An investigation on the conversion of functional materials of fish bones as waste products using surface modification methods

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In this study, fish bones as a waste product in fish industry was investigated whether it is suitable to chemical modification. The surface modification on the surfaces of bone particles was made on two step using silanization and schiff base reaction. Firstly, free amine (-NH2) group was obtained in molecule by silanization of the surfaces of fish bones with 3-aminopropyl-triethoxysilane (S1). Afterwards, amine groups that exist on the surface of bones were reacted with 2-ethyl-2H-pyrazole-3-carbaldehyde (B5) that includes aldehyde groups. The materials gained were analysed with Fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy - energy dispersive X-ray spectroscopy (SEM-EDS) and zeta for their potency. In SEM-EDS analysis, the band of silicon (Si) and nitrogen (N) elements of molecules modified can be clearly seen. Bruanaver Emmet Teller (BET) surface areas of the materials acquired were investigated. BET surface area of the unprocessed apatite is 5.6 m²/g and the surface area of apatite modified with B5 is 1.8 m²/g. The amount of B5 bonded on the surface of bone is calculated as 164 µmol/g. According to the results, it can be said that waste fish bones are applicable for chemical processing and they can be used in various fields.

Keywords: Fish bone, surface modification, 3-aminopropyl-triethoxysilane

Introduction

Fish bones are generally composed of collagen and protein tissues and it was consisted of 30% organic composition and 70% hydroxyapatite (HAP, Ca10(PO4)6(OH)2)1-4. It is known that hydroxyapatite is also one of those cheap and easily recyclable natural wastes. Bones as biogenic apatite are natural source materials with low cost when compared to other synthetic HAP. 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 sodium hydroxide (NaOH), hexane and ethanol are white and the bone powders cleaned with water (H2O), hydrogen peroxide (H2O2) and nitric acid (HNO3) are yellow. After adsorption, it is observed that in copper (Cu²⁺), cobalt (Co²⁺) and nickel (Ni²⁺) solutions white fish bones turned into blue, red, and yellow, respectively2-3. Also, there are significant studies on the treatment factors on the properties of bio-apatite materials using animal bone5. The usage of biological apatite from bones is showed to be important converting meat and fish industry waste into a valuable sorbent material5. Hydroxyapatite (HAP) is generally used for chemical modifications and applications. Hydroxyapatites are known as materials have lots of applications and characteristics as adsorbent, catalyzed and biomaterial. The biologic reactivity of apatite is easily leded in change of ion with organic groups such as CO3²⁻ or RPO4²⁻, RSO4²⁻ and RCOO⁻. Also, P-OH groups, exist on the surface of hydroxyapatite, are very suitable for the adsorption and reaction of mineral and organic types. Some organophosphate products were synthesized on the surface of hydroxyapatite6. Silinization method is frequently one of the most used methods for surface modification7-10. Hydrolyzed silanol groups can be bonded with an end of -OH groups on the surface of the material. 3-aminopropyl-triethoxysilane (APTES) as silinization reactive is widely used for surface modification of the materials. It is known that amine groups such as 3-aminopropyl-triethoxysilane are used in derivatives of organic biomolecules9-10. Pyrazoles

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and its derivatives are used a wide range of applications in medicine\textsuperscript{11}. Therefore, many different derivatives of pyrazole carbaldehydes are known as biologically active compounds\textsuperscript{12}. Some of pyrazole derivatives have been found to have effects as antimicrobial\textsuperscript{13-15}, anti-inflammatory\textsuperscript{16}, antitubercular\textsuperscript{17}, antitumor\textsuperscript{18-19}, antiangiogenesis\textsuperscript{19}, anti-parasitic\textsuperscript{20}, and antimalarial activity\textsuperscript{16}. Some studies have been showed that alkyl and aryl substituted pyrazoles have effects of bacteriostatic, bacteriocidal, fungicidal, analgesic and antipyretic activities\textsuperscript{11}. Therefore, it was investigated and used 2-ethyl-2\textsuperscript{H}-pyrazole-3-carbaldehyde as a derivative of pyrazole carbaldehydes. In this study, the surface modification of the fines bones particles with 3-aminopropyl-triethoxysilane and 2-ethyl-2\textsuperscript{H}-pyrazole-3-carbaldehyde is investigated. The materials were analyzed with FT-IR, SEM-EDS and point of zero charge (Pzc).

**Materials and Methods**

**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 was applied to the waste fish bones provided. According to our previous experiences, the most efficient way for cleansing method is using alkali solutions\textsuperscript{3-4}. Accordingly, unprocessed fish bones were treated with mechanic mixer in 60°C with NaOH solution until they were purified from contamination. Finally, the waste fish bones were dried in the incubator and pulverized with the aid of homogenizer and grinder and it was called as H.

**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. 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), Zetasizer (Malvern, Zetasizer Nano series Nano-ZS) and Olympus Microscope (SZx7, Stereo) were used.

**Functionalization of Bone Surfaces with 3-Aminopropyl-Triethoxysilane**

Fish particles were silinised with the materials 3-aminopropyl-triethoxysilane (Si\textsubscript{1}). Firstly in 50 mL ethanol/water (9/1), solution of 0.2 M silane compound (Si\textsubscript{1}) was prepared for the reactions. It was taken into a 100 mL flask and stirred in room temperature (25 ± 2°C) with magnetic stirrer for 30 minutes. Afterwards, 5 g H\textsubscript{1} was added into the solution and stirred with magnetic stirrer in room temperature for 24 h. Suspense mixture was centrifuged with technical ethanol in 2000 rpm and dried with solid phase, modified by washing 5 times, in 45°C in incubator. The material gained is named as HS\textsubscript{1}. Technical information about the silan compound used for the reaction is given in Table 1.

**Modification with Schiff Base Reaction in Silinised Surfaces**

Firstly, preparing 25 mL ethanol solution of 0.2 M (5×10\textsuperscript{-3} mole) aldehyde compound (B\textsubscript{5}), 50 mL of it was taken into a flask. Afterwards, 2.5 g HS\textsubscript{1} was added into the solution and refluxed in 70 °C for six hours. After refluxing, it was kept in room temperature for 12 hours. The mixture was washed with ethanol 5 times and centrifuged in 2000 rpm. The modified solid phase was dried in 45°C. The materials gained are named as HS\textsubscript{1}B\textsubscript{5}. Aldehyde compounds used for the reactions and technical information about them are given in Table 1.

**Determination of Point of Zero Charge (Pzc)**

Briefly, 0.01 M KNO\textsubscript{3} solutions were firstly prepared in a 100 ml flask. The initial pH of this solution between pH 4 and 10 was adjusted with 0.1 M HCl or NaOH. Then, H, HS\textsubscript{1} and HS\textsubscript{1}B\textsubscript{5} products were added to the prepared solution. The

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**Table 1 — Technical information of Si and B5 molecules**

<table>
<thead>
<tr>
<th>Chemical Molecules</th>
<th>M\textsubscript{A}</th>
<th>Molecular formula</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-aminopropyl-triethoxysilane</td>
<td>221.37</td>
<td>C\textsubscript{9}H\textsubscript{23}NO\textsubscript{3}Si</td>
<td>Si\textsubscript{1}</td>
</tr>
<tr>
<td>2-Ethyl-2\textsuperscript{H}-pyrazole-3-carbaldehyde</td>
<td>124.14</td>
<td>C\textsubscript{6}H\textsubscript{8}N\textsubscript{2}O</td>
<td>B\textsubscript{5}</td>
</tr>
</tbody>
</table>
solution was stirred in constant temperature with a magnetic stirrer for 48 h. The pH of the final solution (PH₅) was measured and recorded after 48 hours. Pzc was determined by taking the graphics of the final pH (pH₅) against the initial pH (pHᵢ). (Ph = pHᵢ-pH₅)⁴.

Results & Discussion
Modification scheme of HS₁ and HS₁B₅ is shown in Figure 1. Silanization is known that is one of the best methods for surface modification. Silanization is performed with triethoxysilane (C₂H₅)₃-O-Si-R, silicon based organic molecule. Silanization is based on the reaction of active ethoxysilane groups (C₂H₅-Si-) with hydroxyl groups (-OH). These practices are successfully applied on silica, magnetic particles and surfaces of various materials²³-²⁴. The surface of bone particles is silanized with 3-aminopropyl-triethoxysilane (S₁), and then it is used for the reaction of Schiff base. Schiff base reactions were performed between amine groups (-NH₂) existing on the surface of HS₁ and aldehyde groups (-CHO).

The results of point of zero charge (Pzc) of H, HS₁ and HS₁B₅ are given in Table 2. The experimental results of H, HS₁ and HS₁B₅ were obtained as 7.2, 7.7 and 7.6, respectively. On the other hand, theoretical Pzc of H, HS₁ and HS₁B₅ were found to be 6.6, 6.9 and 6.2, respectively. Pzc values of HS₁ and HS₁B₅ that have different function groups differ from H.

FT-IR spectra of H, HS₁ and HS₁B₅ compounds are given in Figure 3. According to FT-IR data of the

Table 2—Pzc results of H, HS₁ and HS₁B₅ materials

<table>
<thead>
<tr>
<th></th>
<th>PzcD</th>
<th>PzcT</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>7.2</td>
<td>6.6</td>
<td>0.99</td>
</tr>
<tr>
<td>HS₁</td>
<td>7.7</td>
<td>6.9</td>
<td>0.96</td>
</tr>
<tr>
<td>HS₁B₅</td>
<td>7.6</td>
<td>6.1</td>
<td>0.98</td>
</tr>
</tbody>
</table>

PzcD: Experimental Pzc, PzcT: Pzc and R² values of experimental data calculated with linear regression, *this result was used from a previous study⁴.
compounds, it was observed characteristic weak –OH the peaks in 3243 cm\(^{-1}\), aliphatic–CH\(_2\) in 2984 cm\(^{-1}\), carbonyl (–C=O) in 1655 cm\(^{-1}\), CO\(_3^{2-}\) in 1407 cm\(^{-1}\), intensive PO\(_4^{3-}\) in 1038 cm\(^{-1}\) and weak C-H the peaks in 870 cm\(^{-1}\) on the structure of fish bone as natural hydroxyapatite (H). Additionally, it was observed and slightly increased vibration bands of aliphatic CH\(_2\) groups in 2988 cm\(^{-1}\) and –OH groups in 3261 cm\(^{-1}\) of HS\(_1\). In HS\(_1\) compound, the reason of the increase in the intense of peaks in 1643 and 1557 cm\(^{-1}\) can be explained with the overlap of vibration stretches of carbonyl group (–C=O) and imine (C=N-), and the increase in the intense of peak in 1018 cm\(^{-1}\) can also be explained with the overlap of vibration bands of Si-O and PO\(_4^{3-}\) group. Additionally, the reason of the rise in the intense of peak of PO\(_4^{3-}\) in 1028 cm\(^{-1}\) can be explained with the overlap of vibration bands of PO\(_4^{3-}\) and Si-O. In HS\(_1\)B\(_5\) compound, it was observed that the intense increased due to unsaturated C-C bond stretches (because of aromatic circle) in 1651 cm\(^{-1}\), C=N stretch vibrations, N-H curve vibrations, and the overlap of Si-O vibration bands with the peak of PO\(_4^{3-}\) group in 1026 cm\(^{-1}\).

In Table 3, zeta potential load of all modified materials and surface conductivity values of these materials are given. Zeta potential of unmodified apatite (H) is determined as -20.4 mV. It is also observed that zeta potential differed between 6.1 and -20.4 mV (max) in negative area.

The surface areas of the materials with different methods are given in Table 4. According to BET results, the highest surface area is determined as 5.6 m\(^2\)/g in H. In Langmuir, t-plot and single-pointed models; the highest surface area is determined as 13.6, 6.6 and 5.1 m\(^2\)/g in unmodified apatite (H).

In Table 5, volume of pore and average width of pore results of H, HS\(_1\) and HS\(_1\)B\(_5\) are given. Volume of pore is observed as 14.1, 5.0 and 4.1×10\(^{-3}\) cm\(^3\)/g H, HS\(_1\) and HS\(_1\)B\(_5\), respectively. The highest average width of pore is determined as 53.8 Å in H and the lowest one is determined as 35.8 Å in HS\(_1\).

![Fig. 3 — FTIR spectra of H, HS\(_1\) and HS\(_1\)B\(_5\) products.](image-url)
EDS and SEM images analysis of H, HS₁ and HS₁B₅ compounds are given in Figure 4 & 5. It is seen that surfaces of HS₁ and HS₁B₅ vary according to H, when SEM images are examined. The element compositions of H, HS₁ and HS₁B₅ are given in EDS spectra of Figure 4. As it is seen in EDS analysis, nitrogen (N) element was observed as 6.1 % for HS₁ and 12.3% for HS₁B₅ on the surface, clearly. It is seen that nitrogen (N) and (O) elements that is determinant in S₁ and B₅ molecules have increased distinctively.

The percentage amount of total carbon (C₁) and nitrogen (N¹) in the material, bonded micromole and millimole amount of compound using the percentage of carbon and nitrogen on the bone surface per gram(g) are given in Table 6. C¹(%) and N¹(%) expressions which are given in Table 6 symbolize the percentage amount of analysed carbon and nitrogen in the material. The micromole amounts that calculated using the percentage of carbon and nitrogen elements which were bonded on the bone surface per g were mentioned as CACc and CACn (µmol/g). The values represented by abbreviations in Table 6 are calculated as follows:

\[
C^2 = \frac{([E_R - E_M] \times 1000)}{(100 \times M_{molecule})}
\]

\[
C^2 \text{ and } N^2: \text{ The millimole amount of compound using the percentage of carbon (C) and nitrogen (N) on the bone surface per gram (mmol/g)}
\]

\[
E_R: \text{ Amounts of carbon and nitrogen in unmodified bone}
\]

\[
E_M: \text{ Amounts of carbon and nitrogen in modified bone}
\]

Table 5 — Volume of pore and average width of pore of H, HS₁ and HS₁B₅

<table>
<thead>
<tr>
<th></th>
<th>Volume of pore (cm³/g)</th>
<th>Average width of pore (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>14.1×10⁻³</td>
<td>53.8</td>
</tr>
<tr>
<td>HS₁</td>
<td>5.0×10⁻³</td>
<td>35.8</td>
</tr>
<tr>
<td>HS₁B₅</td>
<td>4.1×10⁻³</td>
<td>45.1</td>
</tr>
</tbody>
</table>

Fig. 4 — EDS results of H (The EDS spectra was taken from a previous study⁴), HS₁ and HS₁B₅ (original spectra) products.
1000: Conversion factor from mole to milimole
100: Conversion factor of elemental analysis results to gram material in terms of percentage

$M_{MS} = \text{The molar mass of carbon and nitrogen (g/mol)}$

$C_{AC}C$ and $C_{AC}N = (C^2 \times N^2 \times 1000) / EM$

Calculated amount of compound ($C_{AC}C$ and $C_{AC}N$): The micromole amount of compound that calculated over total carbon and nitrogen elements in molecules which bonded on surface per bone (µmol/g)

1000: Conversion factor from micromole to milimole

$EM = \text{The number of carbon or nitrogen in the compound that bonded on the surface.}$

According to the data in the Table 6, it was determined 14.2% carbon ($C^1$) and 8.0% nitrogen ($N^1$) in unmodified bone (H). On the other hands, it is seen that total percentage of carbon ($C^1$) is 14.8% for HS$_1$ and 15.9% for HS$_1$B$_5$.

Point of zero charge (Pzc) is given information about the pH or electrical charge density of material surfaces. Where the surface of material has a neutral charge or zero the point of zero charge is that point as pH. When Pzc is above pH 7, the surfaces of particles or materials have a negative or anionic charge. On the others hands, when Pzc is below pH 7, the surfaces of particles or materials have a positive or cationic charge. Therefore, it is attracted anions to the positive surfaces (below pH 7), and vice versa attracted cations to the negative surfaces (above pH 7). In Figure 2, obtained curves between $\Delta pH$ with initial pH were given. It is obviously seen that each of H, HS$_1$ and HS$_1$B$_5$ have different breakpoints in x axis as Pzc. The best regression parameter is observed in H material as 0.99, and the lowest one is observed in HS$_1$ material as 0.96.

### Table 6 — Elemental analysis data of H, HS1 and HS1B5 materials

<table>
<thead>
<tr>
<th></th>
<th>C (%)</th>
<th>C$^2$ (mmol/g)</th>
<th>CACC (µmol/g)</th>
<th>N (%)</th>
<th>N$^2$ (mmol/g)</th>
<th>CACN (µmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>14.2</td>
<td>-</td>
<td>-</td>
<td>8.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HS$_1$</td>
<td>14.8</td>
<td>0.53</td>
<td>178</td>
<td>8.2</td>
<td>0.18</td>
<td>185</td>
</tr>
<tr>
<td>HS$_1$B$_5$</td>
<td>15.9</td>
<td>0.98</td>
<td>164</td>
<td>8.7</td>
<td>0.35</td>
<td>173</td>
</tr>
</tbody>
</table>

$C^1$ (%) $\times N^1$ (%): Percentage rate of carbon and nitrogen amount in material, $C^2$ (mmol/g) and $N^2$ (mmol/g): The bonded milimole amount of compound using the percentage of carbon and nitrogen elements on bone surface per g (mmol/g), CACC and CACN (µmol/g): The bonded micromole amount of compound using the percentage of carbon and nitrogen elements on bone surface per g (µmol/g).
Electro kinetics is the measurement of electricity load that is effective on colloidal dispersions or on the surface of particles and zeta potentials. It gives information about dispersion mechanism of particles on condition of repulsion and draw due to the surface loads of particles in colloidal systems. Measurement of zeta potential enables understanding important characteristics of colloidal, dispersion and aggregate systems, controlling and defining the potential or the load on particles. One of the most important reasons of determining is the measurement of the size of diffuse double layer around the particle. It is seen that HS1 values of modified materials are positive and zeta potential of HS1B5 prepared with Schiff base by using aldehyde groups is -14.7 mV.

In this study, surface areas and other data were determined by doing BET analyses from 11 different points. Measurement of surface area and porosity were performed with the method of BET in 77 K (-196°C) and in liquid nitrogen thanks to the technique of nitrogen gas (N2) adsorption. Using the volume of the gas adsorbed; parameters of multi-pointed surface area (with the method of BET), Langmuir surface area, t-plot surface area, single-pointed surface area, average width of pore, volume of pore was automatically calculated. The fact that the surface area of HS1 and HS1B5 materials is lower than those in H can be explained with the filling of pores on the surface of particle with modification materials. It is seen that the best regression parameter is in t-plot model, generally.

According to SEM image of H, the surface seems as if a tough rock. On the other hand, the surfaces have different layering due to the result of modification of H with S1 (HS1) and B5 (HS1B5) compound. EDS spectra show that they occur on the surface ofapatite with the existence of elements that are determinant in modification. Olympus stereo microscope (SZx7) was used to photograph colored images of the pulverized fish bone and controlled with a digital camera. The microscope was calibrated with the certificated 150 and 50 µm circle diameters. It was given the images of pulverized fish bone particles in Figure 5. The size range of the pulverized fish bone particles was found to be between 5 and 150 µm using microscope. Average particle sizes of pulverized fish bone are calculated as 55 µm.

Considering the elemental analysis results of HS1 material that after silanization process, it was raised from 14.2 to 14.8%. On the other hands, the bonded amount of compound (CACN) on the surface of bone particles per g was calculated as 185 µmol/g using the percentage of N element for HS1 material. The percentage of total carbon (C1) of HS1B4 material was determined as 15.9%. The amount of sulfur in material that modified with 2-ethyl-2H-pyrazole-3-carbaldehyde is an indicator of whether the bonded compound on surface or not. The amount of nitrogen was raised from 8.2 to 8.7% and also the bonded amount of compound on the surface of bone per g with using percentage of nitrogen was calculated as 173 µmol/g.

Nowadays, eliminating waste products has reached to an important extent. Various studies are carried on in order to make them convenient materials by recycling. In this study, recycling of waste fish bones is aimed. Therefore, the convenience of fish bone surfaces with chemical reactions was investigated. Firstly, fish bones were silanized and then 2-ethyl-2H-pyrazole-3-carbaldehyde molecule was bonded with Schiff base. It is observed that modifications occurred with FT-IR. Besides; nitrogen peaks of S1 and B5 molecules modified on the surface of fish bone particles were clearly determined with SEM-EDS analyses. According to the results, chemical modifications on the surface of bones were performed successfully.

Acknowledgements

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References