

# Surface modification of nano-apatite by grafting organic polymer

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## Abstract

Since surface properties of hydroxyapatite (HA) play an important role in its performance, surface modification of HA has gained much attention from researchers. Silane coupling agents have been the focus of the research. In this study, an effective surface modification method was developed using hexamethylene diisocyanate as a coupling agent. Polyethylene glycol ( $M_w = 1500$ ) was successfully coupled to the surface of nano-size apatite particles (nano-apatite). Various methods were used to characterize the surface-modified nano-apatite. Infra-red spectra confirmed the existence of a layer of polymer with both urethane and ether linkage on the surface of nano-apatite. The amount of grafted polymer as determined by total organic carbon analysis (TOC) and thermal gravimetric analysis (TGA) was about 20% in weight. Solid  $^1\text{H}$  MAS NMR spectra indicated that the amount of hydroxyl groups of nano-apatite was decreased by 7.7% after surface grafting reaction. It is concluded that the surface hydroxyl groups of nano-apatite have the reactivity towards isocyanate groups. © 1998 Published by Elsevier Science Ltd. All rights reserved

**Keywords:** Hydroxyapatite; Surface modification; Hexamethylene diisocyanate; Polyethylene glycol; Nano-apatite;  $^1\text{H}$  MAS NMR

## 1. Introduction

Hydroxyapatite (HA), due to its structural similarity to bone mineral, enamel and dentin, has gained much attention from biologists and biomaterial scientists [1–3]. Numerous investigations have dealt with the structure–properties relationship and the application of hydroxyapatite as biomaterial [1–11]. Nowadays, HA has been successfully used as: bone filler [8, 9], coating of orthopaedic implants [7, 10, 11], filler of inorganic/polymer composites [12–15], substrate for the column chromatography of protein [16] and cell culture carrier [17]. In most of the cases, the surface properties of the HA play an important role in its application, as its surface is in direct contact with the environment (body fluids, etc.) when in use. Therefore, to control or to manipulate the surface properties of HA is of great importance [15, 18–25].

Surface modification of HA by organic molecules or polymers will provide an effective means to manipulate

the surface properties of HA. In our point of view, there are two ways to modify the surface of HA by organic molecules. The first method is through surface adsorption. It is known that many polymers and proteins can be firmly adsorbed onto the surface of HA [26–29]. The second approach is to graft organic molecules through covalent bonding to the hydroxyl groups which are available on the crystal surface of HA. The direct application of these surface modification methods will be in the field of HA/polymer composites, column chromatography of protein, cell culture carrier and carrier of catalysts in chemical engineering.

The hydroxyl group present on the surface of HA seems to be a reactive group of which use can be made to graft organic molecules. Silane coupling agents have been the focus of the research. Nishizawa et al. modified the calcium phosphate ceramics by using organic silane coupling agent, and claimed that the silane coupling agent was covalently bonded to the surface of the ceramics through the reaction with surface hydroxyl groups of the ceramics [18]. Labella et al. also speculated that silane could be coupled to the surface of HA through the reaction with the surface hydroxyl group of HA [15]. However, there had been no direct evidence that the surface hydroxyl group was involved in these reactions.

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Since HA has the ability to absorb certain organic molecules through hydrogen bonding, an important issue of our study is to find whether the hydroxyl groups indeed have the ability to react with organic functional groups.

The nature of NMR renders it an effective technique for the structural investigation of calcium phosphate. Previous studies have utilized the favourable NMR properties of  $^{31}\text{P}$  and  $^{19}\text{F}$  nuclei to obtain quantitative structural information from high-resolution solid state NMR spectra of calcium phosphates, including mineralized tissue [30–33]. High-resolution  $^1\text{H}$  NMR techniques have also been used to study the hydroxyl group, structural water, surface adsorbed water, and surface adsorbed proteins in synthetic hydroxyapatite [34–37].

The larger range of proton NMR chemical shifts (ca. 20 ppm) observed for oxygen-bound hydrogen in solid and the sensitivity to hydrogen-bonding effects makes it a very useful technique for studying biologically relevant calcium phosphates. The orientation at the magic angle of  $54.7^\circ$  with respect to the magnetic field (MAS NMR) and high sample spinning speed (can be as high as 8 kHz) can result in highly resolved  $^1\text{H}$  MAS NMR spectra [34]. All those advantages of the  $^1\text{H}$  MAS NMR make it an ideal technique to study the reactivity of hydroxyl groups of HA towards organic functional groups.

In our previous studies, we have shown the necessity of surface modification of HA particles in making HA/Polymer composites [24]. In an attempt to develop an effective and versatile method to modify the surface of HA, we tried to use bifunctional-group organic chemicals as coupling agents to introduce polymers onto the surface of HA. In this study, we looked at the feasibility of using diisocyanate as coupling agent to introduce polyethylene glycol (PEG) to the surface of HA particles. We aimed at improving the interface of HA with a PEG/PBT block copolymer, which has bone-bonding property and can be used as a bone substitute material [38, 39].  $^1\text{H}$  MAS NMR was specially used to study whether the hydroxyl groups of HA can react with isocyanate functional group.

## 2. Materials and methods

Nano-apatite was hydrothermally synthesized as described elsewhere [40] and was freeze-dried. The obtained powder consists of whisker-shaped particles of 300 nm in length and 10–30 nm in width. The specific surface area is  $70\text{ m}^2/\text{g}$  ( $\text{N}_2$ , BET). Polyethylene glycol 1500 was purchased from Aldrich. Hexamethylene diisocyanate (HMDI) was also obtained from Aldrich, and it was distilled and dried before using. Isocyanatoethyl methacrylate (ICEM) was purchased from Polyscience with hydroquinone as a stabilizer, and was used without further purification. Dimethyl formamide (DMF) (Aldrich) was distilled before use.

### 2.1. IR study on the reactivity of surface hydroxyl groups of nano-apatite

To study the reactivity of surface hydroxyl groups of nano-apatite, a mono-isocyanate isocyanatoethyl methacrylate (ICEM) was used.

After thoroughly drying at  $120^\circ\text{C}$  for 48 h, 4 g of nano-apatite was transferred to a 150 ml Erlenmeyer flask together with 75 ml dry DMF, 2 ml ICEM and 0.06 ml dibutyltin dilaurate (catalyst). Hydroquinone (300 ppm) was used as an inhibitor of the polymerization. The suspension was stirred with a magnetic stirrer. Reaction temperature was kept at  $60^\circ\text{C}$  for 5 h under the protection of nitrogen. After that, the powder was separated by centrifugation, washed first with DMF 3 times and then  $\text{CHCl}_3$  2 times to remove DMF. After drying at  $60^\circ\text{C}$ , the sample was used for IR measurement using KBr tablets. The spectra were recorded from  $4000$  to  $200\text{ cm}^{-1}$  in a Perkin–Elmer 783 IR spectrophotometry.

### 2.2. Surface grafting reaction

After thoroughly drying at  $120^\circ\text{C}$  for 48 h, 4 g of nano-apatite was charged to an Erlenmeyer flask together with 75 ml dry DMF, 3 ml dry hexamethylene diisocyanate and 0.06 ml dibutyltin dilaurate. The suspension was stirred with a magnetic stirrer and bubbled with nitrogen. The temperature was increased to  $60^\circ\text{C}$  and kept for 4 h under the protection of nitrogen. Then 20 g PEG ( $M_w = 1500$ ) were added to the suspension together with 20 ml DMF and stirred overnight. The powders were separated by centrifuging and further washed by DMF and ethanol 3 times. Then the powder was precipitated in ether. After decanting the ether, the powders were first dried at room temperature and then dried at  $50^\circ\text{C}$  to fully remove ether.

### 2.3. Characterization of surface-grafted nano-apatite

Transmission electron microscopy (TEM Philips 450) was used to observe the size or shape changes of the particles before and after the nano-apatite was surface-modified by grafting of PEG.

Infrared (IR) spectrophotometry (Perkin–Elmer 783) was used to characterize the surface-grafted polymer and nano-apatite. The spectra were recorded in  $4000$ – $200\text{ cm}^{-1}$  region. The grafted nano-apatite was used directly for IR measurement in KBr tablets. In order to characterize the surface-grafted polymer, a certain amount of grafted nano-apatite was dissolved in 1 M HCl to remove the mineral. The insoluble polymer was separated by filtration and washed 3 times by distilled water. Then the polymer was dried in  $60^\circ\text{C}$  for 24 h and characterized by IR using KBr tablets.

Total organic carbon analysis (DC-190 TOC analyzer) and thermal gravimetric analysis (TGA, Dupont 910) were used to determine the amount of surface-grafted

polymer. For TOC measurement, 0.54 g surface-grafted nano-apatite was put in 100 ml HCl solution (pH = 1), 10 ml of the suspension was used for TOC measurement. The amount of polymer can be calculated from the carbon content of the grafted nano-apatite. TGA measurements were performed at 5°C/min from room temperature up to 700°C. Both grafted and non-grafted nano-apatite was used for these measurements.

Solid NMR was performed in a Bruker solid NMR equipment with magic-angle spinning techniques. Sample spinning speed was 7500 Hz. H<sub>2</sub>O was used as reference substance.

### 3. Results

#### 3.1. IR study on the reactivity of surface hydroxy groups of nano-apatite

Figure 1 gives the IR spectrum of ICEM-treated nano-apatite (a), The  $\text{-CH}_2$  stretch peak and the ester carbonyl peak can be seen at 2960 and 1740  $\text{cm}^{-1}$  respectively. Amide absorption bands appeared at 1660  $\text{cm}^{-1}$  and 1570  $\text{cm}^{-1}$ . The isocyanate peak of ICEM at 2200  $\text{cm}^{-1}$  completely disappeared after the reaction. It is also noticed that there is no noticeable change in the intensity of  $\text{HPO}_4^{2-}$  peak at 875  $\text{cm}^{-1}$ . The existence of amide bands and the disappearance of isocyanate band indicated that the surface reactive groups of nano-apatite indeed reacted with isocyanate groups and resulted in the formation of urethane linkage.

#### 3.2. TEM observation

TEM observation indicated that the size and shape of the nano-apatite remained unchanged after the surface grafting with PEG but the surface grafted nano-apatite had more tendency to aggregate (Fig. 2).

#### 3.3. IR spectra study of PEG grafting

Figure 4 gives the IR spectra of nano-apatite, surface-grafted nano-apatite and the grafted polymer. The polymer on the surface of nano-apatite was identified by its urethane linkage,  $\text{CH}_2$  vibration and  $\text{C-O-C}$  vibration. Some of the main absorption peaks were identified as follows: the peak at 3335  $\text{cm}^{-1}$  belongs to the N-H hydrogen bond. The twin peaks at 2935 and 2861  $\text{cm}^{-1}$  belong to the  $\text{CH}_2$  stretch vibration. The peak at 1734  $\text{cm}^{-1}$  can be assigned to  $\text{-C=O}$ . An amide II band (N-H and C=O stretching vibration) can be observed at 1577  $\text{cm}^{-1}$ . The shoulder at 1100  $\text{cm}^{-1}$  is the  $\text{-C-O-C}$  vibration of PEG. The IR spectra confirms that there is a layer of HMDI coupled PEG on nano-apatite.

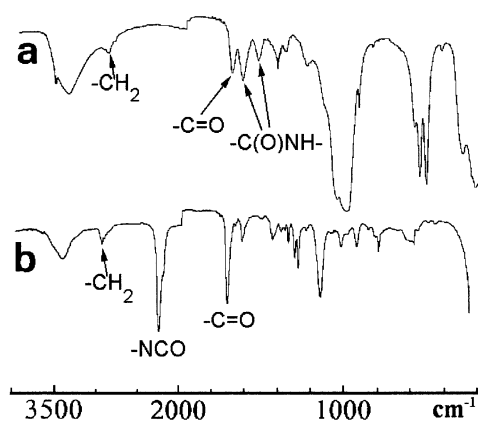


Fig. 1. IR spectra from ICEM-treated nano-apatite (a) and ICEM (b). The  $\text{-CH}_2$  band at 2960  $\text{cm}^{-1}$ , the ester band at 1740  $\text{cm}^{-1}$  and amide absorption bands at 1660 and 1570  $\text{cm}^{-1}$  are all visible. The isocyanate peak at 2200  $\text{cm}^{-1}$  completely disappeared after grafting.

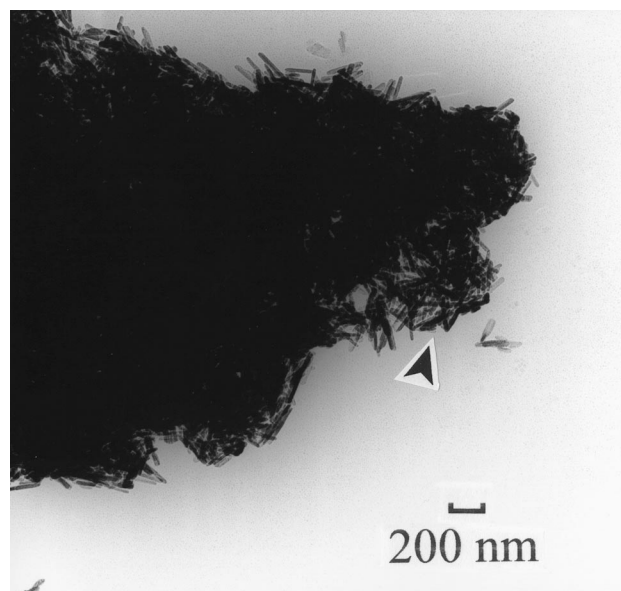


Fig. 2. TEM photograph shows that the nano-apatite still retains the original size and shape after surface modification by PEG grafting, although it has a strong tendency to aggregate due to the surface grafted polymer. Note the needles at the edge of the cluster (bar = 200 nm).

#### 3.4. The amount of grafted PEG

The amount of this layer was measured both by total organic carbon analysis and thermal gravimetric analysis. Both methods gave similar results. The TOC measurement gave 20.6 wt% polymer on grafted nano-apatite. The TGA result (Fig. 3) showed that there was 21.8 wt% organic matter on the surface of grafted nano-apatite.

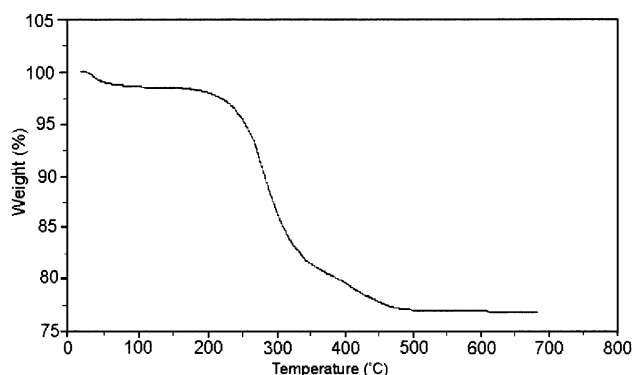


Fig. 3. The TGA graph shows that the organic polymer on the surface of nano-apatite was about 20% by weight. The temperature increase rate was  $5^{\circ}\text{C min}^{-1}$ .

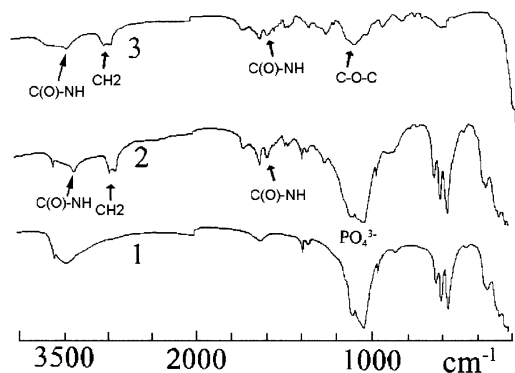


Fig. 4. IR spectra of nano-apatite before surface modification (1), after surface modification (2) and the polymer on the surface of nano-apatite (3). Note the appearance of  $-\text{CH}_2-$  vibration at  $2835$  and  $2861\text{ cm}^{-1}$ , urethane linkage at  $1577\text{ cm}^{-1}$ , and  $-\text{C}-\text{O}-\text{C}-$  vibration at  $1100\text{ cm}^{-1}$  in spectrum 3. The huge  $\text{PO}_4^{3-}$  peak can be seen at  $1040\text{ cm}^{-1}$  from spectrum 1 and 2.

### 3.5. Solid $^1\text{H-NMR MAS}$ study

Fig. 5 gives the  $^1\text{H}$  NMR MAS spectra of PEG surface-grafted nano-apatite and control nano-apatite. Nano-apatite shows several peaks in the spectrum. According to the literature [34, 35], the highest upfield peak usually belongs to the structural hydroxyl group. Therefore, we assigned the peak at  $-0.059\text{ ppm}$  to the structural hydroxyl group of apatite. The downfield chemical shifts at  $6.77$  and  $5.5$  (shoulder) are probably from the two types of surface absorbed water, i.e. loosely bound and firmly bound water. The down field peak at  $13\text{ ppm}$  is from the hydrogen bonding protons in the structure of nano-apatite. It also has to be mentioned that no surface hydroxyl peak is observed from the spectra. Two reasons may be responsible for that [34]: (1) surface hydroxyl resonance may not be resolved from that of bulk hydroxyls; and (2) the surface hydroxyl groups may be in a rapid chemical exchange with the surface-absorbed water.

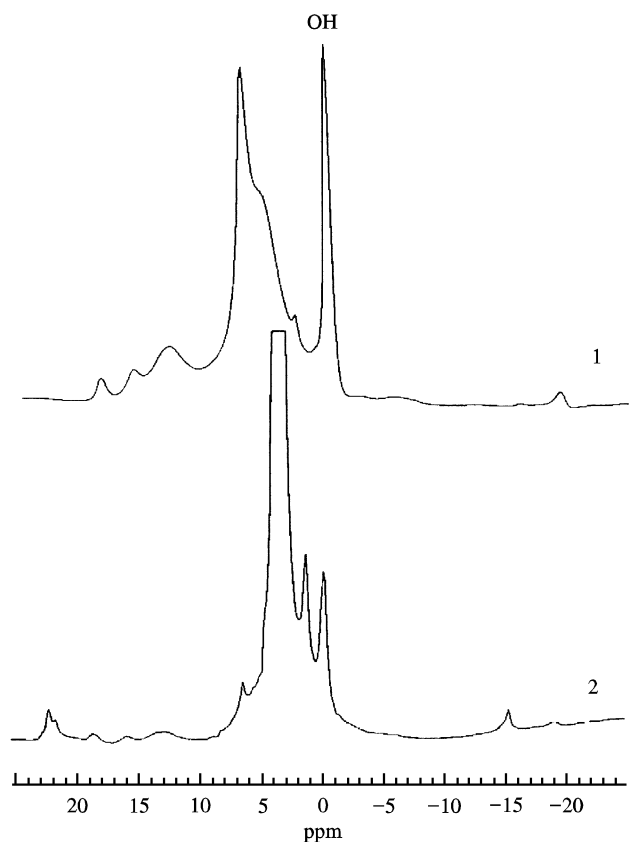


Fig. 5.  $^1\text{H}$  MAS NMR spectra of nano-apatite before (1) and after (2) surface modification. A huge OH peak can be seen at  $-0.059\text{ ppm}$  on nano-apatite. This peak was decreased after the surface modification.

After surface grafting, it can be observed that the relative line height of structural OH decreased drastically due to a huge peak emerged at  $3.58\text{ ppm}$  which was believed coming from the protons of surface-grafted polymer. When carefully calculating the peak height at  $-0.059\text{ ppm}$  using the peaks at  $13$  and  $15.95\text{ ppm}$  as reference, we found that the peak height at  $-0.059$  was decreased by  $7.7\%$  after surface grafting. The decrease in the peak height at  $-0.059$  strongly suggests that the amount of the  $-\text{OH}$  groups was decreased by the reaction of OH with isocyanate group. It is also noted that large portion of the surface absorbed water on nano-apatite was removed by the surface grafting reaction. The assignment of new peak at  $1.39\text{ ppm}$  is still unclear, they are most probably from the surface absorbed polymers through hydrogen bonding.

## 4. Discussion

The nano-apatite used in our study was a nonstoichiometric apatite with a general formula  $\text{Ca}_{10-x}(\text{HPO}_4)_x(\text{PO}_4)_{6-x}(\text{OH})_{2-x}$  ( $0 < x < 2$ ) [41]. Although it has less hydroxyl groups than that of stoichiometric

hydroxyapatite, due to the nano size of the apatite and the large specific surface area ( $70 \text{ m}^2 \text{ g}^{-1}$ ), there are relatively large amounts of atoms on the surface and hence a large fraction of surface hydroxyl groups can be expected. Also Yesinowski and Eckert [34] have suggested in their study that there existed a significant fraction of hydroxyl groups on the surface of hydroxyapatite particles which had a specific surface area of  $37 \text{ m}^2 \text{ g}^{-1}$ . The large specific surface area of nano-apatite makes it possible to study the reactivity of the surface functional groups.

IR spectrum (Fig. 1) indicated that certain functional groups on the surface of nano-apatite have the reactivity towards isocyanate groups. IR spectra (Fig. 4) also confirmed that the polymer on the surface of nano-apatite had the urethane linkage and polyether structure. TGA and TOC results showed the relatively large amount of polymer present on the surface of nano-apatite. The existence of such a large amount of polymer cannot be simply explained by adsorption, since the samples were extensively washed by DMF.

Considering the reactive groups on the surface of nano-apatite, there are possibly three types of reactive species which can react with isocyanate group. They are surface absorbed water,  $\text{HPO}_4^{2-}$ , and  $\text{OH}^-$ . In our preliminary experiment, we have found that when the nano-apatite was not dried enough, it was hard to find any evidence of grafting using IR and TGA. The nano-apatite used for grafting must be dried at  $120^\circ\text{C}$  for 48 h to remove surface-absorbed water molecules. Therefore, we believe that no surface-absorbed water was involved in the grafting reaction when we use dried nano-apatite.  $\text{HPO}_4^{2-}$  is present on the surface of nano-apatite due to its nonstoichiometric structure [40, 41] or the hydrolysis of  $\text{PO}_4^{3-}$  ions on the surface [42]. Arends et al. [35] suggested that a surface-absorbed  $\text{HPO}_4^{2-}$  peak could be at  $875 \text{ cm}^{-1}$  in the IR spectra of hydroxyapatite. When we compared the IR spectra of nano-apatite before and after grafting reaction, we could neither find the change of peak intensity at  $875 \text{ cm}^{-1}$ , nor an emerging of a new peak from P–O–C. Therefore, our data do not support the reaction mechanism between  $\text{HPO}_4^{2-}$  and isocyanate. We thus believe that it is the surface hydroxyl groups of nano-apatite which have the reactivity towards isocyanate groups.

Solid proton NMR MAS spectra of nano-apatite (Fig. 5) showed the peak of structural hydroxyl group and the surface-adsorbed water with positions that are very well in agreement with the literature [34, 35]. After the reaction the peak height of the structural hydroxyl group at  $-0.059 \text{ ppm}$  was decreased by 7.7%. The decrease in peak height at  $-0.059 \text{ ppm}$  clearly indicated that the hydroxyl group of nano-apatite was involved in the reaction. It also confirmed the speculation made by Yesinowski et al. [34] that the surface hydroxyl resonance could not be resolved from that of bulk hydroxyls.

The decrease in the peak height of surface-absorbed water suggests that significant amount of water binding sites were blocked by the surface-grafted polymers. It can be explained by the fact that surface OH groups of nano-apatite may act as water binding sites. The successful bonding of polymer to nano-apatite will provide the possibility to make HA-polymer composites in which HA particles are chemically bound to a polymer matrix, which in turn will result in an improvement of mechanical properties of composites.

Grafting of PEG to nano-apatite did not alter the size and shape of nano-apatite as indicated by TEM. Therefore the grafting reaction was purely a surface reaction.

Grafting organic substances or polymers to the surface of nano-apatite will offer an effective way to modify the surface properties of apatite-like materials which we believe will be very useful in expanding the field of application of these compounds.

## 5. Conclusion

By using hexamethylene diisocyanate, we successfully grafted PEG 1500 molecules to the surface of nano-apatite. We proved that  $-\text{OH}$  groups at the surface of nano-apatite have reactivity towards organic functional groups. Our results also indicate it is possible by surface grafting to modify the surface properties of nano-apatite and hence to manipulate the surface properties of apatite.

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