In Situ Crystallization of Hydroxyapatite on Carboxymethyl Cellulose as a Biomimetic Approach to Biomass-Derived Composite Materials

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ABSTRACT: Nanohydroxyapatite (HAP) was crystallized in an aqueous solution of carboxymethyl cellulose (CMC) to prepare the composites of CMC and HAP with a stable interface between them with the aim of developing a sustainable tough biomass composite material inspired by bone. The temperature (room temperature to 90 °C) and the concentration of CMC (0.83–13.2 g/L) were optimized for the mechanical properties of the composites. The composite containing 67 wt % HAP prepared at 50 °C in the presence of 9.9 g/L CMC exhibited the largest flexural strength of 113 ± 2 MPa and the elastic modulus of 7.7 ± 0.3 GPa. X-ray diffraction showed that nanometer-sized HAP crystals were formed with a large aspect ratio, and energy-dispersive X-ray spectroscopy and infrared spectroscopy revealed that CMC was bound to the surface of HAP through an ionic interaction between Ca²⁺ and COO⁻. Since the composite has a higher flexural strength than polyamide 6 (92 MPa) and a higher elastic modulus than polyamide 6 with 40 wt % glass fiber (5.5 GPa), it can be used as new tough biomass composite material to replace petroleum-derived engineering plastics.

INTRODUCTION

In recent years, the problems of global warming, oil depletion, and marine plastic waste have become more serious, and materials that are environmentally friendly and have excellent mechanical properties are emphasized. Models for such materials include bioceramics such as bones, teeth, and seashells. These are formed under mild conditions and are lightweight, rigid, and tough, making them environmentally friendly materials.1,2 The primary components of the inorganic phases of these bioceramics are carbonated apatite or calcium carbonate such as calcite and aragonite, and those of the organic phases are collagen or chitin.3 Bones contain ca. 65 wt % of a mineral phase, 25 wt % of an organic phase, and 10 wt % of water.4 The mineral phase of bone consists of platelet nanocrystals of carbonated apatite that are 20–50 nm wide.4 Carbonated apatite crystals are formed in the gaps of collagen fibrils with the c-axis of apatite parallel to the collagen fibrils to realize the aligned organic–inorganic composites. Schmidt-Rohr et al. reported that citrate is adsorbed on the {100} surface of hydroxyapatite through carboxylate–Ca²⁺ interactions.5 Reid and co-workers reported that polysaccharides exist at the interface between the organic and mineral components in bone.5 The “brick-and-mortar” structure of bone contributed to the superior mechanical properties.7,8 Currey reported that the bending strengths of bones ranged from 27 to 306 MPa and the elastic moduli from 2 to 33 GPa.9 Understanding of the mechanism of biomineral formation10,11 has prompted us to mimic the mineralization process to develop organic–inorganic composites with similar mechanical performances to bones and teeth.5,12–16

There are several relevant features of bioceramics to mimic for the preparation of bioinspired composite materials.1 First, carbonated apatite crystals hybridized with a polymer are nanometer-sized because (1) fracture is insensitive to crystal defects for nanometer-sized crystals7 and (2) large interface area between the organic phase and the inorganic phase would realize an efficient deformation mechanism when stress is applied to the interface. The polymers should have appropriate functional groups to stabilize the interface between the mineral phase and the organic phase. Phosphorylated proteins have been reported to control HAP crystallization in vivo.11 Therefore, the anionic functional groups in the polymer would assist the nucleation and regulate the crystal growth of the mineral phase.17–20 Second, one of the major functions of these hard tissues is the resistance to external forces, i.e., a high elastic modulus, which cannot be attained by simple organic polymers. The rule of mixtures21 indicates that the elastic modulus of the composite is linearly proportional to the volume fraction of the filler. Bones contain ca. 65–70% of

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carbonated apatite, and the enamel of teeth contains ca. 95–97% of carbonated apatite. Depending on the organic/inorganic ratios, the optimum density of the anionic groups in polymers may vary. In this work, we aimed to prepare composites containing 65–70 wt % of hydroxyapatite, with a composition similar to that of bone. There have been three protocols to hybridize hydroxyapatite and a polymer: (1) mixing of preformed HAP crystals with a polymer,\(^2\) (2) polymerization of a monomer in the presence of a polymer: (1) mixing of preformed HAP crystals with a polymer, and (3) crystallization of HAP in the presence of a polymer. The last protocol is similar to the bone formation process and is a biomimetic approach. Because polar functional groups of organic molecules such as amino acids\(^2\) and carbohydrates\(^3\) can control morphology and the crystal size of HAP, crystallization of HAP in the presence of a polymer with polar functional groups is expected to lead to a structure similar to bone. A facile route to the composite of nanocrystals of apatite and a polymer would be coprecipitation\(^4\) of a polymer solution and a solution containing the inorganic source of the mineral. Coprecipitation can be accomplished in one pot and in a large scale. Both watersoluble polymers\(^5,6\) and amphiphilic polymers\(^7\) can be used for hybridization with coprecipitation. For instance, collagen,\(^7,22\) chitosan,\(^2,32\) starch,\(^2,33–37\) cellulose derivatives,\(^32,40,41\) poly(vinyl alcohol),\(^32,43\) and polyacrylic acid\(^44,45\) have been employed to hybridize with HAP. Several parameters influence the hybridization process such as temperature, the concentrations of polymer and inorganic ions, and the rate of diffusion of the ions. Fang and co-workers\(^34\) reported that chitosan–HAP composites prepared by diffusion through a semi-permeable membrane exhibited a better compressive strength than those prepared by coprecipitation. Kikuchi et al.\(^32\) reported that the coprecipitation temperature affected the mechanical properties of collagen–hydroxyapatite composites.

In the previous papers, we reported that phosphorylated starch\(^46\) and cellulose nanofibers\(^46\) were hybridized with hydroxyapatite by coprecipitation in water. According to these studies, anionic functional groups such as carboxylate or phosphate groups assisted hybridization\(^20,47\) and contributed to better mechanical properties. We employed carboxymethyl cellulose (CMC),\(^48–51\) widely used in medical and industrial applications due to its low toxicity and biocompatibility, as an anionic polymer in this work. CMC is also an ideal biomass material because it can be obtained in large quantities from the abundant forest resources that exist in the earth. Salama reported the review of cellulose/calcium phosphate hybrids.\(^52\) In the review, the hybrids of CMC and HAP have been studied for applications such as bone tissue engineering, drug delivery, and adsorbant for toxic materials. Relatively little is known about the mechanical properties of the CMC–HAP composites. We focused on the coprecipitation conditions such as temperature and polymer concentrations to develop novel bone-inspired biomass composites with high mechanical performances. We report here that coprecipitation at 50 °C in the presence of a high concentration of CMC gave composites whose bending strength and elastic modulus were 113 MPa and 7.7 GPa, respectively. These values were higher than those of petroleum-based engineering plastics such as polyamide 6 and glass fiber reinforced polyamide 6. The temperature and the concentration affected the crystal size of HAP and the stability of the organic–inorganic interface, and their control was important for obtaining bone-like composites. The CMC–HAP composite is a new highly tough biomass material that can contribute to the Sustainable Development Goals (SDGs) to replace petroleum-derived engineering plastics for future use.\(^53\)

## EXPERIMENTAL SECTION

### Materials

Carboxymethyl cellulose sodium salt, with the degree of polymerization of 500, the degree of carboxymethyl substitution of 0.5–0.8, and sodium content of 6.0–8.5%, was purchased from Tokyo Chemical Industry, Co. Ltd., Japan. Calcium chloride dihydrate, disodium hydrogen phosphate, sodium hydroxide, acetone, and potassium bromide were purchased from FUJIFILM Wako Pure Chemical Corporation, Japan. The water used was deionized and distilled.

**Preparation of the CMC–HAP Composite Powder (Addition of Ca\(^{2+}\) to CMC-P0\(_4^{3-}\), Method A).** CMC (1.00 g) and water 199 g were placed in a 500 mL beaker and heated at 90 °C for 30 min. After being cooled to room temperature, an aqueous solution prepared by mixing 0.2 M Na\(_2\)HPO\(_4\) (69.7 mL) and 1 M NaOH (18.6 mL) was then added, and the mixture was stirred at room temperature, 50, 70, or 90 °C for 15 min. Aqueous CaCl\(_2\) (0.2 M, 116.0 mL) was added at a rate of 0.06 mL/s. After the addition was completed, the mixture was stirred at the same temperature for 1 h. After being cooled to room temperature, excess water–acetone (1:1, v/v) was added, and the white precipitates were collected by suction filtration. The white powder was washed with acetone and dried in vacuo at 80 °C for 2 h. The yields of the composites are listed in Table 1.

### Table 1. Yields (g (%)) of the CMC–HAP Composite Powder

| coprecipitation temperature | method A | method B |
|-----------------------------|----------|----------|
| rt                          | 3.28 (98) | 3.18 (95) |
| 50 °C                        | 3.25 (98) | 3.20 (96) |
| 70 °C                        | 3.24 (97) | 3.18 (95) |
| 90 °C                        | 3.25 (98) | 3.11 (94) |

**Preparation of the CMC–HAP Composite Powder (Addition of PO\(_4^{3-}\) to CMC-Ca\(^{2+}\), Method B).** CMC (1.00 g) and water 199 g were placed in a 500 mL beaker and heated at 90 °C for 30 min. After being cooled to room temperature, 0.2 M aqueous CaCl\(_2\) 116 mL was added and the mixture was stirred at room temperature, 50, 70, or 90 °C for 15 min. An aqueous solution prepared by mixing 0.2 M Na\(_2\)HPO\(_4\) (69.7 mL) and 1 M NaOH (18.6 mL) was then added, and the mixture was stirred at the same temperature for 1 h. After being cooled to room temperature, excess water–acetone (1:1, v/v) was added, and the white precipitates were collected by suction filtration. The white powder was washed with acetone and dried in vacuo at 80 °C for 2 h. The yields of the composites are listed in Table 1.

**Preparation of the CMC–HAP Composite Powder in the Presence of Varying Concentrations of CMC.** In a 1 L beaker, 0.5 g of CMC was dissolved in water (200 mL) by heating at 90 °C for 30 min. After being cooled to room temperature, a mixture of aqueous 0.05 M Na\(_2\)HPO\(_4\) (139 mL) and aqueous 0.25 M NaOH (37 mL) was added, and the resulting solution was stirred at 50 °C for 15 min. Then, aqueous 0.05 M CaCl\(_2\) (232.0 mL) was added dropwise at a rate of 0.06 mL/s at 50 °C and aged for 1 h at 50 °C. After being cooled to room temperature, excess water–acetone (1:2, v/v)}
v/v) was added, and the white precipitates were collected by suction filtration. The white powder was washed with acetone and dried in vacuo at 80 °C for 2 h to afford 1.5 g (90%) of the composite powder.

The amounts of CMC were varied (1, 2, 4, 6, and 8 g) while the ratio of CMC to inorganic ions was fixed constant, as listed in Table 2. The volume ratios of the mixed solvent water—acetone used to precipitate the colloidal composites were 1:2 (v/v) for CMC 0.5 and 1 g and 1:1 (v/v) for CMC 2, 4, 6, and 8 g.

### Results and discussion

#### Characterization and Three-Point Bending Test

The X-ray diffraction patterns (XRD) of the composites were obtained on a Rigaku SmartLab diffractometer (Japan), employing Cu Kα radiation operated at 45 kV and 200 mA. The diffraction pattern was collected over a 2θ range of 3−60°. Crystallite size of HAP, L, was calculated from the full width of the diffraction peak at half of the maximum intensity (FWHM) of (002) and (310) peaks, using the Scherrer equation: 

\[ L = \frac{0.94 \lambda}{B \cos \theta} \]

where \( \lambda \) is the wavelength of the X-ray, B measured in radians is the full width at half-maximum of the 2θ peak, and \( \theta \) is the Bragg angle for the peak. Fourier transform infrared spectra (FT-IR) were recorded on a JASCO FT/IR-4600 spectrometer (Japan) as KBr pellets. Thermogravimetric analysis (TGA) was performed on a Shimadzu DTA-60 thermal analyzer (Japan). The hybrid powder was placed in a mold with a rectangular slot with dimensions of 4 mm × 13 mm, and it was uniaxially pressed at 120 MPa at 120 °C in 5 min to obtain a compressed block with dimensions of 4 mm × 13 mm × 1.5−1.7 mm. A three-point bending test was carried out using a bending testing machine MZ-603, Maruto Instrument Co., Ltd., Japan, with a cross-head speed of 0.5 mm/min. Details of the bending test are described in the previous paper. Scanning electron microscopy (SEM) images of the fracture surface and the composite powder were observed with a Hitachi High-Technology SU8020 field emission scanning electron microscope (Japan). The energy-dispersive X-ray spectroscopy (EDS) was performed to detect the elements in the composite samples. The calcium and phosphorus contents in the composites were analyzed with inductively coupled plasma spectroscopy (ICPS), ICPS 8100 Shimadzu (Japan).

#### Table 2. Coprecipitation Conditions and Yields of CMC−HAP Composite Powder

| CMC (g) | CMC (g/L) | Na₂HPO₄ (M) | NaOH (M) | CaCl₂ (M) | yield (%) |
|---------|-----------|-------------|----------|-----------|-----------|
| 0.5     | 0.8       | 0.05        | 0.25     | 0.05      | 1.5       | 90 |
| 1       | 1.6       | 0.1         | 0.5      | 0.1       | 3.15      | 95 |
| 2       | 3.3       | 0.2         | 1        | 0.2       | 6.4       | 97 |
| 4       | 6.6       | 0.4         | 2        | 0.4       | 12.8      | 97 |
| 6       | 9.9       | 0.6         | 3        | 0.6       | 19.2      | 96 |
| 8       | 13.2      | 0.8         | 4        | 0.8       | 26.6      | 100 |

#### Effects of Coprecipitation Temperatures on Hybridization and the Mechanical Properties of the Composites

Calcium chloride, disodium hydrogen phosphate, and sodium hydroxide in a stoichiometric ratio of Ca²⁺/PO₄³⁻/OH⁻ = 10:6:8 were reacted in water in the presence of CMC, according to eq 1.

\[
10\text{CaCl}_2 + 6\text{Na}_2\text{HPO}_4 + 8\text{NaOH} \rightarrow \text{Ca}_{10} (\text{PO}_4)_{6} (\text{OH})_2 + 20\text{NaCl} + 6\text{H}_2\text{O}
\]

The addition of calcium ions to an aqueous solution containing phosphate ions and CMC (Method A) or the addition of an alkaline solution of phosphate ions to an aqueous solution containing calcium ions and CMC (Method B) afforded the CMC−HAP composite powder in a one-pot process. The feed ratio of CMC to inorganic ions was fixed to obtain the composite with CMC:HAP (30:70, wt/wt) in this study to mimic the composition of bone, and the reaction temperatures, the concentrations of the polymer, and the order of mixing were optimized for better mechanical properties. After the addition of Ca²⁺ and PO₄³⁻, a colloidal suspension was obtained. Aqueous acetone was added to precipitate the composite powder, and the powder was collected by suction filtration. The yields of the composite were 94−98%, as listed in Table 1. Coprecipitation occurred effectively, and almost all CMC, Ca²⁺, and PO₄³⁻ were precipitated.

The XRD patterns of the composite powder coprecipitated under various conditions are shown in Figure 1. The XRD patterns indicate that hexagonal hydroxyapatite was formed except for that obtained at room temperature in Method B. As the coprecipitation temperature was higher, the diffraction peaks of (002) and (310) were the most intense, and the L values were the highest.
peaks were sharper, showing that the hydroxyapatite crystal growth was facilitated at higher temperatures. XRD peak broadening originates from many factors such as defects, isomorphous substitution, and low crystallinity in addition to the crystallite size. The crystallite size calculated with the Scherrer equation also reflects these factors, and the crystallite sizes listed in Table 3 should be considered as a measure of the crystallinity of HAP. Table 3 shows that crystallite sizes of hydroxyapatite along the c-direction prepared in Method A were larger than those prepared in Method B. In Method A, a clear solution was formed by premixing CMC and phosphate ions, and the addition of calcium ions resulted in the turbid suspension of the composites. In Method B, premixing of CMC and calcium ions resulted in the turbid suspension due to the cross-linking of CMC chains with the calcium ions. The formation of the gel would disturb the preparation of the well-crystalline hydroxyapatite, particularly at room temperature. Crystallite sizes increased as the coprecipitation temperature was higher. It is worthy to note that, in Method A, the crystallite size was large even if coprecipitation was carried out at room temperature. The average lengths and widths of the platelet crystals of HAP of bone are 50 × 25 nm² and the thickness is 2–3 nm. The larger aspect ratio of hydroxyapatite leads to a larger interface area between HAP and the organic phase and could lead to superior mechanical properties. Table 3 shows that the aspect ratio (002)/(310) was the largest for the composite prepared at 50 °C in Method A. The SEM images of the composite particles are shown in Figures S20–S22. The particles were 2–100 μm and smaller as the coprecipitation temperature was higher.

The TG thermographs of the composite powder are illustrated in Figure 2. The wt % at 950 °C was considered as the wt % of the inorganic phase, and the values are listed in Table 3. The wt % of the inorganic phase was close to the feed ratio of 70%, indicating that coprecipitation occurred quantitatively. There is a trend that the wt % of the inorganic phase increased as the coprecipitation temperature was higher. The Ca/P molar ratios in the composites determined by the ICP spectrometry are shown in Figure S17. The Ca/P ratios are 1.4–1.5, smaller than the value of 1.667 for stoichiometric hydroxyapatite, indicating that calcium-deficient hydroxyapatite.

| temperature | wt % inorg. phase | (002), c | (310), a | (002)/(310), c/a | method |
|-------------|------------------|---------|---------|------------------|--------|
| rt 65       | 23.8             | 7.9     | 3.0     | Method A         |
| 50 °C 67    | 24.0             | 6.2     | 3.9     |                   |
| 70 °C 69    | 26.1             | 7.7     | 3.4     |                   |
| 90 °C 68    | 32.3             | 9.2     | 3.5     |                   |
| rt 65       |                   |         |         | Method B         |
| 50 °C 67    | 18.6             | 5.4     | 3.4     |                   |
| 70 °C 67    | 24.1             | 6.3     | 3.8     |                   |
| 90 °C 68    | 22.3             | 6.4     | 3.5     |                   |

Figure 2. TG thermographs of the HAP–CMC composite powder. The sample weight is shown as a relative value to that at 100 °C.
tite was formed. The Ca/P ratios of the composites prepared with Method A were relatively independent of the coprecipitation temperature, while those of the composites prepared with Method B increased with increasing temperature.

The IR spectra of the composite powder are shown in Figure 3. All of the composites except for the composite prepared at 25 °C by Method B, exhibited characteristic signals of HAP: the $P=O$ stretching vibration at 1030 cm$^{-1}$ and the $O=P=O$ bending vibration at 604 and 562 cm$^{-1}$. The COO$^-$ antisymmetric stretching and symmetric stretching of CMC appeared at 1610–1370 cm$^{-1}$. Carbonated apatite also exhibits signals of carbonate ions at 1579–1414 cm$^{-1}$ in this region. To extract the IR signals of CMC, we calculated the difference spectra by subtracting the IR of HAP (Figure S18), and the spectra are shown in Figure S19. We assigned the signal at 1590 cm$^{-1}$ to the COO$^-$ antisymmetric stretching and those at 1462 and 1420 cm$^{-1}$ to the COO$^-$ symmetric stretching.

The IR spectrum of CMC sodium salt exhibited the COO$^-$ antisymmetric stretching at 1604 cm$^{-1}$ and the COO$^-$ symmetric stretching at 1419 cm$^{-1}$. The relative intensity of the COO$^-$ symmetric stretching at 1420 cm$^{-1}$ varied depending on the coprecipitation conditions. The signal at 1420 cm$^{-1}$ can be assigned to the COO$^-$ group bonded to
Na+ ion, while that at 1462 cm$^{-1}$ can be assigned to the COO$^-$ group bonded to Ca$^{2+}$ (vide infra). Figure 4 shows that the ratios of the absorbance at 1462 cm$^{-1}$ to that at 1420 cm$^{-1}$ decreased with the increasing coprecipitation temperature in Method A. Figure 4 also shows that the ratios of Na/Ca determined by EDS of the fracture surface of the composites were increased as the coprecipitation temperature was higher. This correlation suggests that the HAP crystal growth occurred faster at higher temperatures, and that would result in the cleavage of the carboxylate–Ca$^{2+}$ bond at the interface, with Na$^+$ replacing Ca$^{2+}$ as a countercation of the carboxylate group (Scheme 1).

**Scheme 1. Schematic Representation of the Changes in the Interface Bonding at Higher Coprecipitation Temperatures**

![Scheme 1](image)

The difference in the wavenumbers between the antisymmetric band and the symmetric band of the carboxylate group, $\Delta \nu = \nu_{as} - \nu_s$, has been used to distinguish the coordination mode of the carboxylate to metal ions. The IR spectra of the complex with unidentate binding of carboxylate to a metal ion (see D, Figure 5) exhibit the $\Delta \nu$ values that are much greater than that of the ionic complexes (A, Figure 5). The IR of the complex with chelating (bidentate) binding (F, Figure 5) exhibits $\Delta \nu$ values that are significantly less than that of the ionic complex. The $\Delta \nu$ values for bridging complexes (G, Figure 5) are greater than those of chelating complexes and close to the ionic values. The ionic complex of the sodium salt of CMC showed the $\Delta \nu$ value of 185 cm$^{-1}$, while the composites showed the $\Delta \nu$ values of 170 cm$^{-1}$ ($\nu_{as} = 1420$ cm$^{-1}$) and 128 cm$^{-1}$ ($\nu_s = 1462$ cm$^{-1}$). The signal at 1420 cm$^{-1}$, the major signal of CMC-Na$^+$, can be ascribed to the ionic complex, in which the carboxylate group is not bound to Ca$^{2+}$.

Tasumi and co-workers reported the $\nu_{as}$ and $\nu_s$ values of various coordination complexes of acetate by ab initio molecular orbital study, and the wavenumbers of carboxylate stretching vibration of various coordination modes are illustrated in Figure 5. If we assign the symmetric stretching vibration of the composite at 1420 cm$^{-1}$ to the coordination mode A or B in Figure 5, then the signal at 1462 cm$^{-1}$ can be assigned to the coordination mode E, F, or G. However, the value of $\Delta \nu$ (128 cm$^{-1}$) is too small if we assign this signal to the coordination mode E. Therefore, it is likely that the signal at 1462 cm$^{-1}$ is assigned to the bidentate form F or the bridging complex G. Papageorgiou et al. reported that sodium alginate showed $\Delta \nu$ of 192 cm$^{-1}$ while calcium alginate $\Delta \nu$ of 179 cm$^{-1}$. We suggest that the signal at 1462 cm$^{-1}$, which was seen only for the composites, is assigned to the carboxylate coordinated to Ca$^{2+}$ (F or G in Figure 5) and the signal at 1420 cm$^{-1}$ to the carboxylate coordinated to Na$^+$ (A or B in Figure 5).

**Effects of Coprecipitation Temperatures on the Density of the Compatibles.** The dried composite powder was uniaxially pressed at 120 MPa at 120 °C for 5 min in a mold to obtain a compressed block. In Figure 6a, the density of the composites was plotted against coprecipitation temperatures. Composites prepared in Method B showed higher densities than those prepared in Method A. This can be attributed to the smaller crystallite sizes of HAP in the

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**Figure 5.** Values of antisymmetric stretching ($\nu_{as}$), symmetric stretching ($\nu_s$), and $\Delta \nu$ of acetate coordinated to sodium (A–C) and calcium ions (D–G).

| Mode | $\nu_{as}$ (cm$^{-1}$) | $\nu_s$ (cm$^{-1}$) | $\Delta \nu$ (cm$^{-1}$) |
|------|-----------------------|---------------------|---------------------------|
| A    | 1568                  | 1373                | 195                       |
| B    | 1578                  | 1399                | 179                       |
| C    | 1560                  | 1409                | 151                       |
| D    | 1602                  | 1342                | 260                       |
| E    | 1588                  | 1391                | 197                       |
| F    | 1542                  | 1440                | 102                       |
| G    | 1556                  | 1393                | 163                       |
Mechanical Properties of the Compressed Composites. Figure 6b–e shows the bending strengths, the strains at break, the elastic moduli, and the fracture energies of the compacts of the composites as a function of coprecipitation temperatures. Standard errors of the means based on three to five determinations are also shown. The mechanical properties of the composites prepared in Method A were superior than those prepared in Method B. In particular, the bending strength and the fracture energy of the composite prepared in Method A at 50 °C were the largest. The elastic moduli of the composites increased as the coprecipitation temperature was higher (Figure 6d). The SEM images of the fracture surface are shown in Figures S1–S8. The composites coprecipitated at 50 and 80 °C in Method A showed no crack on the fracture surface, while other composites showed some cracks on the fracture surface. We previously reported that the cellulose nanofibers–HAP composites prepared by Method A showed better mechanical properties than those prepared by Method B. As shown in Figure 6b–e, the dependence of the bending strength on the coprecipitation temperature was similar to that of the strain at break. It is interesting to note that the aspect ratio of HAP is largest for the composite precipitated at 50 °C in Method A (Table 3). There is a positive correlation between the aspect ratios of hydroxyapatite crystals and the bending strength and the elastic moduli of the compacts. As discussed above, the coprecipitation temperature determined the crystallite size of HAP and the ion distribution in the organic–inorganic interface. We speculate that these parameters governed the elastic deformation limit, and eventually the bending strength of the composites.

Two factors governing the mechanical properties of the composites are that the large crystal of HAP would help the HAP crystals act as an efficient filler and the adsorption of CMC to the surface of HAP crystals would resist the deformation of the compact by the stress. The XRD results demonstrate that the crystal sizes of HAP were larger as the coprecipitation temperature was higher. The IR and EDS studies indicate that the bonding of the carboxylate to Ca²⁺ is
weakened as the coprecipitation temperature was too high. The optimum temperature represents a compromise between HAP crystal growth and the binding of the polymer to HAP at the interface.

**Effects of the Concentrations of CMC and Inorganic Ions on Hybridization and the Mechanical Properties of the Composites.** Using the optimum conditions of coprecipitation, that is, Method A at 50 °C, the effects of the concentrations of CMC and inorganic ions in the coprecipitation on the mechanical properties were studied. Table 4 lists the wt % of HAP in the composites coprecipitated in the presence of varying concentrations (0.8−13.2 g/L) of CMC. For all concentrations of CMC, the wt % of HAP was close to the feed ratio of 70%. As shown in Figure 7, the XRD patterns of the composites revealed that the mineral phase was hydroxyapatite for all of the CMC concentrations. The composite coprecipitated at 0.8 g/L CMC showed broader peaks than the other composites prepared in the presence of higher concentrations of CMC. At 0.8 g/L of CMC, both homogeneous and heterogeneous nucleations could occur to give poorly crystalline HAP. Therefore, CMC at a concentration of 1.6 g/L or higher helped nucleation and crystal growth of HAP at 50 °C. Crystallite sizes and the aspect ratios are listed in Table 4. The aspect ratio (002)/(310) was increased with the increase in the CMC concentration to 2.5 g/L but decreased with a further increase in the CMC concentration.

The IR spectra of the CMC−HAP composites coprecipitated in the presence of varying concentrations of CMC at 50 °C, Method A, are shown in Figure 8. There was no clear correlation between the signal intensity ratio at 1462−1420 cm⁻¹ and the CMC concentrations.

The densities of the compressed composites are plotted against the CMC concentrations in Figure 9a. A high density of the compact prepared under the condition of 0.83 g/L CMC indicates that the small crystallite size of HAP leads to a dense compact. There is a correlation between the crystallite size along the c-direction and the density of the compacts: larger crystallite resulted in less dense compacts.

The mechanical properties of the compacts are shown in Figure 9b,c. The bending strengths, elastic moduli, and fracture energies were increased as the CMC concentrations were higher. The strain at break increased from the CMC concentration of 0.83−1.6 g/L, reaching a plateau at 4−13 g/L of CMC. The bending strength and the elastic modulus of the composite coprecipitated in Method A, at 50 °C in the presence of 9.9 g/L of CMC were 113±2 MPa and 7.7±0.3 GPa, respectively, the highest values obtained in this study. The bending strength was larger than that of cellulose nanofibers−HAP composites (57 MPa)⁴⁰ and of starch−HAP composites (37 MPa)⁴⁶ prepared in a similar fashion. The larger bending strength of the CMC−HAP composites can be attributed to the larger number of carboxylate groups in the polymer to help binding to the surface of HAP. To realize the binding of the carboxylate groups to HAP, coprecipitation temperature and concentrations of both polymer and inorganic precursors should be carefully controlled. The mechanical properties of the CMC−HAP composites are in the range of the bending strengths of 30−320 MPa and of the elastic

![Table 4. Weight Percentage of the Inorganic Phase in the Composites Determined by TGA and Crystallite Sizes (nm) of Hydroxyapatite Determined by XRD](image)

In the presence of varying concentrations (0.8−13.2 g/L) of CMC. For all concentrations of CMC, the wt % of HAP was close to the feed ratio of 70%. As shown in Figure 7, the XRD patterns of the composites revealed that the mineral phase was hydroxyapatite for all of the CMC concentrations. The composite coprecipitated at 0.8 g/L CMC showed broader peaks than the other composites prepared in the presence of higher concentrations of CMC. At 0.8 g/L of CMC, both homogeneous and heterogeneous nucleations could occur to give poorly crystalline HAP. Therefore, CMC at a concentration of 1.6 g/L or higher helped nucleation and crystal growth of HAP at 50 °C. Crystallite sizes and the aspect ratios are listed in Table 4. The aspect ratio (002)/(310) was increased with the increase in the CMC concentration to 2.5 g/L but decreased with a further increase in the CMC concentration.

The IR spectra of the CMC−HAP composites coprecipitated in the presence of varying concentrations of CMC at 50 °C, Method A, are shown in Figure 8. There was no clear correlation between the signal intensity ratio at 1462−1420 cm⁻¹ and the CMC concentrations.

The densities of the compressed composites are plotted against the CMC concentrations in Figure 9a. A high density of the compact prepared under the condition of 0.83 g/L CMC indicates that the small crystallite size of HAP leads to a dense compact. There is a correlation between the crystallite size along the c-direction and the density of the compacts: larger crystallite resulted in less dense compacts.

The mechanical properties of the compacts are shown in Figure 9b,c. The bending strengths, elastic moduli, and fracture energies were increased as the CMC concentrations were higher. The strain at break increased from the CMC concentration of 0.83−1.6 g/L, reaching a plateau at 4−13 g/L of CMC. The bending strength and the elastic modulus of the composite coprecipitated in Method A, at 50 °C in the presence of 9.9 g/L of CMC were 113±2 MPa and 7.7±0.3 GPa, respectively, the highest values obtained in this study. The bending strength was larger than that of cellulose nanofibers−HAP composites (57 MPa)⁴⁰ and of starch−HAP composites (37 MPa)⁴⁶ prepared in a similar fashion. The larger bending strength of the CMC−HAP composites can be attributed to the larger number of carboxylate groups in the polymer to help binding to the surface of HAP. To realize the binding of the carboxylate groups to HAP, coprecipitation temperature and concentrations of both polymer and inorganic precursors should be carefully controlled. The mechanical properties of the CMC−HAP composites are in the range of the bending strengths of 30−320 MPa and of the elastic

![Figure 7. XRD patterns of CMC−HAP composite powder coprecipitated at varying concentrations of CMC at 50 °C in Method A.](image)

![Figure 8. IR spectra of the CMC−HAP composites coprecipitated in the presence of 0.83−13.2 g/L CMC. IR spectrum of CMC is shown in black.](image)
moduli of 2–35 GPa of bone.\textsuperscript{9} Furthermore, the composite has a higher flexural strength than polyamide 6 (92 MPa)\textsuperscript{61} and a higher elastic modulus than polyamide 6 with 40 wt % glass fiber (5.5 ± 1.2 GPa),\textsuperscript{62} so it can be expected to be used as a new tough biomass composite materials to replace petroleum-derived engineering plastics.

At a low concentration of CMC of 0.8 g/L, the addition of Ca\textsuperscript{2+} to a solution of CMC and phosphate ions results in both homogeneous and heterogeneous nucleation of HAP. The crystallite size was small, and the mechanical properties were poor. As the concentration of CMC increases, heterogeneous nucleation of HAP was a major process, and the tight binding of CMC to HAP is anticipated. On the one hand, at a high concentration of CMC, we speculate that the viscous solution could prevent the formation of well-aligned HAP crystals along the polymer chain, reducing the mechanical properties. The argument is supported by the XRD studies, in which the aspect ratio of HAP exhibited bell-shaped dependence on the CMC concentrations (Table 4). The particle size of the composite powder was minimum at a CMC concentration of 3.3 g/L (Figure S22). These observations suggest that crystal growth of HAP proceeded rapidly at the CMC concentration of 3.3 g/L.

\section{CONCLUSIONS}

Crystallization of hydroxyapatite in water in the presence of CMC (0.8–13.2 g/L) at room temperature to 90 °C by either the addition of calcium ions to phosphate ions or vice versa was carried out, and the resulting CMC–HAP composites were evaluated with TG, XRD, IR, EDS, and a three-point bending test. The addition of calcium to CMC–phosphate (Method A) gave a larger HAP crystallite as well as a larger bending strength than the addition of phosphate to CMC–
calcium (Method B). As the coprecipitation temperature was higher, the crystal of HAP was larger, while the carboxylate—Ca$^{2+}$ bond in the organic—inorganic interface was dissociated. Thus, the optimum temperature of coprecipitation to obtain the composites with high mechanical performances was 50 °C. The bending strength and the elastic modulus of the composites were also dependent on the CMC concentration in the coprecipitation process. A low concentration of CMC tends to lead to homogeneous nucleation of HAP and efficient hybridization is hampered. A concentration of CMC of 9.9 g/L gave the composite compact with the largest bending strength. The composite prepared under the optimum conditions (Method A, 50 °C, 9.9 g/L CMC) showed the bending strength of 113 MPa and the elastic modulus of 7.7 GPa, which are in the range of the bending strengths (30–320 MPa) and of the elastic moduli (2–35 GPa) of natural bone. Furthermore, the composite has a higher flexural strength than polyamide 6 (92 MPa) and a higher elastic modulus than polyamide 6 with 40 wt % glass fiber (5.5 ± 1.2 GPa), which means that they can contribute to Sustainable Development Goals (SDGs) as a new tough biomass composite materials that can replace the petroleum-derived engineering plastics used in car and airplane bodies.

**ASSOCIATED CONTENT**

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c00423.

SEM images and EDS of the fracture surface of the composites; stress–strain curves of the three-point bending test; Ca/P ratios of the composites; FT-IR spectra of HAP and the composites; and SEM images of the composite powder (PDF)

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Notes
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