure water (SG, Ultra Clear 2001-B), pH meter (InoLab pH 7110), and mechanic mixer (WiseStir HS-120A) were used. Histidine (Sigma) was used in the experiments. In the analysis of the products acquired Scanning Electron Microscope (SEM-EDS, Jeol) was used.

Functionalization of Bone Surfaces with Organic Acids

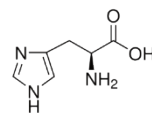
Reaction was occurred between hydroxyapatite -OH group of bone apatite and carboxyl group (-COOH) of histidine. Reaction was carried out 0.1 M (2.5×10^{-3} mole) A₄ in 25 mL water in 50 mL flask. Afterwards, 2.5 gr H was added to the solution and refluxed in boiling point for 8 hours in inert atmosphere. Then, the mixture was set in the room temperature. The mixture was then centrifuged with water at 2000 rpm, washed five times and finally the modified solid phase was dried at 45 °C in incubator (Tan et al. 2014). Technical information of histidine is given in Table 1.

Determination of Point of Zero Charge (PZC)

For determination of PZC, 0.01 M KNO₃ solutions were firstly prepared in a 100 ml flask. The initial pH of this solution (PH_B) 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_S) was measured and recorded after 48 hours. PZC was determined with obtained curve against initial pH between the difference of the initial pH (pH_I) and the final pH (pH_F) ($pH = pH_I - pH_F$) (Tan et al. 2014).

Table 1. The technical information of histidine.

Chemical	Molecules	M _A	Molecular Formula	Code
Histidine		155,15	C ₆ H ₉ N ₃ O ₂	A ₄

Results and discussion

Modification scheme of Histidine is shown in Figure 1. Esterification is the reaction that occurs between -OH and carboxylic acid (-COOH) groups. In this study esterification reaction took place in an acidic environment by occurring H₂O over hydroxy apatite -OH group and carboxyl group. Such kinds of surface modification are successfully applied with hydroxyapatite synthesized in the laboratory and aliphatic flat chain organic acids (Li and Weng, 2008; Qiu et al. 2005).

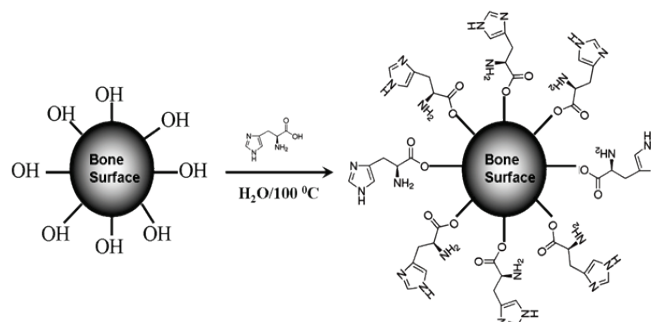


Figure 1. Demonstration of chemical modification of Histidine on the bone surface.

The point of Zero Charge of H and HA₄ is given in Figure 2 and Table 2. It has been obviously seen that H and HA₄ had different points in x axis. It was also seen that HA₄ had a better linear curve than H. The points of Zero Charge of H and HA₄ were 7.25 and 6.98, respectively.

Table 2. PZC results of H and HA₄ products

	PZC _D	PZC _T	R ²
H ⁺	7.25	6.69	0.993
HA ₄	6.98	6.12	0.998

EDS spectrum and element composition of H and HA₄ are given in Figure 3. As it could be seen in EDS analysis on the surface of HA₄, the amount of N is 8.4 % of weight. In the molecule of histidine, it was clearly seen that the elements nitrogen (N) and oxygen (O) have especially ascended in HA₄

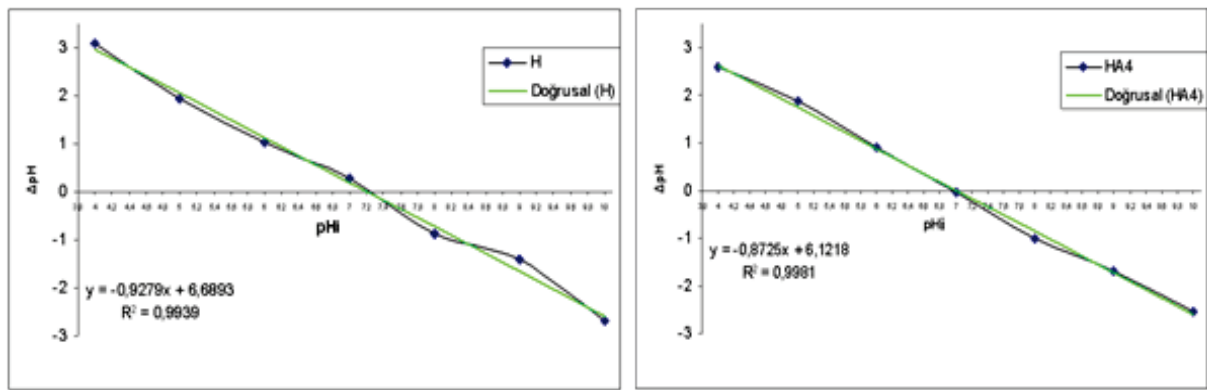


Figure 2. PZC curves of H (H spectrum was taken from a previous study according to Tan et al. 2014) and HA₄ products.

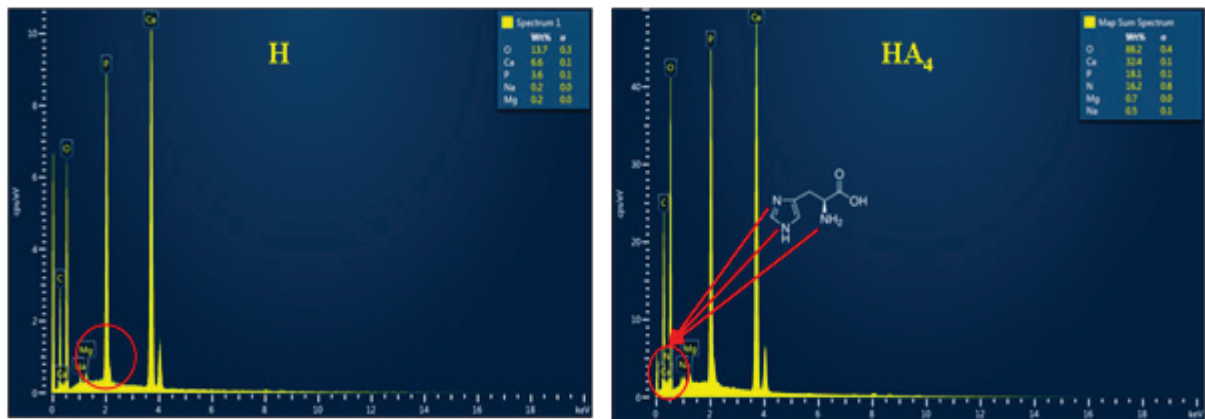


Figure 3. EDS spectrums of H (H spectrum was taken from a previous study according to Tan et al. 2014) and HA₄.

spectrum. It was also observed that in H spectrum, nitrogen element that exists in the structure of histidine could not be seen, but in HA₄ material, it was clearly seen. Besides, it was determined that the percentage of oxygen element has increased in HA₄ material. According to EDS spectrum, it was observed that the modification on the surface of apatite took place by the existence of the elements, which were determinant, in histidine molecule.

In Figure 4 analysis of SEM-EDS; Ca, P, O, C, Mg, Na and N mapping of the section and SEM image of the area on HA₄ surface were given. EDS analysis of nitrogen element in Figure 4, nitrogen areas of histidine molecule could be seen. According to EDS spectrum results, it was obviously seen that the modification had occurred.

Conclusion

Environmental pollution has recently reached a hazardous level with the increasing world population, technological development and uncontrolled industrialization. Therefore, many waste products can be evaluated such as this study.

Fish bones are biogenic sources of apatite and they appear as an industrial waste. Within this study recycling of the fish bones, as more efficient and functional materials, was aimed by chemical modification. In the shade of experimental results, PZC of H and HA₄ is determined as 7.25 and 6.98. Besides, nitrogen element that took place in the structure of histidine was clearly seen. According to

these results; the surface of apatite particles, acquired from the fish bones, were successfully modified.

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References

- 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.
- 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.
- Tan, E., Kizilkaya, B., Üçyol, N., Ormancı, H.B. and A. Oral. 2014. Surface modification with P-aminohippuric acid on biogenic apatite (fish bones) particles. *Marine Science and Technology Bulletin*, 3(2):45-50.
- El Hammari, L., Laghzizil, A., Saoiabi, A., Barboux, P. and M. Meyer. 2006. Chemical modification of porous calcium

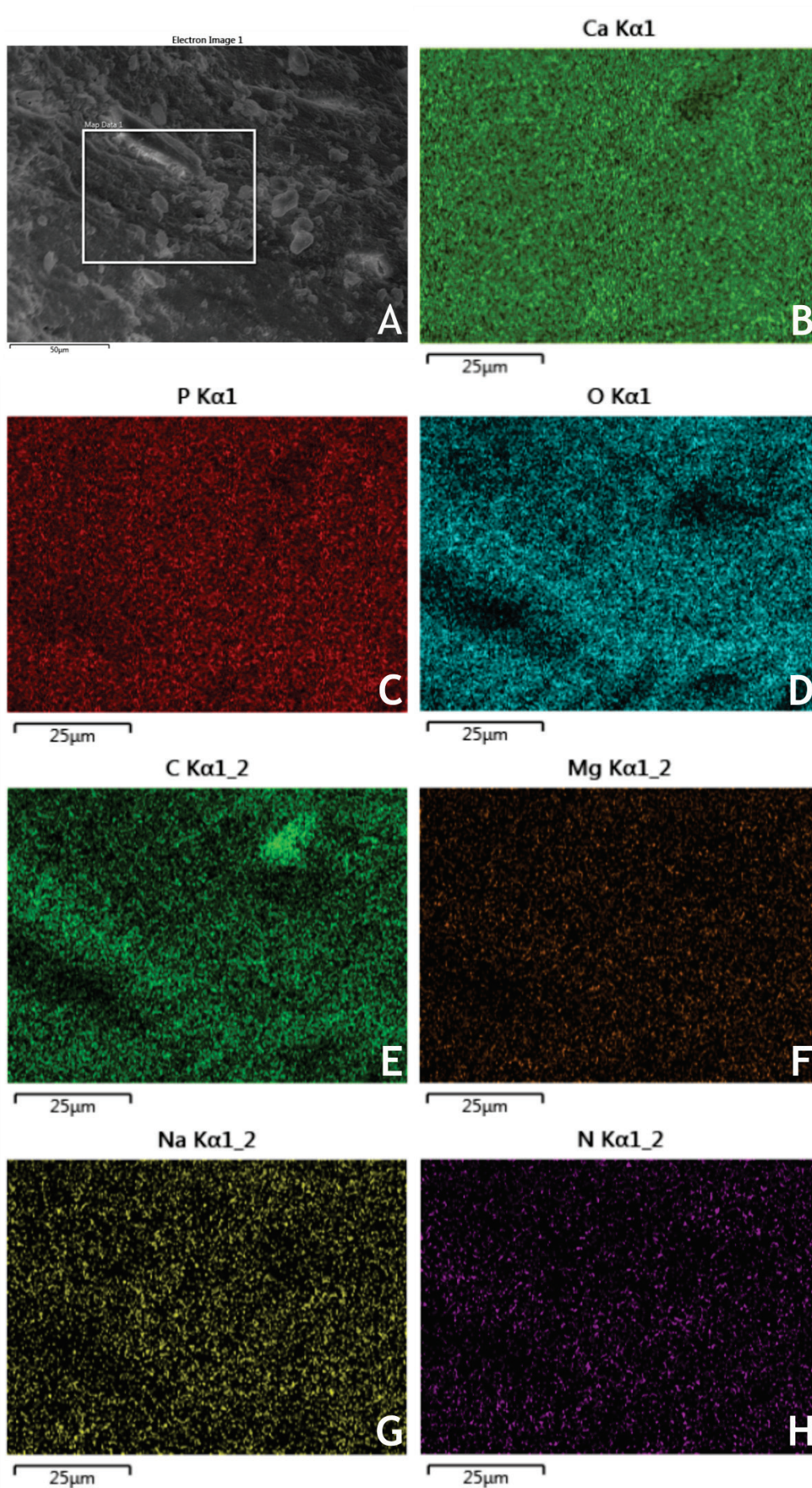


Figure 4. HA₄ (A) according to EDS analysis, SEM image of the area that EDS mapping would be applied; (B) Ca, (C) P, (D) O, (E) C, (F) Mg, (G) Na and (H) N mapping.

- hydroxyapatite surfaces by grafting phenylphosphonic and phenylphosphite acids. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, **289**:84-88.
- Murugan, R. and S. Ramakrishna. 2004. Coupling of therapeutic molecules onto surface modified coralline hydroxyapatite. *Biomaterials*, **25**:3073-3080.
- Smiciklas, I., Dimovic, S., Plecas, I. and M. Mitric. 2006. Removal of Co^{2+} from aqueous solutions by hydroxyapatite. *Water Research*, **40**:2267-2274.
- Janq, S.H., Jeonq, 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.
- 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.
- Zhu, R., Yu, R., Yao, J., Mao, D., Xing, C. and D. Wang. 2008. Removal of Cd^{2+} from aqueous solutions by hydroxyapatite. *Catalysis Today*, **139**:94-99.
- Li, Y. and W. Weng. 2008. Surface modification of hydroxyapatite by stearic acid: Characterization and in vitro behaviours. *Journal of Materials Science: Materials in Medicine*, **19**:19-25.
- Qiu, X., Chen, L., Hu, J., Sun, J., Hong, Z., Liu, A., Chen, X. and X. Jing, 2005. Surface-modified hydroxyapatite linked by L-lactic acid oligomer in the absence of catalyst. *Journal of Polymer Science Part A: Polymer Chemistry*, **43**:5177-5185.