ADSORBABILITY OF ALIZARIN RED S ON Fe(III)- AND Pb(II)-TREATED HYDROXYAPATITITES IN WATER

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Keywords: Adsorption, Alizarin red S, Iron, Lead, Hydroxyapatite

Abstract: We studied on adsorbability of alizarin red S (ARS) on Fe(III)- and Pb(II)-treated hydroxyapatites in distilled water (H2O) and phosphate buffer solution (PBS). The Fe(III)-treated apatites indicated high adsorption capacity from their isothermic curves both in H2O and PBS: The capacities in PBS were larger than those in H2O. On the other hand, adsorption capacities of Pb(II)-treated apatites in PBS were much lower than those in H2O. Surface analysis of those treated apatites by electron probe micro analysis and FT-IR microspectroscopy affords significant information on surface element (P, Ca, Fe, or Pb) distribution and interaction patterns between ARS and the corresponding metal (Fe or Pb) sites. Eventually, predominant adsorption mechanisms were elucidated as follows, chelate form adsorption for Fe(III)-treated apatites and salt form adsorption for Pb(II)-treated apatites.

1. INTRODUCTION

Hydroxyapatite indicates a unique property, exchange of Ca2+ and OH− for outer cations1-7 and anions.8, 9 In the previous paper,10 we reported that reaction of heavy metal ions such as Pb2+, Fe3+, Fe2+, Cu2+, Cd2+, Cr3+, Co2+, Ni2+, and Zn2+ with stoichiometric hydroxyapatite Ca10(PO4)6(OH)2, HAP-300 (Ca/P=1.68, slightly containing CO3), and Ca-deficient hydroxyapatite Ca10-x(HPO4)x(PO4)6-x(OH)2-x, HAP-400 (Ca/P=1.38), afforded the corresponding metal-treated hydroxyapatites. Particularly, complete inclusion of heavy metal ions along with stoichiometric elution of Ca2+ were recognized in the following combinations, Fe3+ versus HAP-300, Fe3+ versus HAP-400, and Pb2+ versus HAP-400. The reaction of Fe3+ with HAP-300 and HAP-400 involved erosion of these apatites to afford Fe(III)-treated hydroxyapatite, HAP-300-Fe and HAP-400-Fe, respectively, as hybridized materials having amorphous structures consisting of much iron (III) phosphate FePO4 phase and a little original apatite phase. The reaction of Pb2+ with HAP-400 involved not only exchange of Ca2+ for Pb2+ on the surface but also new formation of lead apatite Pb(PO4)2(OH)2 crystals, affording Pb(II)-treated hydroxyapatite, HAP-400-Pb, as a mixture. Although metal consumption was low, ion exchange of Ca2+ for Pb2+ occurred stoichiometrically in the case of HAP-300 to afford Pb(II)-treated hydroxyapatite, HAP-300-Pb, as a single crystal material without new lead apatite formation.

We had an attractive attention to elucidation of surface physicochemical property, or possible application of the four apatites HAP-300-Fe, HAP-300-Pb, HAP-400-Fe, and HAP-400-Pb as adsorbents for organic molecules. Therefore, we investigated adsorption of alizarin red S (ARS, referred to SCHEME 1) on those treated apatites in distilled water (H2O) and phosphate buffer solution (PBS), compared with HAP-300 and HAP-400. ARS is often used as a born-staining agent11, 12 and indicates strong interaction with metal ions.13 Adsorption of ARS on HAP-300 has already been reported,14, 15 in which the adsorption mechanism was defined. We assumed that the Fe(III)- and Pb(II)-treated apatites have a possibility to exhibit large capacities and/or specific behaviors in adsorption of ARS unlike the original raw apatites. In addition, we elucidated ARS adsorption mechanisms by surface analysis of Fe(III)- and Pb(II)-treated apatites and those adsorbing ARS using an electron probe micro analyzer (EPMA) and an FT-IR microspectroscope.

2. MATERIALS AND METHODS
SCHEME 1 Structure of alizarin red S (ARS). The numerals mean carbon numbers (C-1 to C-10).

2.1. Materials

Hydroxyapatites, HAP-300 and HAP-400, which were synthesized from CaHPO$_4$·2H$_2$O and CaCO$_3$ and from CaHPO$_4$·2H$_2$O and NaOH,$^{16,17}$ respectively, were supplied from Taihei Chemical Industrial Co., Ltd. Alizarin red S (ARS), lead (II) nitrate Pb(NO$_3$)$_2$ (purity 99.9%), iron (III) chloride hexahydrate FeCl$_3$·6H$_2$O (purity 99.9%), distilled water (H$_2$O), phosphate buffer solution (PBS) at pH 6.86 containing 25 mM KH$_2$PO$_4$, 25 mM Na$_2$HPO$_4$, and about 0.1% NaN$_3$, and 0.1 M NaOH solution were purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan. All chemicals were used without further purification.

2.2. Preparation of Fe(III)- and Pb(II)-treated hydroxyapatites

According to the previous paper$^{10}$, Fe(III)- and Pb(II)-treated hydroxyapatites were synthesized as follows: To 0.50 g of hydroxyapatite (HAP-300 or HAP-400) with a stirrer tip in a 200 mL eggplant-shaped flask was added 100 mL of 25 mM aqueous solution of Pb(NO$_3$)$_2$ or FeCl$_3$·6H$_2$O. The mixture was shaken at 25°C for 4 h in a concussive water incubator, M-100N, (TAITEC Co., Ltd., Saitama, Japan), and centrifuged at 3000 rpm with a centrifuge, Model B (Shimadzu Co., Kyoto, Japan) to recover product material. This material was washed three times with H$_2$O and dried at 150°C overnight in a drying oven, DZ-54 (Yamato Scientific Co., Ltd., Tokyo, Japan) to obtain Fe(III)-treated hydroxyapatites, HAP-300-Fe (Ca 6.27%, P 15.8%, Fe 28.15%, (Ca+Fe)/P = 1.29) and HAP-400-Fe (Ca 4.39%, P 17.76%, Fe 28.51%, (Ca+Fe)/P = 1.08), and Pb(II)-treated hydroxyapatites, HAP-300-Pb (Ca 24.24%, P 7.80%, Pb 27.09%, (Ca+Pb)/P = 2.92) and HAP-400-Pb (Ca 8.41%, P 5.83%, Pb 56.19%, (Ca+Pb)/P = 2.56). Chlorine element was not detected in the Fe(III)-treated apatites, and no strong IR absorptive band for NO$_3$ stretching between 1520-1280 cm$^{-1}$ was detected in Pb(II)-treated hydroxyapatites (referred to FIGURE 7 and the previous report$^{10}$). Specific surface area (see in Section 3.3) and X-ray diffraction patterns of these product materials (referred to FIGURE 1 as representative XRD patterns, HAP-300-Fe and HAP-300-Pb) were in good accordance with the previous report.$^{10}$

![FIGURE 1 XRD patterns of HAP-300-Fe (a) and HAP-300-Pb (b).$^{10}$ In the pattern (b), peaks with asterisk marks are for Pb apatite, Pb$_{10}$(PO$_4$)$_{16}$(OH)$_2$, and the other peaks are for the original hydroxyapatite, HAP-300. X-ray target, Cr.](image-url)

2.3. Adsorption test

A typical adsorption test using ARS was investigated as follows: To 10 mg of the metal-treated hydroxyapatite (HAP-300-Pb, HAP-300-Fe, HAP-400-Pb, or HAP-400-Fe) in a brownish glass tube bottle was added 10 mL of ARS solution in H$_2$O or PBS, of which concentration was...
changed from 0.04 to 0.26 mM. After the bottle was tightly closed with a screw cap, the mixture was vigorously shaken at 25°C for 30 h in the concussive water incubator. The mixture was filtered via a PTFE membrane filter with 0.45 µm pores, DISMIC-13HP (ADVANTEC Toyo Roshi Kaisha, Ltd., Tokyo, Japan), and the filtrate was analyzed with a total organic carbon (TOC) analyzer, TOC-VCSN (Shimadzu Co., Kyoto, Japan) to give carbon content of remaining ARS. From the TOC data, adsorption quantities (µmol/g) were evaluated by using a working curve method and the following Equation 1, where Q is adsorption quantity (µmol/g), V is solution volume (mL), C₀ is initial concentration of ARS (mM), and W is adsorbent weight (mg), to afford isothermic curves (FIGURE 3 and 4). For checking elution of ions (Ca²⁺, Fe³⁺, Pb²⁺, PO₄³⁻), or corresponding elements (Ca, Fe, Pb, P) from the adsorbents during adsorption in water, the filtrates were simultaneously analyzed by an inductively coupled plasma atomic emission spectrometer (ICP), SPS3100 (Seiko Instruments Inc., Tokyo, Japan).

\[ Q = \frac{V(C_0 - C)}{W} \times 10^3 \]  

(1)

2.4. Desorption test

For preparation of metal-treated hydroxyapatites adsorbing ARS, to HAP-300-Fe, HAP-300-Pb, HAP-400-Fe, or HAP-400-Pb (10 mg) in a brownish glass tube bottle was added 10 mL of 0.2 mM ARS solution in H₂O, and closed with a screw cap. The mixture was vigorously shaken at 25°C for 30 h in the concussive water incubator. A portion of supernatant was analyzed by TOC analysis to evaluate adsorption quantity. The rest material adsorbing ARS was recovered by suction filtration via a PTFE membrane filter (0.5 µm, ADVANTEC Toyo Roshi Kaisha, Ltd., Tokyo, Japan), washed three times with H₂O, and lyophilized to give the aimed ARS-adsorbed material, ARS-HAP-300-Fe, ARS-HAP-300-Pb, ARS-HAP-400-Fe, or ARS-HAP-400-Pb.

The obtained ARS-adsorbed materials were contributed to the following desorption test: To one of the ARS-adsorbed materials (10 mg) in a brownish glass tube bottle was added 10 mL of PBS, and its screw cap was tightly closed. The mixture was shaken at 25°C for 30 h in the concussive water incubator. Similarly to the above test (Section 2.3), the mixture was filtered via the PTFE membrane filter, and the filtrate was analyzed by TOC to evaluate desorption (%) by using Equation 2, where Mₑₜ is ARS quantity eluted in 10 mL of each test solution (mol), and Mₐₜ is ARS quantity adsorbed on 10 mg of each adsorbent. For checking the structure of ARS eluted, the recovered ARS solution was simultaneously analyzed by a UV-VIS spectrophotometer, U-2000 equipped with a temperature controller, SDR-30 (Hitachi Ltd., Tokyo, Japan).

\[ \text{Desorption} \% = \frac{M_{\text{eluted}}}{M_{\text{adsorbed}}} \times 100 \]  

(2)

2.5. Visualization of surface elemental distribution of metal-treated hydroxyapatites

A little pieces of the prepared Fe(III)- or Pb(II)-treated hydroxyapatites was put on a cut of adhesive carbon tape and made gold vapor deposition in order to prepare a photogenic sample. This sample was put in an electron probe micro analyzer (EPMA), S-2380N connecting an option system EMAX-7000 (Hitachi Ltd., Tokyo, Japan) to give the SEM photographs and the surface element distributions of P, Ca, Fe, and Pb. The accelerated voltage in EPMA was 15 kV.

2.6. FT-IR microspectroscopic analysis of HAP-400-Fe and HAP-400-Pb adsorbing ARS

The ARS-adsorbed samples, ARS-HAP-400-Pb and ARS-HAP-400-Fe described in Section 2.4, were analyzed by using a FT-IR microspectrometer AutoIMAGE System equipped with System 2000 FT-IR (Perkin-Elmer Co., Wellesley, MA). In this analysis, a little quantity of each sample was put on a gold-plated mirror of a plastic slide and analyzed by a reflective method along with 100 times scans. The obtained spectra were subtracted by the spectrum of HAP-400-Pb or HAP-400-Fe, and converted to the corresponding difference spectra (FIGURE 6) with a computer software, Spectrum 2000 for the Windows (Perkin-Elmer Co., Wellesley, MA).

3. RESULTS AND DISCUSSION

3.1. Preliminary adsorption test

For finding out equilibrium time for adsorption of ARS on metal-treated hydroxyapatites, an
adsorption investigation using 0.08 mM ARS (10 mL) and HAP-400-Fe (10 mg) was carried at 25°C for 48 h in H2O. This investigation resulted in affording a time-adsorption curve as shown in FIGURE 2, which indicates that it takes time more than 24 h to reach the adsorption equilibrium. Consequently, we confirmed that it is suitable to employ 30 h as adsorption time in the present study.

![FIGURE 2](image2.png)

**FIGURE 2** A time-adsorption curve in adsorption of 0.08 mM ARS on HAP-400-Fe (10 mg) at 25°C in H2O.

### 3.2. Adsorption behaviors of ARS in H2O and PBS

For elucidation of physicochemical behaviors of ARS on the prepared apatites, we investigated adsorption of ARS at 25°C for 30 h in H2O (pH 7) and PBS (pH 6.86) by using HAP-300-Fe, HAP-300-Pb, HAP-400-Fe, and HAP-400-Pb as adsorbents, compared with raw hydroxyapatites, HAP-300 and HAP-400. The initial concentration of ARS was changed from 0.04 to 0.26 mM, while adsorbent quantity was fixed. The results are shown as isothermic curves in FIGURE 3 for HAP-300 series and FIGURE 4 for HAP-400 series. In H2O (solid lines), all isothermic curves can be regarded as typical Langmuir-typed (L-typed) ones. Among them, HAP-300-Fe, HAP-300-Pb, and HAP-400-Fe exhibit higher adsorption quantities than the corresponding raw hydroxyapatites at lower ARS concentrations. However, HAP-400-Pb indicated less adsorption than HAP-400 at any ARS concentration. In comparison between HAP-300 and HAP-400 series, the former series can adsorb larger amounts of ARS molecules: The difference between adsorption capacities of the raw hydroxyapatites, HAP-300 > HAP-400, seemingly remains even after metal substitution, namely HAP-300-Fe > HAP-400-Fe and HAP-300-Pb > HAP-400-Pb. After adsorption test, the recovered filtrates were simultaneously analyzed by ICP to elucidate elution of components of the adsorbents. Table 1 shows that contents of Ca, P, Fe, and Pb in the filtrates (Ca and P: < 10 ppm, Fe and Pb: < 1 ppm), implying that a little elution of Ca2+ (< 0.11 mM) and PO43- (< 0.26 mM) was observed, and that slight elution of Fe3+ (< 0.006 mM) and Pb2+ (< 0.001 mM) was confirmed. We found that ARS adsorption occurred predominantly under the present condition.

### TABLE 1 Contents of Ca, P, Fe, and Pb eluted in the adsorption test solutions.

<table>
<thead>
<tr>
<th>Adsorbent</th>
<th>Ca (ppm)</th>
<th>P (ppm)</th>
<th>Fe (ppm)</th>
<th>Pb (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAP-300-Fe</td>
<td>4.37</td>
<td>7.98</td>
<td>0.22</td>
<td>–</td>
</tr>
<tr>
<td>HAP-300-Pb</td>
<td>1.51</td>
<td>1.35</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td>HAP-400-Fe</td>
<td>2.36</td>
<td>5.15</td>
<td>0.29</td>
<td>–</td>
</tr>
<tr>
<td>HAP-400-Pb</td>
<td>1.89</td>
<td>3.28</td>
<td>–</td>
<td>0.72</td>
</tr>
</tbody>
</table>

(a) initial concentration of ARS, 0.05 mM (in H2O); temperature, 25°C; time, 30 h. (b) detected by ICP. ND = not detected.

![FIGURE 3](image3.png)

**FIGURE 3** Isothermic curves in adsorption of ARS on HAP-300 series adsorbents (HAP-300-Fe, HAP-300-Pb, HAP-300) at 25°C for 30 h in H2O (solid lines) and PBS (hashed lines).
FIGURE 4  Isothermic curves in adsorption of ARS on HAP-400 series adsorbents (HAP-400-Fe, HAP-400-Pb, HAP-400) at 25°C for 30 h in H₂O (solid lines) and PBS (hashed lines).

Further, FIGURE 5 reveals UV-VIS spectra of ARS left (or non-adsorbed under equilibrium) in the present adsorption test solution. Adsorption of ARS with the Pb(II)-treated apatites (line B and E) afforded spectral lines with λ<sub>max</sub> values (334 and 518 nm) similar to the original ARS solution (line A), whereas the case with the Fe(III)-treated apatites (line C and D) afforded spectral lines with shifted λ<sub>max</sub> values (323 and 533 nm). The spectral shift of ARS in the latter case is probably attributed to interaction between the existing ARS and Fe<sup>3+</sup> ion eluted from the Fe(III)-treated apatites. Judging from the result of the line B in FIGURE 5 and TABLE 1, interaction between ARS and Pb<sup>2+</sup> affords no effect for spectral shifting. However, we believe that decomposition of ARS never occurs in the present adsorption condition.

In PBS (hashed lines in FIGURE 3 and 4), HAP-300-Fe and HAP-400-Fe indicated remarkable increase in their adsorption quantities, whereas the other adsorbents indicated terrible decrease. The decrease is possibly ascribed to disturb adsorption<sup>21</sup> of ARS molecules by PBS-constituent phosphate ions (H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, HPO<sub>4</sub><sup>2-</sup> and PO<sub>4</sub><sup>3-</sup>), which possibly have strong affinity to Pb sites similarly to Ca sites.<sup>18, 22, 23</sup> In addition, S-shaped isothermic curves appear obviously, which arises from adsorption inhibition by solvent molecules.<sup>19, 20</sup> Although HAP-300-Fe in PBS indicated the S-shaped curve (FIGURE 3), or such adsorption inhibition at relatively low ARS concentration (initial concentration C<sub>0</sub> < 0.10 mM), its adsorption quantity was dramatically and eventually improved at the higher concentration. We considered that HAP-300-Fe possibly has two different adsorption sites: One is subjected to phosphate ion inhibition, and the other is disrelated to that.

To evaluate adsorption constants from the isothermic curves (FIGURE 3 and 4), the Freundlich<sup>24</sup> and Langmuir<sup>25</sup> isothermic equations were employed and transferred to Equation 3, where n means an affinity constant (no unit), and K<sub>F</sub> means a constant including both affinity and capacity ((mol/g)(L/mol)<sup>-1/n</sup>), and to Equation 4, where Q<sub>S</sub> means saturated adsorption quantity (µmol/g), and K<sub>L</sub> means an equilibrium constant (L/mol), respectively.

In the both Equations, Q is adsorption quantity and C is equilibrium concentration.

\[
\log Q = \log K_F + \left(\frac{1}{n}\right) \log C \quad (3)
\]

\[
\frac{1}{Q} = \frac{1}{Q_S} + \left(\frac{1}{K_L Q_S}\right) \frac{1}{C} \quad (4)
\]

By plotting lines with Equation 3 and 4,<sup>14, 15, 26, 27</sup> the constants K<sub>F</sub> and Q<sub>S</sub> were derived from the corresponding y-intercepts, and n and K<sub>L</sub> were derived from the corresponding slopes. These values were summarized in TABLE 2. All the plot lines afforded high correlation coefficients, r<sup>2</sup> values, or good linear relationships. Freundlich constants were completely evaluated. All the n constants were more than 1 (n>1) in the case of H₂O.
However, n values for HAP-300-Pb, HAP-300, and HAP-400 were obtained less than 1 (n<1) in PBS. This means that affinity of these adsorbents to ARS is weak in PBS. The K_f constants for HAP-300 series adsorbents were about 2 to 10 times larger than those for HAP-400 series in the case of H_2O. In PBS, K_f values having four-digit and huge values for HAP-300-Pb, HAP-300, and HAP-400 were evaluated nevertheless of the low n values (n<1), and maybe due to the S-shaped isothermic curves (FIGURE 3 and 4). Langmuir constants Q_S and K_L for all adsorbents could be evaluated in the case of H_2O. Similarly to the case of Freundlich constants, Langmuir constants for HAP-300 series was larger than those for HAP-400 series. Compared with the raw hydroxyapatite HAP-300 and HAP-400, both Q_S and K_L values for HAP-300-Pb and HAP-400-Fe increased by metal treatment, while lowering Q_S but improving K_L was observed in the cases of HAP-300-Fe and HAP-400-Pb. Unfortunately, in the case using PBS as a solvent, all adsorbents except for HAP-400-Fe afforded minus Langmuir constants attributed to the corresponding minus y-intercepts, of which linear lines were figured with data on the S-shaped isothermic curves (FIGURE 3 and 4). On the other hand, the rest HAP-400-Fe afforded meaningful Langmuir constants in PBS, which were larger than those in H_2O. Although HAP-300-Fe exhibited incorrect Langmuir constants in PBS, it also could have extended its affinity and capacity constants in PBS, judging from FIGURE 3.

3.3. Surface analysis of Fe(III)- and Pb(II)-treated hydroxyapatites

We presumed that the difference in ARS adsorption behaviors described above is due to surface properties of the adsorbents used. The order of surface areas of the present adsorbents has been reported as follows: HAP-300 (81 m^2/g) > HAP-300-Pb (69 m^2/g) > HAP-400 (37 m^2/g) > HAP-400-Pb (23 m^2/g) > HAP-300-Fe (17 m^2/g) > HAP-400-Fe (15 m^2/g). However, this order is inconsistent with that of the present evaluated adsorption constants, particularly the Langmuir Q_S constants (TABLE 2). We considered that adsorbability of ARS is not always dependent on the surface area sizes.

Alternatively, surface element distributions (Ca, Fe, Pb, P) of the Fe(III)- and Pb(II)-treated hydroxyapatites were analyzed by EPMA. FIGURE 6 shows each SEM photographs along with each element distribution with element dots in the isomorphic size. In Fe(III)-treated hydroxyapatites, HAP-300-Fe indicated both thick Fe and Ca dots (FIGURE 6a). HAP-400-Fe indicated thick Fe dots but obscure Ca dots (FIGURE 6b). Appearance of P dots in HAP-400-Fe was much clearer than that in HAP-300-Fe. Therefore, HAP-300-Fe has many Fe and Ca sites but a few PO_4 sites on the surface, while HAP-400-Fe has many Fe and PO_4 sites but rare Ca sites.

<table>
<thead>
<tr>
<th>Adsorbent (Solvent)</th>
<th>Freundlich</th>
<th>Langmuir</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>K_f (x 10^3)</td>
</tr>
<tr>
<td>HAP-300-Fe (H_2O)</td>
<td>3.59</td>
<td>1.34</td>
</tr>
<tr>
<td>(PBS)</td>
<td>1.49</td>
<td>102</td>
</tr>
<tr>
<td>HAP-300-Pb (H_2O)</td>
<td>2.65</td>
<td>4.45</td>
</tr>
<tr>
<td>(PBS)</td>
<td>0.76</td>
<td>7829</td>
</tr>
<tr>
<td>HAP-300 (H_2O)</td>
<td>1.96</td>
<td>13.1</td>
</tr>
<tr>
<td>(PBS)</td>
<td>0.75</td>
<td>6016</td>
</tr>
<tr>
<td>HAP-400-Fe (H_2O)</td>
<td>4.18</td>
<td>0.60</td>
</tr>
<tr>
<td>(PBS)</td>
<td>3.89</td>
<td>1.09</td>
</tr>
<tr>
<td>HAP-400-Pb (H_2O)</td>
<td>3.83</td>
<td>0.56</td>
</tr>
<tr>
<td>(PBS)</td>
<td>1.05</td>
<td>150</td>
</tr>
<tr>
<td>HAP-400 (H_2O)</td>
<td>3.09</td>
<td>1.18</td>
</tr>
<tr>
<td>(PBS)</td>
<td>0.78</td>
<td>5732</td>
</tr>
</tbody>
</table>

(a) based on data from isothermic curves in FIGURE 2 and 3. (b) Equation 3. (c) Equation 4. (d) no unit. (e) (mol/g) (L/mol)^1/n. (f) correlation coefficient. (g) µmol/g. (h) L/mol. (i) affording a minus value at y-intercept on Equation 4 in spite of a good correlation coefficient (r^2).
FIGURE 6 SEM photographs and surface element distributions (P, Ca, Fe, Pb) of HAP-300-Fe (a), HAP-400-Fe (b), HAP-300-Pb (c), and HAP-400-Pb (d) by using EPMA. The areas in orange circles in the SEM photo (d) indicate newly formed lead apatite crystals.

In Pb(II)-treated hydroxyapatites, HAP-300-Pb indicated both thick Pb and Ca dots and somewhat
thin P dots on the surface (FIGURE 6c). HAP-400-Pb indicated lopsided thick Pb dots, which come from newly formed lead apatite crystals, as emphasized with orange circles in the SEM photo of FIGURE 6d. Simultaneously, it indicated thick Ca and P dots and thin Pb dots in the other area, which comes from the surface of original HAP-400 crystals (the outside of the orange circles as shown in FIGURE 6d). Therefore, HAP-300-Pb has uniform distribution of Pb, Ca, and PO₄ sites on the surface, whereas HAP-400-Pb has lopsided distribution of those sites. We considered that such specific surface site distributions possibly breed the characteristic ARS adsorption patterns, as shown in FIGURE 3 and 4. The S-shaped isothermic curves for HAP-300-Fe in PBS (FIGURE 3) possibly arises from the thick site distributions of Fe and Ca (FIGURE 6a), which should exhibit a different binding behaviors to an ARS molecule on the surface, respectively. The L-typed isothermic curve for HAP-400-Fe even in PBS (FIGURE 4) is possibly due to the predominantly thick Fe site distribution (FIGURE 6b).

We have reported that adsorption of ARS on raw hydroxyapatite occurs by salt form superior to chelate form: The former means electrostatic interaction of two ionized C–O groups at C-1 and C-2 of an ARS molecule with a Ca site of apatite surface; The latter means chelated and resonated interaction of two negatively charged C–O groups at C-1 and C-9 of ARS with a Ca site. Judging from the results of isothermic curves (FIGURE 3 and 4) and adsorption constants (TABLE 2), Fe and Pb sites on the present adsorbents unambiguously act as adsorption sites. Therefore, for observation of actual ARS-adsorbing status, Fe(III)- and Pb(II)-treated apatites adsorbing ARS were recovered after adsorption tests and analyzed by FT-IR microspectroscopy, compared with the original non-adsorbed cases. As representative samples, ARS-adsorbed HAP-400-Fe (ARS-HAP-400-Fe) and ARS-adsorbed HAP-400-Pb (ARS-HAP-400-Pb) were employed since HAP-400-Fe indicated the L-typed isothermic curves both in H₂O and PBS and the predominant Fe distribution on the surface, and HAP-400-Pb indicated the lopsided Pb sites and about twice-higher Pb content than that of HAP-300-Pb (referred to Section 2.2). FIGURE 7 shows comparison of spectra between adsorbed and non-adsorbed cases, namely (a) ARS-HAP-400-Fe versus HAP-400-Fe and (b) ARS-HAP-400-Pb versus HAP-400-Pb. In these FIGURES, the bands of surface OH stretching mode (A), which is common to HAP-400-Fe and HAP-400-Pb, and internal OH stretching mode (B), which is exclusive to HAP-400-Pb, obviously retain even after ARS adsorption. This implies that OH site is never concerned with the present adsorption and that the adsorbent structures are possibly preserved even after ARS adsorption.

![FIGURE 7](https://via.placeholder.com/150)

**FIGURE 7** FT-IR spectra of ARS-HAP-400-Fe and HAP-400-Fe (a) and those of ARS-HAP-400-Pb and HAP-400-Pb. The band A means surface OH stretching, and the band B means internal OH stretching.

The bands between 1800 and 1100 cm⁻¹, of which region affords information of functional groups of ARS, were significantly changed after ARS adsorption. Therefore, difference spectra informed of ARS adsorption status were derived by subtraction of a spectrum for non-adsorbed apatite from that for the corresponding ARS-adsorbed apatite, as shown in FIGURE 8, which reveals significant spectra based on naked ARS-structural information as well as interaction patterns of ARS with Fe and Pb sites. According to Kiel’s and Puchtler’s methods, the bands observed in those spectra were identified as follows: FIGURE 8a
([ARS-HAP-400-Fe] – [HAP-400-Fe]) shows a C=O stretching band at C-10 position of ARS, ν(10-C=O), (1656 cm\(^{-1}\)) and a strong complex ring band, which features six-membered ring chelation between Fe and two negatively charged C–O groups both at the C-1 and C-9 positions (1511 cm\(^{-1}\)), featuring chelate form structure; FIGURE 8b ([ARS-HAP-400-Pb] – [HAP-400-Pb]) shows the other C=O stretching band at the C-9 position, ν(9-C=O), (1631 cm\(^{-1}\)) and a weak complex ring band (1502 cm\(^{-1}\)), featuring salt form structure. The other bands were common to FIGURE 8a and 8b: aromatic C=C stretching bands, ν(Ar C=C), 13, 29 a OH bending band, δ(OH), 29 a combination band from C–O and C=C stretching modes at the C-1 and C-2 positions, ν(C=O)-1,30, 31 another combination band from C–O stretching and O–C=C bending modes at the same positions, ν(C=O)-2,30, 31 and bands for stretching and bending modes of three carbon framework centering C=O carbon on the aromatic ring, carbonyl C–C–C.29

FIGURE 8  Difference IR spectra of ARS adsorbed on HAP-400-Fe (a) and HAP-400-Pb (b).

SCHEME 2 Possible schematic mechanisms for ARS adsorption on Fe(III)-treated apatites (a) and Pb(II)-treated apatites (b).

3.4. Adsorption mechanism

From the physicochemical study and the surface analyses, adsorption patterns of ARS on the Fe(III)- and Pb(II)-treated hydroxyapatites were depicted in SCHEME 2. The Fe sites almost all interact with ARS molecules by chelate form (SCHEME 2a), but hardly by salt form. Particularly, HAP-400-Fe with thin surface distribution of Ca sites (FIGURE 6b) can exclusively make chelate form on the surface both in H\(_2\)O and PBS, featuring L-typed isothermic curves at any condition (FIGURE 4). HAP-300-Fe with clear Fe and Ca distributions (FIGURE 6a) also makes chelate form on the Fe sites, but it partially makes salt form on the Ca sites, featuring the S-shaped isothermic curve in PBS (FIGURE 3). The presence of Ca sites affects the surface property of HAP-300-Fe to some extent. The improvement of adsorption capacity of HAP-300-Fe and HAP-400-Fe in PBS is possibly ascribed to exclusive and strong chelation of ARS molecules to the Fe sites, since phosphate ions adsorb and hide Ca sites and interfere interaction of ARS with Ca.18, 21-23 The Pb sites mainly interact with ARS molecules by salt form.
(SCHEME 2b), and partially by chelate form, similarly to the case of raw hydroxyapatite. Pb sites are subjected to the adsorption interfere by phosphate ions. Thereby, HAP-300-Pb and HAP-400-Pb revealed the S-shaped isothermic curves in PBS (FIGURE 3 and 4) nevertheless of featuring different Pb and Ca distributions (FIGURE 6c and 6d).

TABLE 3 Desorption of ARS from ARS-adsorbed apatites by using PBS as a solvent at 25°C for 30 h.

<table>
<thead>
<tr>
<th>ARS-adsorbed apatite</th>
<th>Desorption (%)&lt;sup&gt;(a)&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARS-HAP-300-Fe</td>
<td>8</td>
</tr>
<tr>
<td>ARS-HAP-300-Pb</td>
<td>74</td>
</tr>
<tr>
<td>ARS-HAP-300</td>
<td>72</td>
</tr>
<tr>
<td>ARS-HAP-400-Fe</td>
<td>9</td>
</tr>
<tr>
<td>ARS-HAP-400-Pb</td>
<td>99</td>
</tr>
<tr>
<td>ARS-HAP-400</td>
<td>90</td>
</tr>
</tbody>
</table>

(a) Equation 2.

For reconfirming the adsorption mechanisms mentioned above, desorption of ARS from ARS-adsorbed apatites was investigated at 25°C for 30 h in PBS. If ARS interacts with an adsorbent by the chelate form, low desorption from the adsorbent must be observed in PBS, but if by the salt form, much desorption must be observed. Although H<sub>2</sub>O and NaOH aqueous solution may be also pointed out as desorption solvents, they can be excluded because of affording low desorption effect (< 20%). The result of desorption test with PBS was summarized in TABLE 3. Apparently, the Fe(III)-treated hydroxyapatites indicated low desorption in less than 10%, which is ascribed to desorption from the remaining Ca sites rather than the Fe sites. The Pb(II)-treated hydroxyapatites as well as the raw hydroxyapatites indicated high desorption in 72-99%, which implies that adsorption on them involves predominant salt form and minor chelate form. In addition, the ARS molecules eluted from ARS-HAP-300-Pb and ARS-HAP-400-Pb featured UV-VIS spectra bearing λ<sub>max</sub> 334 and 518 nm at pH 6.86 in PBS (FIGURE 9), of which shape is consistent with the case of original ARS (referred to FIGURE 5). The other spectra (C and D, overlapped) for ARS-HAP-300-Fe and ARS-HAP-400-Fe also indicate λ<sub>max</sub> 518 nm unlike the case of adsorption test showing the spectral shifts by interaction of ARS with Fe<sup>3+</sup> ions eluted from the Fe-treated apatites (FIGURE 5). FIGURE 9 implies that ARS never decomposed by adsorption as well as desorption. Thereby, we could reconfirm that ARS molecules almost (> 90%) adsorb on Fe sites by the chelate form, and that they adsorb on Pb sites mainly by the salt form.

FIGURE 9 UV-VIS spectra of eluted ARS from ARS-HAP-300-Pb (A), ARS-HAP-400-Pb (B), and overlapped spectra from ARS-HAP-300-Fe and ARS-HAP-400-Fe (C and D). The enlarged spectrum C or D (100 times) is also sited in the upper blank area.

CONCLUSION

As mentioned above, we elucidated predominant ARS adsorption mechanisms for the Fe(III)- and Pb(II)-treated hydroxyapatites, chelate form and salt form, respectively. Further, we considered that the metal-treated apatites have applicable possibilities as adsorbents or catalysts. For example, the Fe(III)-treated apatites may be applied as adsorbents for cleaning colored wastewater containing much phosphate ions, and as catalysts for Fe-catalyzed organic reactions. The Pb(II)-treated apatites may be applied as recyclable adsorbents for removing dyes by using a proper solvent such as PBS.

ACKNOWLEDGMENT

We express an appreciation to all staffs at Department of Mechanical Engineering, Osaka Municipal Technical Research Institute, Japan for using an electron probe micro analyzer (EPMA) and giving much good advice to us.

REFERENCES