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Photocatalysis by Calcium Hydroxyapatite Modified with Ti(IV): Albumin Decomposition and Bactericidal Effect

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Colloidal calcium hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$; HAP) particles doped with Ti(IV) ions in different atomic ratios, $\text{Ti}/(\text{Ca} + \text{Ti}) = X_{\text{Ti}}$, by a coprecipitation method were characterized by TEM, UV, FTIR, XPS, and ICP–AES. The photocatalytic activity of the modified HAP particles was examined by decomposition of acetaldehyde and albumin and a bactericidal test by colon bacillus. Ca(II) of HAP was one-to-one substituted by Ti(IV) at $X_{\text{Ti}} \leq 0.1$. When doped at $X_{\text{Ti}} > 0.1$, irregular particles of amorphous titanium phosphate were formed besides long rectangular particles of HAP. X_{Ti} of the surface phase of the particles was much less than that of the whole particle; Ti(IV) is less contained in the surface phase than in the bulk one. A UV beam was absorbed by Ti(IV)-modified HAP particles but not by the unmodified particles. The decomposition of acetaldehyde and albumin by Ti(IV)-doped particles was found under UV irradiation, while the unmodified HAP particles were inactive for the decomposition of both materials. Further, different from TiO_2 , Ti(IV)-doped HAP showed a bactericidal function in the dark.

1. Introduction

Recently, titanium dioxide TiO_2 has received much attention as a photocatalyst which photooxidizes organic substances in both water and air.^{1,2} Synthetic calcium hydroxyapatite $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, denoted as HAP, is biologically important and is used in bioceramics and adsorbents for biomaterials, because it was established that HAP shows an excellent affinity to biomaterials such as proteins. Hence, it is of interest to prepare composite materials of TiO_2 and HAP for photocatalytic decomposition of biomaterials, such as proteins and lipids. Nonami et al. proposed a new structure for TiO_2 –HAP composite films in which porous HAP layer forms on TiO_2 layer on a substrate in a pseudo body solution.³ However, this composite structure is inefficient for photocatalysis which requires UV light irradiation on the TiO_2 layer. We attempted to develop a novel photocatalyst with a high affinity to biomaterials by an atomic level composite of photocatalytic material and HAP, which could be realized by modifying HAP particles with Ti(IV). The modification of HAP particles was performed by coprecipitation and ion-exchange methods. Suzuki et al. have found that Ca(II) of HAP can be exchanged with various metal ions in aqueous media.^{4–12} However, the surface structure and

property of the metal-substituted HAP have not been clarified. Ribeiro et al. have studied the surface of HAP modified with Ti(IV) ions by immersion methods¹³ and indicated the formation of a titanium phosphate, $\text{Ti}(\text{HPO}_4)_2 \cdot n\text{H}_2\text{O}$ ($n = 1–3$), which probably has a double layered structure.¹⁴ Weiser et al. have coated metal Ti with HAP by doping and diffusing of Ca and P elements into the metal before the coating to obtain a high-quality HAP film on the metal Ti surface.¹⁵ On the other hand, Zeng et al. have studied the adsorption of albumin on calcium phosphate (CaP) and Ti films deposited on a germanium ATR crystal by ion beam sputter deposition from HAP and metal Ti targets and found that the CaP film adsorbs a greater amount of albumin than the surface of metal Ti; CaP shows a higher affinity to protein than metal Ti.¹⁶ Since HAP surface is known to show a high affinity to albumin,^{21,22} HAP is used as an adsorbent of column chromatography for separating proteins. The bactericidal effects of Ag–HAP thin films on alumina have been reported by Feng et al.²³ Although Ti(IV)-modified

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HAP is anticipated to show a bactericidal effect, there has been no study on the bactericidal character of this material.

Wakamura et al. previously investigated the surface structure and composition of the HAP particles modified with various metal ions by coprecipitation and ion-exchange methods.^{17–19} In the present study, HAP particles modified with Ti(IV) by a coprecipitation method were characterized by various techniques. Photocatalytic activities of the well-characterized, modified HAP particles were examined by decomposition reactions of acetaldehyde and albumin. On the basis of the obtained results, we discussed the surface structure and photocatalysis of the modified HAP particles. Further, the bactericidal function of the materials was examined using a colon bacillus²⁴ and a noble photocatalyst was able to be developed.

2. Experimental Section

Materials. Colloidal HAP particles doped with Ti(IV) were prepared by coprecipitation. $\text{Ca}(\text{NO}_3)_2$ and $\text{Ti}(\text{SO}_4)_2$ were dissolved in 1 dm³ deionized–distilled water free from CO₂ at different atomic ratios $\text{Ti}/(\text{Ca} + \text{Ti}) = X_{\text{Ti}}$ from 0 to 0.8. The total amount of Ca and Ti in the solutions was held at 0.1 mol; 0.060 mol of H₃PO₄ was added to the solutions and the solution pH was adjusted to 9 by adding a 15 mol dm^{−3} NH₄OH solution. The resulting suspension was aged in a capped Teflon vessel at 100 °C for 6 h. The resulted precipitates were filtered off, washed with 5 dm³ deionized–distilled water, and finally dried in an air oven at 70 °C. Ninhydrin and albumin supplied by Wako Pure Chemicals were used as received.

Characterization. The HAP particles thus modified with Ti(IV) were examined by various conventional methods as follows. The morphology of the particles was observed using a JEOL transmission electron microscope (TEM) at an accelerating voltage of 200 kV. The samples for TEM were prepared by a dispersing method. X-ray diffraction (XRD) patterns were taken by a powder method using a Shimadzu high-intensity diffractometer with a rotating cathode using Cu K α radiation (50 kV and 200 mA). Transmission IR spectra were recorded in vacuo using a Perkin-Elmer Fourier transform infrared (FTIR) spectrophotometer by a self-supporting disk method in a vacuum cell. The sample powders (30 mg) were pressed into disks of 1 cm diameter under 572 kg cm^{−2}. Before the spectra were taken, the sample disks were outgassed at 300 °C for 2 h. Reflection UV–vis spectra were taken using a UV spectrometer (JASCO V-560) at 200–400 nm. The specific surface area was calculated by applying the BET equation to the N₂ adsorption isotherm measured at −196 °C using an automatic volumetric apparatus.

Chemical Analysis. Ti and P contents were determined by a Perkin-Elmer induced coupled plasma spectrometer (ICP–AES) employing wavelengths of 317.933 (Ca), 334.941 (Ti), and 213.618 nm (P). The samples for ICP–AES were dissolved in a dilute HNO₃ solution. X-ray photoelectron spectroscopy (XPS) was done using a Perkin-Elmer spectrophotometer with Mg K α radiation (20 kV and 30 mA), where the samples were mounted on the sample holder by a carbon tape.

Acetaldehyde Decomposition Test. The photocatalytic activities were estimated from the decomposition of acetaldehyde vapor under 1 mW cm^{−2} UV irradiation. The samples used for photocatalysis were HAP modified at $X_{\text{Ti}} = 0$ and 0.1. The sample weight was decided by adjusting the surface area of the samples as a constant value of 85 m² using the BET specific surface area. The samples were settled in the bottom of a 500 cm³ cylindrical glass vessel sealed with a quartz plate with 5 mm thickness using an O-ring. A mixed gas (N₂, 80%; O₂, 20%) was introduced into the vessel through the gas inlet to replace air by the mixed gas, and then acetaldehyde vapor was injected into the vessel, followed by keeping it in the dark to achieve equilibrium. Then ultraviolet irradiation (1 mW cm^{−2}) and holding in the dark were repeated at 24 h intervals. After the reaction, the concentrations of acetaldehyde and CO₂ in the reaction vessel were determined by a gas chromatograph (FID).

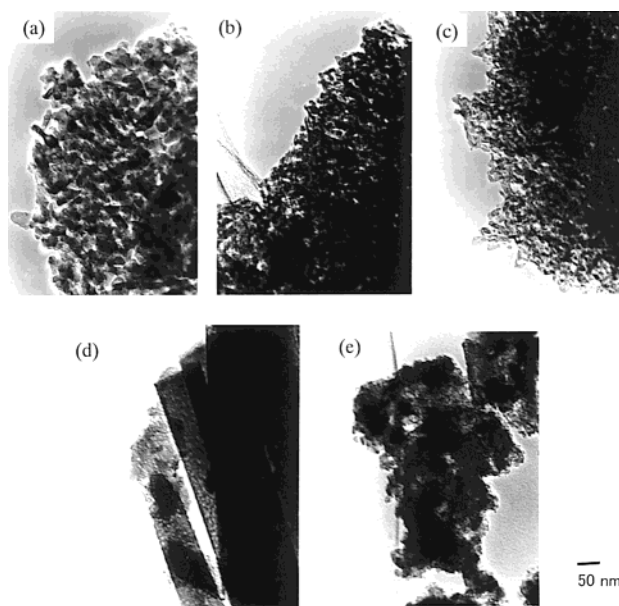


Figure 1. TEM pictures of the particles formed at varied X_{Ti} values by coprecipitation method: $X_{\text{Ti}} =$ (a) 0, (b) 0.03, (c) 0.1, (d) 0.5, and (e) 0.8.

Ninhydrin Test of Albumin Decomposition. The affinity to biomaterials and photocatalytic activities were estimated from adsorption and decomposition of albumin. The ninhydrin test was employed to identify albumin before and after the decomposition reaction. Samples of 1 g of HAP modified at $X_{\text{Ti}} = 0$ and 0.1 and 1 g of TiO₂ were immersed in 1 g dm^{−3} albumin solutions at 30 °C for 12 h. The particles were filtered, washed with 10 dm³ deionized–distilled water, and dried in an air oven at 50 °C. Each sample was divided into two parts (each 0.5 g); one was kept in a dark box and the other was irradiated by 1 mW cm^{−2} UV light for 24 h at room temperature. Finally, the samples were sprayed with a ninhydrin indicator and dried in an air oven at 150 °C.

Bactericidal Test by *Escherichia coli* (*E. coli*). The suspensions of HAP modified at $X_{\text{Ti}} = 0$ and 0.1 and TiO₂ homogeneously dispersed in silica sols were deposited on a glass plate (5 × 5 cm) by a spin-coating method. The films on glass were sterilized by drying at 180 °C for 30 min. *E. coli* cells (IFO 3310 strain) were grown aerobically in 2.5 cm³ of nutrient broth (“Daigo”, Nippon Seiyaku) at 30 °C for 16–18 h. The cells were centrifuged at 4000 rpm and suspended in sterilized water with appropriate dilution. An *E. coli* cell suspension (150×10^{-6} dm³, 2×10^5 cells cm^{−3}, total 3×10^4 cells) was pipetted onto glass plates coated with HAPs (modified at $X_{\text{Ti}} = 0$ and 0.1) and TiO₂, spread out to give a liquid film of approximately 1 cm in diameter and placed in an airtight illumination chamber to prevent drying. This chamber was illuminated with a 15 W black light, and the light intensity with peaks around 360 nm was 1.0 mW cm^{−2}, which was measured using a UV radiometer (UVR-36, Tepeco) at the sample position. After the illumination, the cells were removed using a gauze patch and collected in a 0.15 mol dm^{−3} NaCl solution. This solution was spread onto nutrient agar medium (Standard Method Agar “Nissui”, Nissui Seiyaku) and incubated for 24 h in order to determine the number of viable cells in terms of colony-forming units.

3. Results and Discussion

Figure 1 displays TEM micrographs of Ti(IV)-doped HAP particles. The HAP particles formed at $X_{\text{Ti}} \leq 0.1$ are short rods. The particles formed at $X_{\text{Ti}} \geq 0.5$ are a mixture of long rectangular and irregular particles, although (d) and (e) show only long rectangular and irregular particles, respectively. The irregular particles increased with increase of X_{Ti} . Although the detailed reason for the formation of the long particles remains unclear, the charge difference between Ca(II) and Ti(IV) seems to influence

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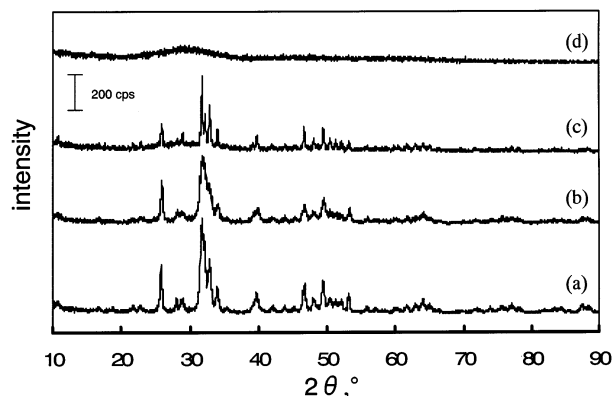


Figure 2. XRD patterns of the products at varied X_{Ti} values by coprecipitation method: X_{Ti} = (a) 0, (b) 0.1, (c) 0.5, and (d) 0.8.

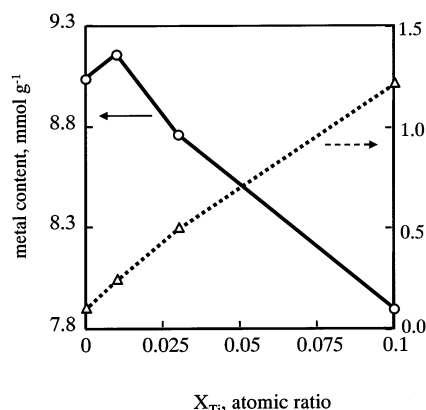


Figure 3. Plots of Ca and Ti contents against X_{Ti} : \circ , Ca content; \triangle , Ti content.

the particle formation of HAP as found in the previous study on the modification with Cr(III).¹⁹

Figure 2 displays XRD patterns of the products at different X_{Ti} values. Their peak intensity is lowered by increasing X_{Ti} at $X_{\text{Ti}} \leq 0.5$, and the product at $X_{\text{Ti}} = 0.8$ is poorly crystallized. Except for the product at $X_{\text{Ti}} = 0.8$, the patterns are characteristic of HAP (JPCDS 9-432), verifying that the long rectangular particles formed at $X_{\text{Ti}} \geq 0.5$ are well-crystallized HAP. This finding suggests that HAP crystals can be doped with Ti(IV) up to $X_{\text{Ti}} = 0.1$.

To determine the composition of the products, Ca, Ti, and P in the formed particles were assayed by ICP-AES. Figure 3 plots the contents of Ca(II) and Ti(IV) ions against X_{Ti} by open circles and triangles, respectively. The increase of Ti(IV) in the formed particles accompanies an equivalent decrease of Ca(II), which means that Ti(IV) in the crystal of HAP is present as a divalent ion, such as $[\text{Ti}(\text{OH})_2]^{2+}$ and $[\text{Ti}(\text{HPO}_4)]^{2+}$. Figure 4 plots X_{Ti} of the whole particle and the surface phase of particles determined by ICP-AES and XPS, respectively. For all the materials X_{Ti} of the surface phase (noted as X_s) was less than that of the whole particle (noted as X_w), which can be interpreted by considering that Ti(IV) is more easily precipitated than Ca(II) to be more contained in the inner part of particle. X_w is larger than X_{Ti} in the starting solutions, implying that Ti(IV) is incorporated into the particles more than Ca(II). These results are ascribed to the higher hydrolysis constant of Ti(IV) than Ca(II).

Figure 5 shows FTIR spectra of the HAP particles modified with Ti(IV). The spectrum of the sample formed at $X_{\text{Ti}} = 0$ gives rise to three bands at 3680, 3673, and 3657 cm^{-1} . We previously assigned these three bands to

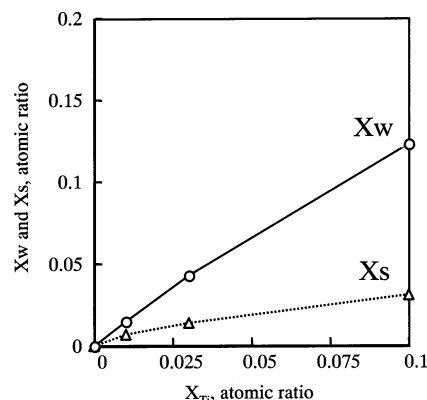


Figure 4. Atomic ratio in the whole particle (X_w ; \circ) and in the surface phase (X_s ; \triangle) of Ti(IV)-modified HAP particles.

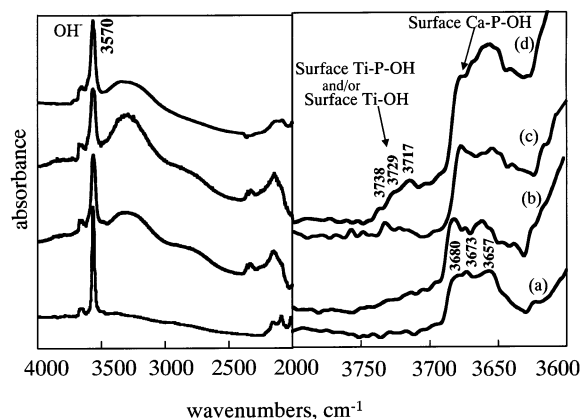


Figure 5. IR spectra of HAP particles modified with Ti(IV) by coprecipitation at various X_{Ti} values: X_{Ti} = (a) 0, (b) 0.01, (c) 0.03, and (d) 0.1.

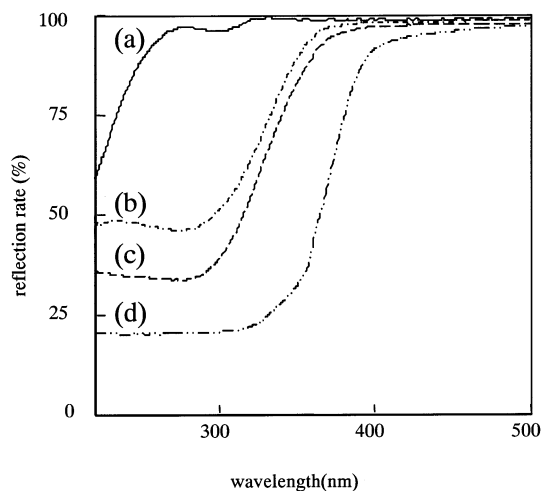


Figure 6. Reflection UV-vis spectra of HAP particles modified with Ti(IV) by coprecipitation at various X_{Ti} values and TiO_2 : X_{Ti} = (a) 0, (b) 0.01, and (c) 0.1. (d) TiO_2 .

the O-H stretching vibration modes of surface P-OH groups which are considered to form due to protonation of surface PO_4^{3-} ions to balance the surface charge.²⁰ The spectrum of the samples formed at $X_{\text{Ti}} = 0.1$ shows three additional IR bands at 3738, 3729, and 3717 cm^{-1} that would be ascribed to surface Ti-OH groups.

Figure 6a-c illustrates the reflection UV-vis spectra of the products at varied X_{Ti} along with that of TiO_2 (Figure 6d). The UV absorption above ca. 370 nm is observed in spectra b and c of the samples modified at $X_{\text{Ti}} = 0.01$ and 0.1 but not in spectrum a of the unmodified HAP. With

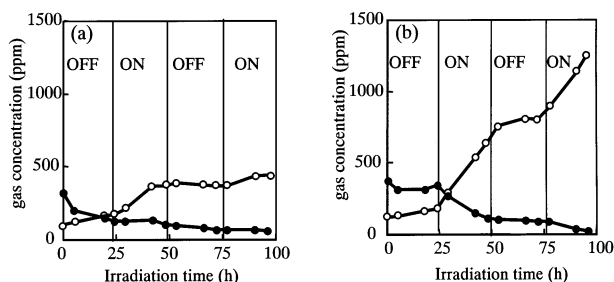


Figure 7. Concentration of acetaldehyde (closed symbols) and CO₂ (open symbols) vs UV irradiation time: (a) unmodified HAP; (b) Ti(IV)-modified HAP at $X_{Ti} = 0.1$.

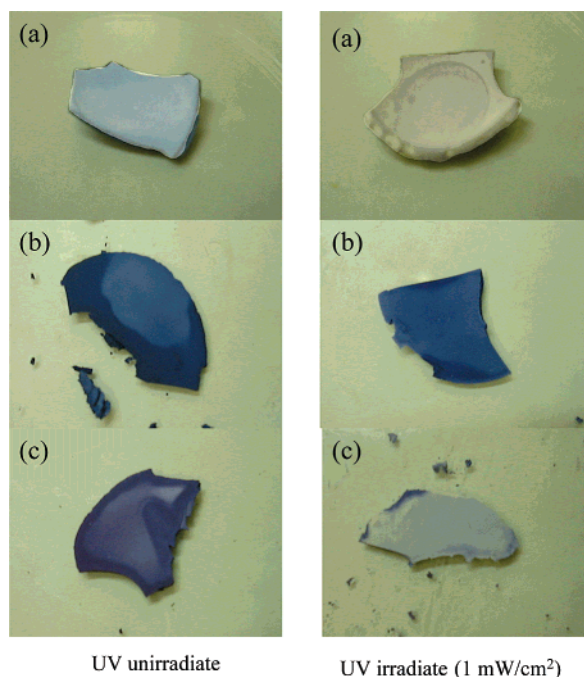


Figure 8. Results of adsorption and photocatalysis decomposition of albumin obtained by ninhydrin colored tests: (a) TiO₂; (b) HAP; (c) Ti(IV)-modified HAP.

increasing X_{Ti} the absorbance rises to that of TiO₂. These results clearly imply that the surface of HAP particles is modified by substituting with Ti(IV).

Figure 7 plots the concentrations of acetaldehyde and CO₂ against the UV irradiation time. It is clearly seen in Figure 7b that CO₂ is increased (open circles) and acetaldehyde is decreased (closed circles) on irradiating the UV beam to the material modified at $X_{Ti} = 0.1$ and on stopping the irradiation the concentrations of acetaldehyde and CO₂ are essentially not changed. However, as shown in Figure 7a, the unmodified sample shows less concentration change of CO₂ and acetaldehyde than the modified sample. Therefore, it can be confirmed that Ti(IV)-modified HAP exhibits higher activity in photocatalysis than the unmodified HAP.

Figure 8 shows the results of the ninhydrin colored test: the photographs of TiO₂ (a), the unmodified HAP (b), and Ti(IV)-modified HAP (c) unirradiated and irradiated by UV after albumin adsorption. TiO₂ (a) shows no ninhydrin color under UV irradiation and unirradiation, although a slight blue color is detected due to albumin weakly adsorbed. The unmodified HAP (b) is colored by

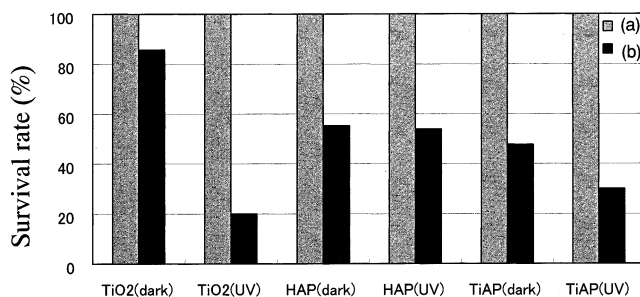


Figure 9. Results of bactericidal test using colon bacillus: (a) 0 h; (b) after 4 h.

ninhydrin under UV irradiation and unirradiation. Ti(IV)-modified HAP (c) is colored only under UV unirradiation. These results indicate that TiO₂ does not strongly adsorb albumin, whereas the unmodified HAP adsorbs albumin but shows no activity of photocatalytic decomposition and Ti(IV)-modified HAP adsorbs albumin and decomposes it by photocatalysis.

Figure 9 compares the results of the bactericidal test by colon bacilli. TiO₂ gives rise to essentially no photocatalysis in the dark and kills less than 10% of the colon bacilli, while TiO₂ kills 80% of the bacilli under UV irradiation. On the other hand, Ti(IV)-modified and unmodified HAPs show bactericidal effects even in the dark, killing ca. 50% of the bacilli. It should be noted that the bactericidal effect of Ti(IV)-modified HAP was enhanced by UV irradiation and only ca. 30% of the bacilli survive after UV irradiation. These findings indicate that Ti(IV)-modified HAP exhibits a higher bactericidal effect than TiO₂ both in the dark and under UV irradiation. The Ti(IV)-modified HAP generate positive holes (h^+) as well as TiO₂ catalysts. The formed positive holes interact with adsorbed H₂O to yield hydroxyl radicals (OH^+) with a strong oxidation ability which would decompose various organic materials to show the bactericidal effect. To verify the details of this mechanism, further analysis of the band structure of Ti(IV)-modified HAP is required.

4. Conclusion

From the obtained results, we can draw the following conclusions. HAP has a feature that Ca(II) ions in the crystal are exchanged with Ti(IV) up to $X_{Ti} = 0.1$ in atomic ratio. The added Ti(IV) exists in the HAP crystals as divalent cations such as $[Ti(OH)_2]^{2+}$ and $[TiHPO_4]^{2+}$. Ti(IV)-substituted HAP has surface OH groups originating from HPO_4^{2-} and OH^- coordinating to Ti(IV). The HAP particles penetrated by Ti(IV) into the crystals absorb UV beam at a wavelength less than 380 nm, so that it exhibits a photocatalytic activity in oxidation and decomposition of acetaldehyde. This character of Ti(IV)-modified HAP resembles the case of oxidative decomposition of organisms by TiO₂. However, HAP has a high affinity to organisms such as proteins although TiO₂ shows no affinity to organisms. Therefore, distinct from TiO₂ photocatalysts, Ti(IV)-modified HAP has both adsorption affinity and photocatalytic activity for organisms. To make matters better, Ti(IV)-modified HAP shows the bactericidal effect even in the dark although TiO₂ shows it only under UV irradiation.

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