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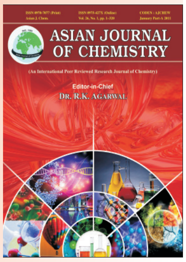

Molecular Mechanism of Nano-Hydroxyapatite Surface Changes from Hydrophilic to Hydrophobic†

XU RAN*, XUEYING YU, JIN HE and ZHE XIAO

School of Materials Science and Engineering, Changchun University of Technology, Changchun 130012, P.R. China

*Corresponding author: Tel: +86 15526853785; E-mail: ranxu@mail.ccut.edu.cn

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The hydroxyapatite was modified *via* a stearic acid with different content. The microstructure, surface structure and surface element binding energy of the modified hydroxyapatite were investigated. Furthermore, the activation ratio of the modified hydroxyapatite was also tested. The FTIR and XPS detection revealed that the stearic acid molecules had been grafted onto the hydroxyapatite's surface through ionic bonds, which had been formed through a reaction between the carboxyl groups of the stearic acid molecules and the calcium ions on the hydroxyapatite's surface. The result of the activation ratio test demonstrated that in the presence of a critical value of stearic acid, the hydroxyapatite's surface was then modified to a nearly complete hydrophobic state.

Keywords: Hydroxyapatite, Hydrophilicity change, Molecular mechanism.

INTRODUCTION

Hydroxyapatite (HA), of molecular formula $Ca_{10}(PO_4)_6(OH)_2$ is a major inorganic component of a human's hard tissue. It possesses advantageous qualities such as excellent biocompatibility and bioactivity. However, its mechanical qualities are significantly poorer, in particular its extreme brittleness. As a result, hydroxyapatite cannot be used for load-bearing implants in clinical applications. Thus, it can only be applied for unload-bearing or low-loaded implants, such as powders, coating and porous implants[1]. To combat this problem, various hydroxyapatite/polymer composites were widely constructed with hydroxyapatite and some polymers, such as polyethylene[2], polylactic acid[3], polymethylmethacrylate[4], chitosan[5], *etc*. These hydroxyapatite/polymer composites successfully remedied the issues associated with hydroxyapatite's mechanical properties. They are still highly biocompatible but are now much less brittle. Therefore, they can be used for bone replacement and bone tissue engineering[6,7]. Nevertheless, the interfacial bonding is very weak between the hydroxyapatite and the polymer matrix because of their significantly difference hydrophilicities. Because of this, their interface can be easily destroyed from stress or a physiological fluid when it is implanted into the body[8]. Therefore, if the hydroxyapatite/polymer compounds fail, then implant failure would subsequently take place.

Usually, the interfacial compatibility of hydroxyapatite with a polymer matrix can be improved through hydroxyapatite surface modification. The hydroxyapatite/polymer composites' mechanical properties can by significantly enhanced through hydroxyapatite surface modification, such as compressive modulus[9,10], bending strength[11,12] and tensile strength[3].

Thus far, various surface modifiers have been used to modify the hydroxyapatite, such as silane coupling agent[10,11], dodecyl alcohol[13], L-lactic acid oligomer[12], polymethyl methacrylate[14] poly (L-phenylalanine)[15] *etc*. However, each of these surface modifiers has their own associated disadvantages. For example, the silane molecules adversely affect cell morphology after hydrolyzation and can even lead to cell damage at a high concentration[16]. In addition, the processes of most modifications are complicated and the related parameters are hard to control. Therefore, their practical applications are limited.

However, stearic acid (SA) offers a practical solution for hydroxyapatite modification. Stearic acid is a typical surfactant and is widely used in industry. It is harmless after decomposition because it is elementally composed of only C, H and O. Furthermore, it can become metabolism end products. For this reason, stearic acid is the ideal surfactant to use for hydroxyapatite modification.

Although, surface modification of hydroxyapatite with stearic acid has been previously reported[17], the molecular mechanism behind surface modification is still unclear. In this

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paper, a convenient and efficient method has been developed to modify hydroxyapatite with stearic acid. Furthermore, the mechanism behind the surface modification was also systematically investigated. This paper is focused on analyzing why the hydroxyapatite surface changes from hydrophilic to hydrophobic after it has been molecularly modified.

EXPERIMENTAL

Hydroxyapatite with 97.5 % purity was purchased from Nanjing Emperor Nano Material Co., Ltd. (Jiangsu, China). AR grade stearic acid (SA) was purchased from Xilong Chemical Co., Ltd. (Guangdong, China). hydroxyapatite and stearic acid were dried before they were used. AR grade absolute ethanol was purchased from Bejing Chemical Works (Beijing, China).

Stearic acid (0.5, 1.0, 1.5, 2.0, 2.5 and 3.5 g) was added into 500 mL of absolute ethanol and then stirred continuously until a clear stearic acid solution was formed. Then, a given mass of hydroxyapatite powders (relative to the amount of hydroxyapatite powders, content of stearic acid was 1, 2, 3, 4, 5 and 7 wt %, respectively) was added to the solution, shaken and ultrasonicated for 20 min in order to form a homogeneous, mixed solution. The solutions were heated in a constant temperature water bath at 70 ºC, with stirring. The powders were dried after the solvent evaporation. Finally, the powders were ground to form modified hydroxyapatite powders.

Fourier transform infrared (FTIR) spectra were recorded on a Shimadzu 8400s spectrometer as KBr pellets in the 4000-400 cm^{-1} region. X-Ray photoelectron spectroscopy (XPS) analyses were carried out using a Thermo Escalab-250 equipment using an AlKα radiation source ($h\nu = 1486.6$ eV) operated at 200 W in vacuum $< 10^{-7}$ Pa. 5 g of hydroxyapatite powder were dispersed into 100 mL of distilled water and then stirred for 3 min, ultrasonicated for 10 min and left to stand for 2 h. After placement in order to get clear delamination, the powder that was floating on the upper layer was cleared, and the sinking powder, located at the bottom, was filtered, dried and weighted.

RESULTS AND DISCUSSION

FTIR analysis: Fig. 1 shows the FTIR spectra of the hydroxyapatite powders before and after surface modification. In the spectrum of the hydroxyapatite (Fig. 1a), the peaks located at 472, 565, 603, 963, 1035 and 1094 cm^{-1} can be attributed to the P-O stretching bands of the PO_4^{3-} ions and the peak at 3570 cm^{-1} was due to the stretching vibration of the OH group. The peak at 1636 cm^{-1} and the wide peak at about 3447 cm^{-1} came from the absorption of H_2O[18]. The peaks at 1417 and 1456 cm^{-1} were the characteristic vibration bands of CO_3^{2-}[19].

After modification, the peak located at 878 cm^{-1} is a result of the reaction of PO_4^{3-} with the H^+ that came from the COOH group. The peaks at 2850 and 2920 cm^{-1} due to the stretching vibration of the C-H that was located in CH_2 and the peak at 2957 cm^{-1} can be attributed to the stretching vibration of the C-H that was in the CH_3 group. It showed that the hydroxyapatite particles' surfaces were covered by carbon chains of stearic acid molecules. The new peak at 1558 cm^{-1} (Fig. 1b) was attributed to the stretching vibration of COO. It confirmed that the ionic bonds were formed by the reactions between the COOH and Ca^{2+} on the hydroxyapatite surface and thus, stearic acid molecules were grafted onto the surfaces of the hydroxyapatite particles. The peaks at 2341 and 2358 cm^{-1} corresponded to the stretching vibration of the P-OH in the HPO_4^{2-} group. There was a distinct increase in its intensity in comparison to pre-modification. The two weak peaks at 3674 and 3734 cm^{-1} (Fig. 1c) were attributed to the stretching of the OH in P-OH[19,20].

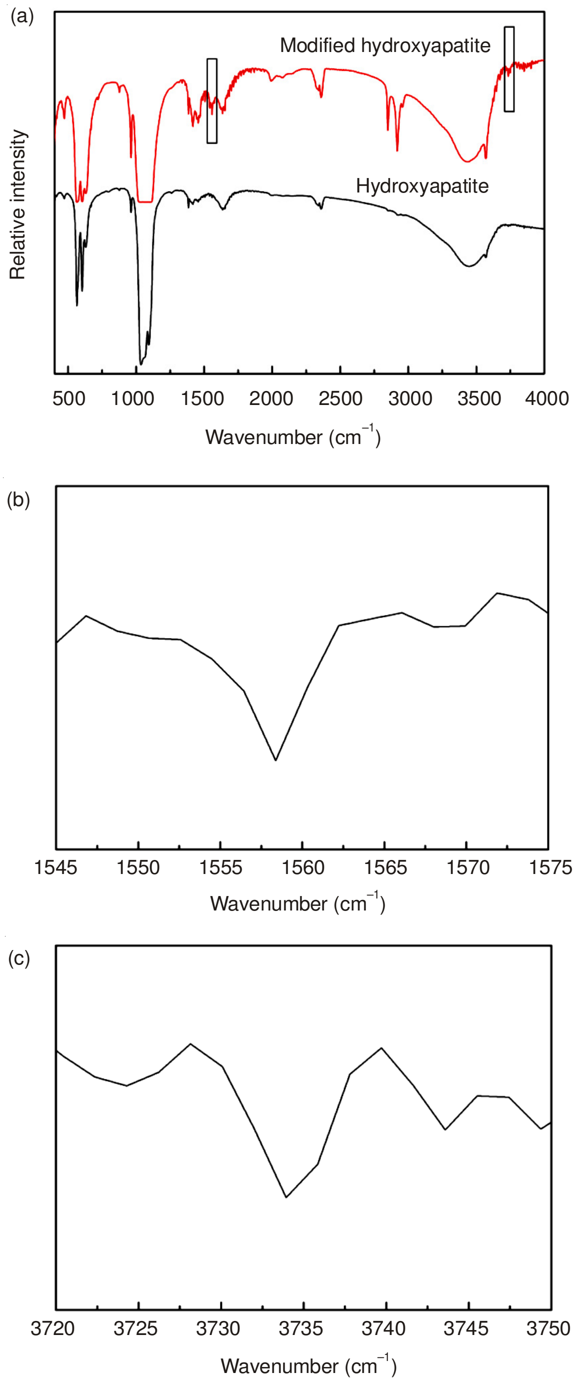

Fig. 1. FTIR spectra of hydroxyapatite powders: (a) FTIR spectra of hydroxyapatite powders; (b) and (c) enlarged views of rectangle areas in (a)

XPS analysis: After surface modification, the chemical environment for the atoms located on the hydroxyapatite surface should be different and, therefore, lead to a binding energy change. XPS was used to monitor the binding energy change. Fig. 2 shows the Ca2p XPS spectra. Before modification, the Ca2p XPS spectra were located at 350.55 and 347.00 eV. After modification, however, the Ca2p XPS spectra moved to a higher binding energy and was then located at 351.25 and 347.80 eV. This confirms that the chemical environment for the Ca atom was changed by the modification.

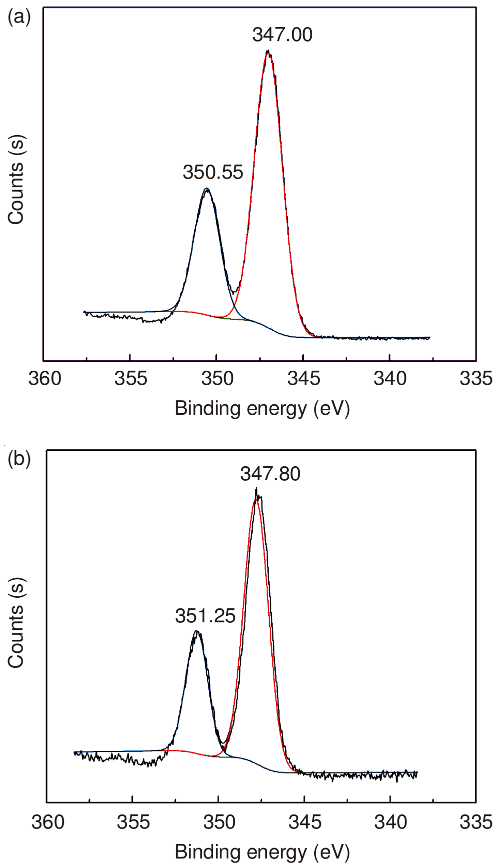

Fig. 2. XPS spectra of Ca2p before (Fig. a) and after (Fig. b) surface modification

An ionic bond was formed by the combination of the Ca^{2+} on the hydroxyapatite surface and the COO^- in $CH_3(CH_2)_{16}COO^-$ and, therefore, the Ca was then located in a new environment. Because an O atom (3.5) has a greater electronegativity than a Ca atom (1.0), the electrons were more attracted to the O atom. As a result, the electron density of calcium was reduced, following the binding energy increase.

There are two different adsorption sites on the hydroxyapatite surface that are capable of adsorbing some ions or groups[21]. The first adsorption sites are generated instantaneously through the loss of Ca_I ions on the hydroxyapatite surface. When Ca_I ions are located on surface c of the hydroxyapatite crystal, a part of the Ca_I ions are connected to three negatively charged O atoms. In the solution, a weaker adsorption site can be generated by losing a Ca_I ion from the site. Other Ca_I ions are connected to six negatively charged O atoms and a stronger adsorption site, named the P site, can be generated. This site can adsorb some cations, such as K^+, Sr^+, *etc*.

The second adsorption sites are instantaneously generated by the loss of OH^- ions from the hydroxyapatite surface. The OH^- ions are connected to two positively charged Ca_{II} ions when the OH^- ions are located on surface a (or b) of the hydroxyapatite crystal. An adsorption site, named the C site, is formed instantaneously by losing OH^- in the solution. It can adsorb the carboxyl and PO_4^{3-} group of the macromolecule.

The P2p XPS spectra are shown in Fig. 3. As illustrated, the peak located at 133.10 eV is from before modification. The peak of the P2p XPS spectrum after modification could be divided to two spectra by fitting, which are located at 134.00 and 133.34 eV. The peak located at 134.00 eV of the spectrum has been assigned to the P2p that was from PO_4^{3-} and the peak located at 133.34 eV has been assigned to the P2p that was from HPO_4^{2-}. A stearic acid molecule can be divided into $CH_3(CH_2)_{16}COO^-$ and H^+ through the modification process. An ionic bond was formed by the combination of the $CH_3(CH_2)_{16}COO^-$ with Ca^{2+} and the P-OH bond was formed by the combination of the H^+ with PO_4^{3-}. The binding energy of the P2p increased after the modification due to an electron density decrease around the P from the combination of H^+ with PO_4^{3-}.

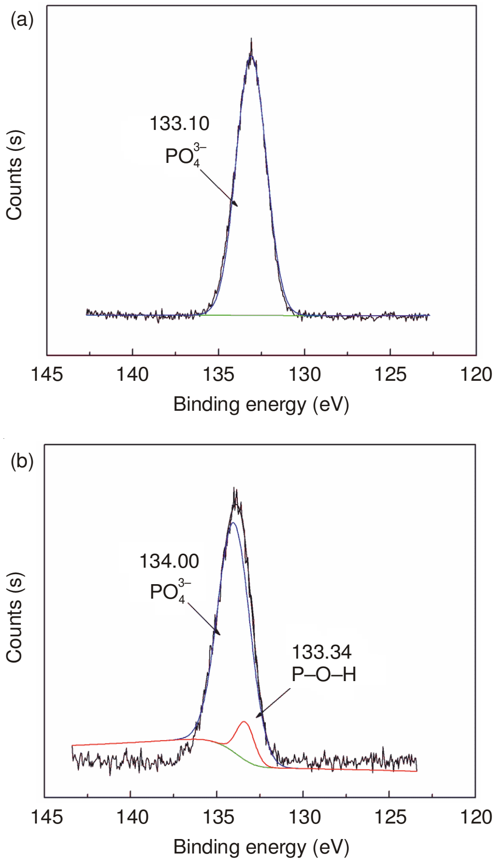

Fig. 3. XPS spectra of P2p before (Fig. a) and after (Fig. b) surface modification

Because of the loss of Ca^{2+} from the hydroxyapatite surface from a change in the chemical environment, the protonation of the surface PO_4^{3-} occurred in order to maintain the surface charge balance of the hydroxyapatite particle. As far as the protonation process was concerned, a HPO_4^{2-} with one OH^- could have been formed by a PO_4^{3-} combining with one H^+. However, the possibility that a $H_2PO_4^-$ with two OH^- groups could have been formed by a PO_4^{3-} combining with two H^+ should not be excluded[22]. This can be used to interpret the increase in the OH adsorption peak intensity of the FTIR spectrum of the modified hydroxyapatite compared with the less intense peak of the unmodified hydroxyapatite.

In the modification process, a strong ionic bond was formed by the combination of the $CH_3(CH_2)_{16}COO^-$ in stearic acid with a Ca ion on the hydroxyapatite surface. At the same time, protonation was also generated between the H^+ and PO_4^{3-}.

Fig. 4 shows the structural change from hydrophilic to hydrophobic on the hydroxyapatite surface[20,22]. The left figure shows the hydrophilic state of the unmodified hydroxyapatite. The calcium ions combined with water molecules by chemical bonds and the P-OHs on the hydroxyapatite surface combined with water molecules by hydrogen bonds. Therefore, the hydroxyapatite was hydrophilic. After modification, however, strong ionic bonds could be formed by the combination of the calcium ions on the hydroxyapatite surface and the $CH_3(CH_2)_{16}COO^-$. Additionally, P-OHs combined with stearic acid molecules by hydrogen bonds. In this way, all adsorption sites for water molecules on the hydroxyapatite surfaces were currently occupied. Consequently, hydroxyapatite then changed from a hydrophilic state to a hydrophobic state.

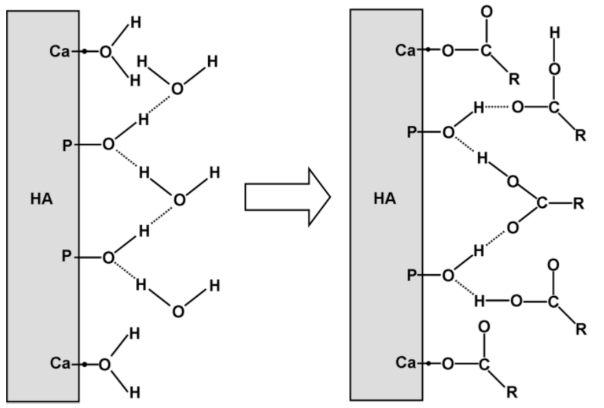

Fig. 4. Model of the structures of hydroxyapatite (HA) surface change from hydrophilic state (left) to hydrophobic state (right), R is $CH_3(CH_2)_{16}$ of stearic acid

Activation ratio of hydroxyapatite: In order to estimate the effect of modifying the surface of hydroxyapatite with stearic acid composed of different compositions, an activation ratio of modified hydroxyapatite has been tested. hydroxyapatite's hydrophilicity change could be directly reflected. Table-1 presents the activation ratios of modified hydroxyapatite powders. The activation ratio was very low when the content of stearic acid was 1 wt %. Then, it rose rapidly when the content of stearic acid was greater than 2 wt %. The activation ratio exceeded 99 % when the content of stearic acid was greater than 3 wt % and then remained a relatively steady value.

TABLE-1
ACTIVATION RATIOS OF MODIFIED HYDROXYAPATITE POWDERS

Stearic acid content (wt %)	1	2	3	4	5	7
Activation ratio (%)	2.5	7.9	99.3	99. 8	99.7	99.7

Photographs of modified hydroxyapatite powders which had been dispersed into distilled water are shown in Fig. 5. As shown in Fig. 5, the activation ratios were very low when the content of stearic acid was 1 and 2 wt %, so as a result, most of the hydroxyapatite powders settled on the bottom [as show in ellipse below (a) and (b)]. In contrast, when the content of stearic acid was 3-7 wt %, the activation ratios was very high. As a result, most of the hydroxyapatite powders floated on the upper layer [as show in ellipse above (c) to (f)] and the water was clean except that (c) was slightly turbid.

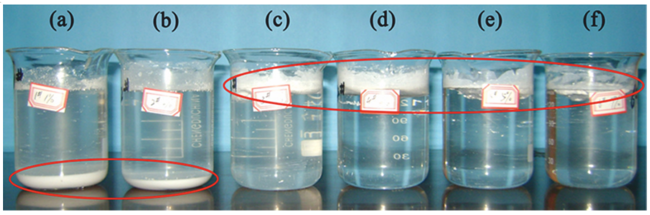

Fig. 5. Photographs of modified hydroxyapatite powders with stearic acid of different contents were dispersed in the distilled water: (a) 1 wt %, (b) 2 wt %, (c) 3 wt %, (d) 4 wt %, (e) 5 wt %, (f) 7 wt %

When the stearic acid content was 1 wt %, there were not enough stearic acid molecules to provide one stearic acid molecule for one hydroxyapatite particle on average, so a lot of the hydroxyapatite particles were in the "naked state". The stearic acid molecules that had grafted onto the hydroxyapatite particles surface were not enough to change the hydroxyapatite particles into a hydrophobic state. As a result, the activation ratio was very low. Afterwards, the numbers of stearic acid molecules increased exponentially with an increasing content of stearic acid. Therefore, more stearic acid molecules began to graft onto the hydroxyapatite particles that were in the "naked state". Moreover, the number of stearic acid molecules that had been grafted on to the hydroxyapatite particles surface further increased. At that time, the hydrophilic state of the hydroxyapatite particles started to obviously change to the hydrophobic state. The number of stearic acid molecules reached a saturation value of hydrophobicity when the concentration of stearic acid was 3 wt %, which then determined as the "critical value". Perhaps, the "critical value" was not an accurate value. Hydroxyapatite particles had completely changed from the hydrophilic state to the hydrophobic state once the "critical value" had been reached. After that point, the activation ratio remained stable and only the cover area of the stearic acid molecules on the hydroxyapatite surface increased with an increasing stearic acid content.

Conclusion

The modification reaction is only a surface reaction in which stearic acid molecules are grafted onto the hydroxyapatite surface by both ionic and hydrogen bonds, but it was the ionic bonds that played the critical role in the modification. After modification, the adsorption sites on the hydroxyapatite surface for water molecules were occupied by stearic acid molecules. There is a determined critical value that represents the required amount of stearic acid molecules necessary to change the hydroxyapatite particles from hydrophilic to hydrophobic. The hydroxyapatite is almost entirely in the hydrophobic state when the numbers of stearic acid molecules is greater than the critical value. Further research will be carried out on an investigation of the accurate point of the "critical value" and the relation between the hydrophobicity and the numbers of stearic acid molecules present on the hydroxyapatite particle surface.

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