

Reaction of sodium calcium borate glasses to form hydroxyapatite

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Abstract This study investigated the transformation of two sodium calcium borate glasses to hydroxyapatite (HA). The chemical reaction was between either $1\text{CaO} \cdot 2\text{Na}_2\text{O} \cdot 6\text{B}_2\text{O}_3$ or $2\text{CaO} \cdot 2\text{Na}_2\text{O} \cdot 6\text{B}_2\text{O}_3$ glass and a 0.25 M phosphate (K_2HPO_4) solution at 37, 75 and 200 °C. Glass samples in the form of irregular particles (125–180 μm) and microspheres (45–90 and 125–180 μm) were used in order to understand the reaction mechanism. The effect of glass composition (calcium content) on the weight loss rate and reaction temperature on crystal size, crystallinity and grain shape of the reaction products were studied. Carbonated HA was made by dissolving an appropriate amount of carbonate (K_2CO_3) in the 0.25 M phosphate solution. X-ray diffraction, Fourier transform infrared spectroscopy, and scanning electron microscopy were used to characterize the reaction products. The results show that sodium calcium borate glasses can be transformed to HA by reacting with a phosphate solution. It is essentially a process of dissolution of glass and precipitation of HA. The transformation begins from an amorphous state to calcium-deficient HA without changing the size and shape of the original glass sample. Glass with a lower calcium content ($1\text{CaO} \cdot 2\text{Na}_2\text{O} \cdot 6\text{B}_2\text{O}_3$), or reacted at an elevated temperature (75 °C), has a higher reaction rate. The HA crystal size increases and grain shape changes

from spheroidal to cylindrical as temperature increases from 37 to 200 °C. Increase in carbonate concentration can also decrease the crystal size and yield a more needle-like grain shape.

Introduction

Synthetic hydroxyapatite (HA, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) has been studied extensively as it closely resembles the mineral component of human bone, accounting for approximately 70 wt% of natural bone and 50% of its volume. However, human bone mineral or biological apatites, differ from pure HA in stoichiometry, composition, and crystallinity and in other physical and mechanical properties [1]. Biological apatites are usually calcium deficient (Ca/P molar ratio below 1.67) and always contain additional ions (CO_3^{2-} , F^- , Cl^-), of which the carbonate ion is the most abundant species (3–8 wt%) [2].

Due to its biocompatibility, HA has been used in medicine and dentistry for more than 20 years as a biomaterial for bone repair, augmentation, and substitution. The development and commercialization of HA for use in biomedical applications was based on the rationale that bone mineral has been usually described as HA and followed the observation by Hench [3] that the chemical bonding between natural bone and bioactive glasses was through a calcium phosphate-rich layer.

The so-called bioactive glasses are usually based on the $\text{NaO}_2\text{--CaO--P}_2\text{O}_5\text{--SiO}_2$ system. The first, and most well-studied bioactive glass, known as 45S5, has the composition 45% SiO_2 , 24.5% Na_2O , 24.4% CaO , and 6% P_2O_5 in weight percent. It is believed that its dental and orthopedic

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applications depend on the formation of HA at the initial implantation stage [1]. Such glasses have been actively studied *in vitro* and *in vivo* since the late 1960s, but borate glasses have not been investigated in this way.

The main objective of the present work was to study the transformation of sodium calcium borate glass into HA by reaction with a phosphate solution and to investigate the effects of calcium content, reaction temperature, and phosphate concentration on the transformation process and the nature of the reaction products. Samples in the form of irregular glass particles and microspheres were used to understand the reaction mechanism. Carbonated HA was also made by reacting the sodium calcium borate glasses with a phosphate solution that contained an appropriate amount of carbonate ions.

Materials and methods

Glass preparation

Two sodium calcium borate glasses were made with a molar ratio of $1\text{CaO} \cdot 2\text{Na}_2\text{O} \cdot 6\text{B}_2\text{O}_3$ and $2\text{CaO} \cdot 2\text{Na}_2\text{O} \cdot 6\text{B}_2\text{O}_3$, respectively, which are hereafter designated as 1-2-6 and 2-2-6 glass. The weight and mole percent compositions are shown in Table 1. The starting batch consisted of a mixture of reagent grade calcium carbonate (CaCO_3), sodium carbonate (Na_2CO_3), and boric acid (H_3BO_3) powders that melted at 1,000 °C for 1 h in a platinum crucible.

The melted glass was quenched into a thin plate between two cold stainless steel plates to prevent crystallization. The quenched glass was crushed in a steel mortar and pestle and sieved into a size range of 125–180 and 45–90 μm . The irregular glass particles were spheridized using a vertical tube furnace or a propane torch. The vertical tube furnace had a more uniform and longer hot zone, and thus gave a good yield of microspheres. However, clumping of the particles was a serious problem for particles smaller than 90 μm . A propane torch is better for spheridizing particles smaller than 90 μm .

Glass microspheres with a diameter range of 125–180 μm were made with a vertical tube furnace at 950 ± 10 °C. Irregular glass particles were dropped from a vibrating spatula into a vertical mullite tube (ID 6.5 mm),

which extends into a larger dense mullite tube (ID 85 mm) in the furnace. The irregular shaped glass particles melted and formed microspheres due to surface tension as they fell through the hot zone of the furnace. The microspheres were collected in a glass jar at the bottom of the larger tube. The bottom of the tube was sealed to prevent vertical drafts (chimney effect) in the tube. Collected microspheres were sieved to a size range of 125–180 μm .

Microspheres with a diameter of 45–90 μm were made using a propane torch. Irregular glass particles were dropped into a propane flame, where the particles melted and became spherical. The molten microspheres were carried by the flame into a stainless cylinder for collection.

Reaction with a phosphate solution

Irregular glass particles were reacted for a specified time at 37, 75, and 200 °C in a 0.25 M phosphate solution that was prepared by dissolving $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$ in distilled water. Solution pH was adjusted to 9.0 ± 0.05 with dilute HCl.

The reaction of the irregular glass particles at 37 and 75 °C was done in glass beakers. The 0.25 M phosphate solution was maintained at a constant temperature by placing the beakers on a heated sand bath at 37 ± 1 °C and on a hotplate at 75 ± 3 °C. A Teflon pressure vessel was used to react the particles at 200 °C. Irregular glass particles were also reacted at 37 °C in a 0.25 M phosphate solution that contained either 0.1 M or 0.2 M K_2CO_3 at pH 10.7.

Glass microspheres were reacted at only 37 °C in a 0.25 M phosphate solution (pH 9.0). In all reactions, the amount of the phosphate in solution was about 20 times more than the calcium in the glass with regard to forming stoichiometric HA, so the reaction to form HA was limited by the calcium in the glass.

After reaction, the phosphate solution was decanted and the reaction products were rinsed first with distilled water and then alcohol. After drying at 70 °C in an oven, the samples were stored in a desiccator.

Weight loss

The weight loss was measured for irregular particles of both glasses in the size range of 125–180 μm . After drying at 70 °C, approximately 300 mg of glass particles were placed in a 25-mL glass vial, which was filled with 20 mL of the 0.25 M K_2HPO_4 solution at pH 9.0. The glass particles reacted in the static solution at 37 and 75 °C for time intervals ranging from 1 to 48 h. The phosphate solution was decanted, the reaction products were carefully rinsed in the vial with distilled water, dehydrated with alcohol and dried at 70 °C before weighing. The weight loss was measured on a minimum of three samples from which the average weight loss was calculated.

Table 1 Weight and mole percent composition of 1-2-6 and 2-2-6 glass (%)

Glass	Weight percent			Mole percent		
	CaO	Na ₂ O	B ₂ O ₃	CaO	Na ₂ O	B ₂ O ₃
1-2-6	9.3	20.7	70.0	11.1	22.2	66.7
2-2-6	17.1	18.9	64.0	20.0	20.0	60.0

Analysis of reaction process and products

Real time video microscopy (RTVM)

Microspheres (45–90 μm) of the 2-2-6 glass were photographed by RTVM as they reacted with a 0.25 M K_2HPO_4 solution (pH 9.0) at 37 °C. The RTVM has an inner reaction chamber, which holds the microspheres and the solution. An optical microscope was used to view the microspheres through a transparent glass slide that forms the bottom of the reaction chamber. Temperature control (± 1 °C) was achieved using heated water that surrounded the reaction cell and the temperature was determined with a thermocouple.

Scanning electron microscopy (SEM)

The appearance and microstructure of the reacted irregular particles and microspheres were determined by SEM. Carbon tape or paint was placed on metal SEM mounts and the samples were placed onto the mounts. After the paint was dry, the samples were sputter coated with gold/palladium to form a conductive layer. The samples were analyzed using a Hitachi S570 SEM or JOEL T330 SEM for magnifications below 2,000 \times . A Hitachi S-4700 field emission SEM, equipped with an EDAX Phoenix system, was used for images with magnification up to 60,000 \times and energy dispersive spectroscopy (EDS) analysis of carbon coated samples.

Fourier transform infrared spectroscopy (FT-IR)

The infrared spectra of the reaction products were measured by FT-IR spectroscopy. Approximately 5 mg of the reacted materials was mixed with 150 mg of IR-grade KBr powder in a mortar and pestle. The mixture was then pressed in a stainless steel pellet die for 1 min at 700 MPa to form a translucent disc for FT-IR measurements. The absorption spectra were measured from 400 to 4,000 cm^{-1} with a resolution 0.48 cm^{-1} using a Nicolet-Magna-IR 750 spectrometer. A nitrogen purge was used to remove water and carbon dioxide from the sample chamber. The spectra recorded were the average of 32 scans.

X-ray diffraction (XRD)

XRD patterns of the reacted materials, ground into a fine powder, were obtained with a Scintag 2000 X-ray diffractometer. The XRD patterns were collected over a 2θ range from 10° to 90° at a scan rate of 0.03°/s with a counting time of one second. The phases in the material were identified by comparing the XRD patterns with JCPDS standards (Joint Committee Power Diffraction Standard).

X-ray photoelectron spectroscopy (XPS)

The irregular particles (125–180 μm) of the 2-2-6 glass that had been reacted in a 0.25 M K_2HPO_4 solution and a 0.25 M K_2HPO_4 + 0.1 M K_2CO_3 solution at 37 °C for 24 h were analyzed on a Kratos Axis 165 XPS spectrometer. The sample was analyzed using a Mg X-ray source at 1253.6 eV. The sputter rate was approximately 1.5 nm/min.

Results

Reaction of irregular glass particles

Reaction progress

To monitor the reaction progress and identify the reaction product, FT-IR spectra were collected for 2-2-6 glass particles (125–180 μm) that had been reacted for 0 h, 1 h, 3 h, 6 h, 24 h, 6 days and 30 days at 37 °C in a 0.25 M K_2HPO_4 solution with pH 9.0 (Fig. 1).

The identification of HA using FT-IR spectra is based on the peaks corresponding to phosphate (PO_4^{3-}) and hydroxyl (OH^-) ions. The FT-IR spectrum of a commercial HA (Sigma Co., Cat. # H-0252) is shown by the upper line in the figure

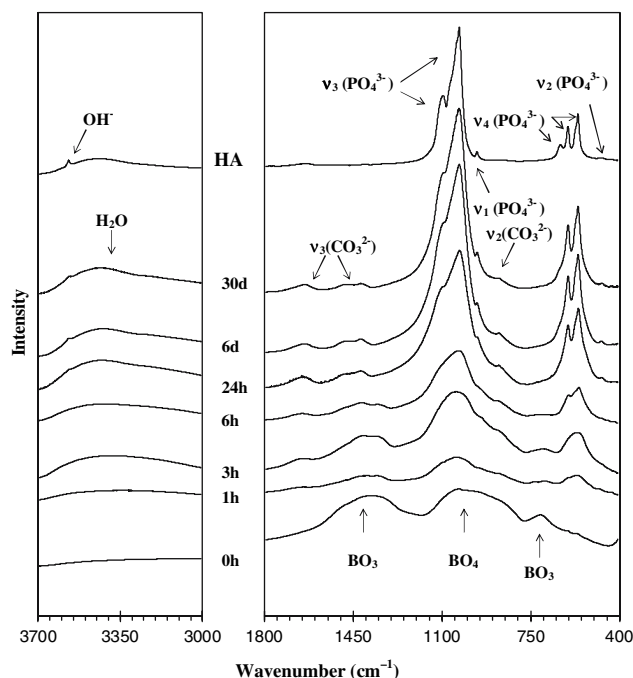


Fig. 1 FT-IR spectra for 2-2-6 glass particles (125–180 μm) that had been reacted for 0 h, 1 h, 3 h, 6 h, 24 h, 6 days and 30 days at 37 °C in a 0.25 M K_2HPO_4 solution at pH 9.0. Successive spectra have been shifted upwards. No peaks were observed in the region of 3,000–1,800 cm^{-1} so that region has been omitted to show the regions of interest. The FT-IR spectrum for a commercial HA (Sigma Co., Cat. # H-0252) is shown by the upper line in the figure

(Sigma Co., Cat. # H-0252) is shown by the upper line in Fig. 1. As labeled, there are four vibrational modes present for phosphate ions (PO_4^{3-}), ν_1 , ν_2 , ν_3 , and ν_4 that are IR active [4]. The most dominant bands for HA are the phosphate ν_3 band, centered at 1,094 and 1,032 cm^{-1} , and the phosphate ν_4 band with peaks at 632, 603, and 564 cm^{-1} . There is also a weak shoulder, which corresponds to the phosphate ν_1 band, located at 962 cm^{-1} . The ν_2 band is very weak and can barely be observed at 472 cm^{-1} . Hydroxyl bands are observed at 3,570 cm^{-1} , which are often masked by broad water absorption. Table 2 summarizes the assignment of the FT-IR peaks for HA in reference to several authors [4–7]. Since the carbonate ion is often present in HA, its peak assignment is also included.

The FT-IR spectrum for the as-made (unreacted) glass particles (0 h) is shown by the bottom curve in Fig. 1. There were three broad absorption bands, one centered at 1,600–1,200 cm^{-1} and one centered at 750–600 cm^{-1} , both of which correspond to vibrations of the trigonal BO_3 groups. The other one centered at 975 cm^{-1} is due to the vibration of tetrahedral BO_4 groups in the borate glass [8].

The 1h FT-IR spectrum resembled that of the as-made glass, but the peaks in the vicinity of 1,050 and 600 cm^{-1} due to phosphate ions were barely visible. After the glass particles had reacted for 3 h, these phosphate ν_3 and ν_4 bands were sharper and larger.

At 6 h, the phosphate ν_4 band showed the first signs of splitting into two peaks. As the reaction time increased, the peaks of the phosphate ν_3 bands began to split and became more distinct, a weak ν_1 band appeared at the shoulder of ν_3 band, the ν_4 band split into two peaks and hydroxyl stretching mode and phosphate ν_2 band appeared at 24 h. The splitting of the ν_4 band is a sign of low site symmetry, indicating that there is more than one distinct site for the phosphate group [4].

After the glass particles had reacted for 24 h, the FT-IR spectrum contained peaks characteristic of HA, although indicating low crystallinity compared with the spectrum for commercial HA. In spite of a slight increase in hydroxyl intensity, the 6-day and 30-day spectra basically were the same as that for 24 h and no further splitting or new peaks

Table 2 A summary of common peak positions in FT-IR spectra of HA

Ions	Phosphate (PO_4^{3-})	Carbonate (CO_3^{2-})	Hydroxyl(OH^-)
Band positions (cm^{-1})	ν_1 962	–	3570
	ν_2 472	ν_2 873	–
	ν_3 1032,1094	ν_3 1400–1650	–
	ν_4 564, 603, 632	–	–

appeared. The reaction went from starting glass to HA in 24 h at 37 °C.

A broad peak centered at 3,400 cm^{-1} , due to ν_1 symmetrical stretching and ν_2 asymmetrical stretching of physically adsorbed water, was evident in some of the spectra shown in Fig. 1. Carbonate ν_2 and ν_3 peaks could also be seen in the region of 1,650–1,400 cm^{-1} and at 873 cm^{-1} , respectively, which could be caused by atmospheric carbon dioxide that dissolved in the phosphate solution. Since water also has bending mode at around 1,600 cm^{-1} , it should at least partly contribute to the intensity of the carbonate ν_3 mode [1, 6].

The XRD patterns (Fig. 2) for the 1-day, 6-day and 30-day reaction products confirmed that the starting glass had transformed to HA. The identification of HA is primarily based on the two most prominent peaks at 26°, (002), and at 32–34°, a result of overlap of (211), (112), and (300) as shown in the standard XRD pattern, JCPDS 72-1243, at the bottom of Fig. 2. All three XRD patterns matched HA and showed little change in the number and sharpness of the peaks for reaction times between 1 day and 30 days. The reaction products were stable after 1 day of reaction.

Reaction rate

The weight loss is an indication of the reaction rate. The weight loss of the 1-2-6 and 2-2-6 glass was measured for

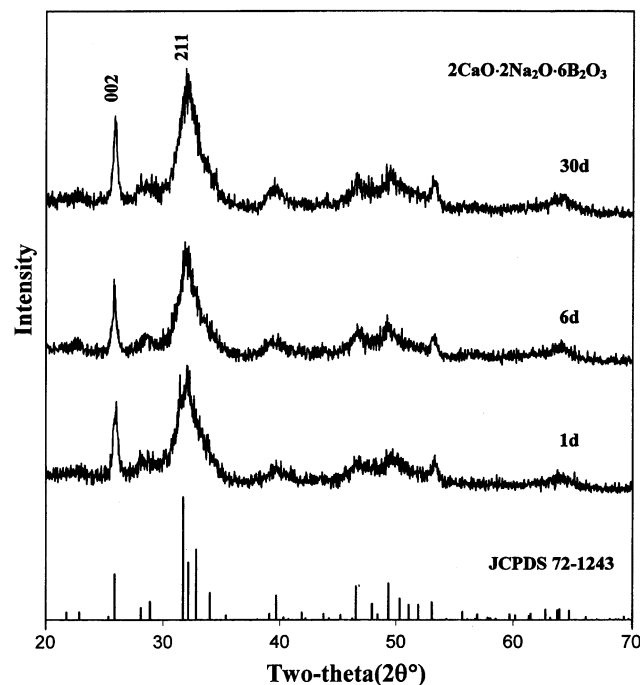


Fig. 2 XRD patterns for 2-2-6 glass particles (125–180 μm) reacted for 1 day, 6 days, and 30 days at 37 °C in a 0.25 M K_2HPO_4 solution at pH 9.0. Standard XRD pattern for HA, JCPDS 72-1243, is shown by the lines at the bottom of the figure

irregular particles (125–180 μm) reacted at 37 and 75 °C in a 0.25 M K₂HPO₄ solution at pH 9.0. As shown in Fig. 3, both glasses experienced a rapid weight loss for about 8–10 h and only a small additional weight loss thereafter.

The weight loss of the 1-2-6 glass leveled off at around 80% whereas the weight loss of the 2-2-6 glass leveled off at around 60%. The 1-2-6 glass, which had a lower calcium content, had a higher weight loss rate as indicated by the steeper initial slope. For the first 6 h, the average percent weight loss for the 1-2-6 glass and 2-2-6 glass was 12 and 7%/h, respectively. The maximum weight loss and weight loss rate depended on the calcium content of the glasses as expected. After 24 h, both glasses had reached their maximum weight loss, which did not change at longer times. This indicates completion of the reaction, which is in agreement with the FT-IR spectra in Fig. 1.

At 75 °C, the time (1 h) for reaching the maximum weight loss for both glasses was an order of magnitude faster than that at 37 °C. Table 3 lists the weight loss for irregular particles of both glasses after they had reacted in a 0.25 M K₂HPO₄ solution at pH 9.0 for 1, 3, and 6 h at 37

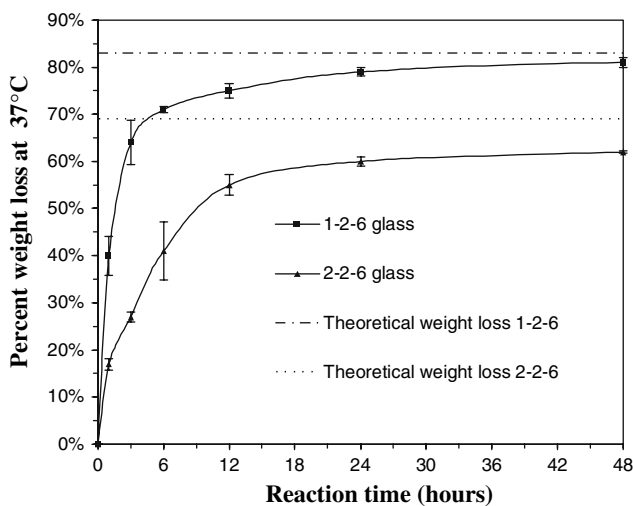


Fig. 3 Weight loss of 1-2-6 and 2-2-6 glass particles (125–180 μm) reacted at 37 °C in a 0.25 M K₂HPO₄ solution at pH 9.0

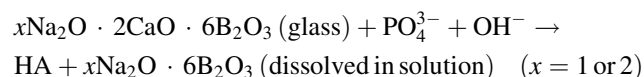
Table 3 Measured percent weight loss of 1-2-6 and 2-2-6 irregular glass particles (125–180 μm) after reacting in a 0.25 M K₂HPO₄ solution at pH 9.0 for 1, 3, and 6 h at 75 and 37 °C

Time (h)	1-2-6	2-2-6
1	76(40) ^a	62(17) ^a
3	81(64) ^a	64(27) ^a
6	80(71) ^a	63(41) ^a
Theoretical	83	69

^a Numbers in parentheses are for 37 °C

and 75 °C, respectively. For both glasses, the maximum weight loss was reached in about 1 h at 75 °C. A comparison of the FT-IR spectra for 1-2-6 glass particles reacted at 37 and 75 °C for 6 h is shown in Fig. 4. The spectrum at 37 °C still showed features of amorphous calcium phosphate (ACP) [6], broad bands of moderate intensity at phosphate ion ν₃ and ν₄ mode positions, and was still in the middle stage of the reaction. The spectrum at 75 °C had more pronounced peaks and clearly resembled the FT-IR spectrum for commercial HA. After 6 h at 75 °C, the glass had fully reacted, which agrees with the weight loss data.

The theoretical weight loss values in Table 3 were calculated by assuming that all of the Na₂O and B₂O₃ in each glass completely dissolved and went into solution, while all the CaO in the glass reacted with the phosphate ion and hydroxyl in solution to form stoichiometric HA (Ca₁₀(PO₄)₆(OH)₂).



Without exception, the measured weight loss at both 37 and 75 °C was only about 3–6% less than the theoretical values. This small difference is most likely due to the difference in composition of the transformed HA and that for stoichiometric HA.

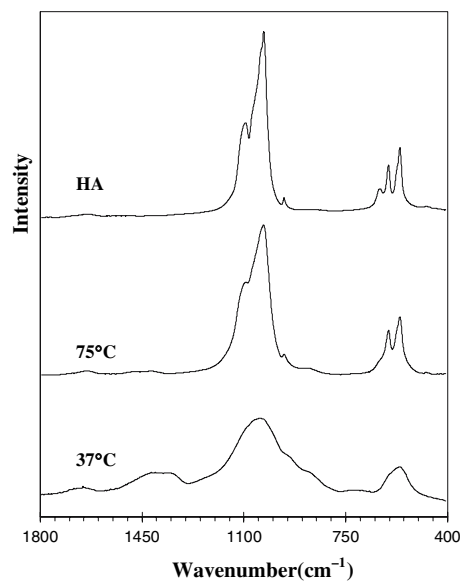


Fig. 4 FT-IR spectra for 1-2-6 glass particles (125–180 μm) reacted for 6 h at 75 and 37 °C, respectively, in a 0.25 M K₂HPO₄ solution at pH 9.0. The FT-IR spectrum for a commercial HA (Sigma Co., Cat. # H-0252) is shown by the upper line in the figure

Effect of temperature

Temperature not only affected the reaction rate, but also affected the crystallinity, crystal size and grain shape of the transformed HA.

The FT-IR spectra of 2-2-6 irregular glass particles (125–180 μm) reacted in a 0.25 M K_2HPO_4 solution (pH 9.0) at 37 $^\circ\text{C}$ for 24 h and at 75 and 200 $^\circ\text{C}$ for 6 h are shown in Fig. 5. It is evident from the spectra that as the temperature increased from 37 to 200 $^\circ\text{C}$, the spectra more closely resembled that for commercial HA, indicating that the crystallinity improved with increasing reaction temperature.

At 37 $^\circ\text{C}$, only two peaks for the phosphate ν_4 mode were visible in the spectrum, with an indication of a third peak in the spectrum for 75 $^\circ\text{C}$. Three ν_4 peaks could be clearly seen in the spectrum at 200 $^\circ\text{C}$. The phosphate ν_1 band at 962 cm^{-1} became sharper as the temperature increased. At the same time, the ν_3 mode became narrower and changed from two barely distinguishable peaks at 37 $^\circ\text{C}$ to two distinct peaks at 200 $^\circ\text{C}$. The hydroxyl band at 3,570 cm^{-1} changed from being barely noticeable to obvious. The carbonate bands, 1,650–1,400 and 873 cm^{-1} , due to dissolved atmospheric carbon dioxide in the phosphate solution, were most clearly seen at 37 $^\circ\text{C}$ and diminished as the temperature increased.

The XRD patterns of the 2-2-6 irregular glass particles (125–180 μm) reacted in the 0.25 M K_2HPO_4 solution (pH 9.0) at 37 $^\circ\text{C}$ for 24 h and at 75 and 200 $^\circ\text{C}$ for 6 h are

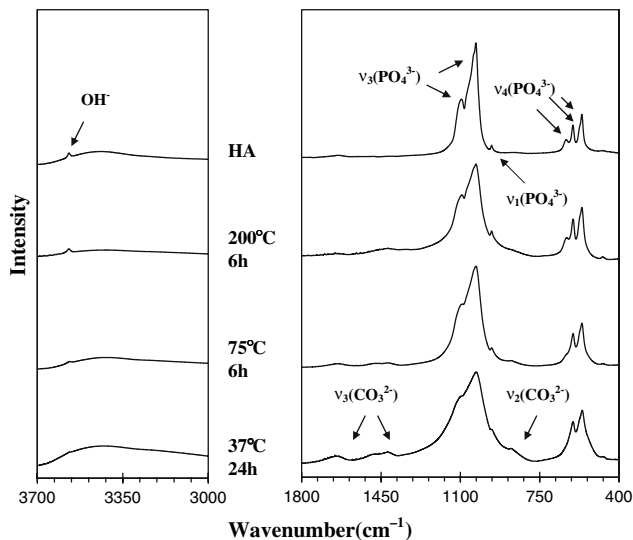


Fig. 5 FT-IR spectra for 2-2-6 glass particles (125–180 μm) reacted in a 0.25 M K_2HPO_4 solution (pH 9.0) at the temperatures and for the times shown. No peaks were observed in the region of 3,000–1,800 cm^{-1} so that region has been omitted to show the regions of interest. The FT-IR spectrum for a commercial HA (Sigma Co., Cat. # H-0252) is shown by the upper line in the figure

shown in Fig. 6. The XRD pattern for the particles reacted at 37 $^\circ\text{C}$ contained only two broad peaks, corresponding to the (002) and (211) planes, which indicate that the reaction products had a low crystallinity and that the crystal size was small [9–12]. The XRD pattern at 75 $^\circ\text{C}$ showed signs of new peaks and the peak shape became sharper and narrower. At 200 $^\circ\text{C}$, the XRD pattern showed almost all the major peaks for HA and resembled that for commercial HA closely as shown in Fig. 6.

Peak broadening is a result of decrease in crystal size. The crystal size of the reaction products increased as the temperature increased. The effect of temperature on the crystal size of HA synthesized from aqueous solutions has been observed previously and most studies reported a broadening of XRD patterns at temperatures below 80 $^\circ\text{C}$ [5, 13]. The same conclusion can be made for the reaction between sodium calcium borate glass and a phosphate solution.

SEM images of reaction products on the surface of the 1-2-6 and 2-2-6 glass particles reacted at 37 $^\circ\text{C}$ for 24 h and 75 and 200 $^\circ\text{C}$ for 6 h are shown in Fig. 7. In general, the surface at the lower reaction temperature (37 $^\circ\text{C}$), or lower calcium content (1-2-6 glass), had relatively open structure. As the temperature increased, the structure

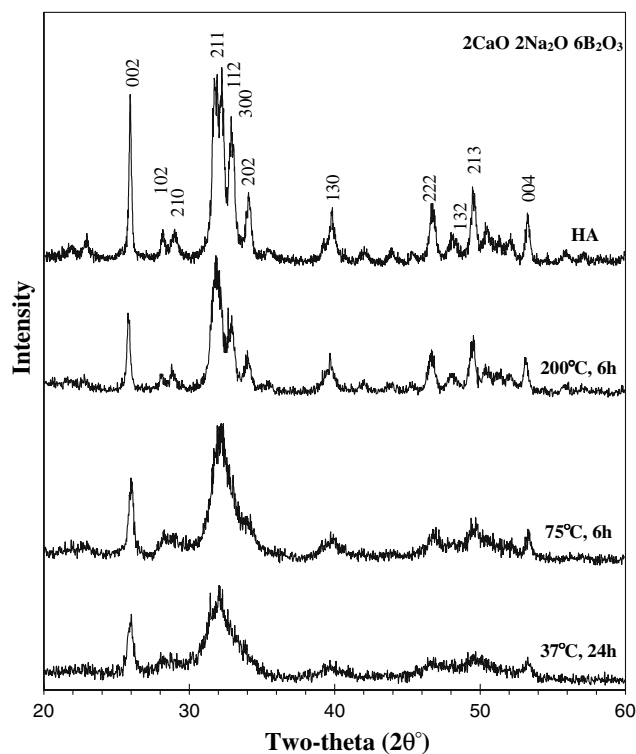
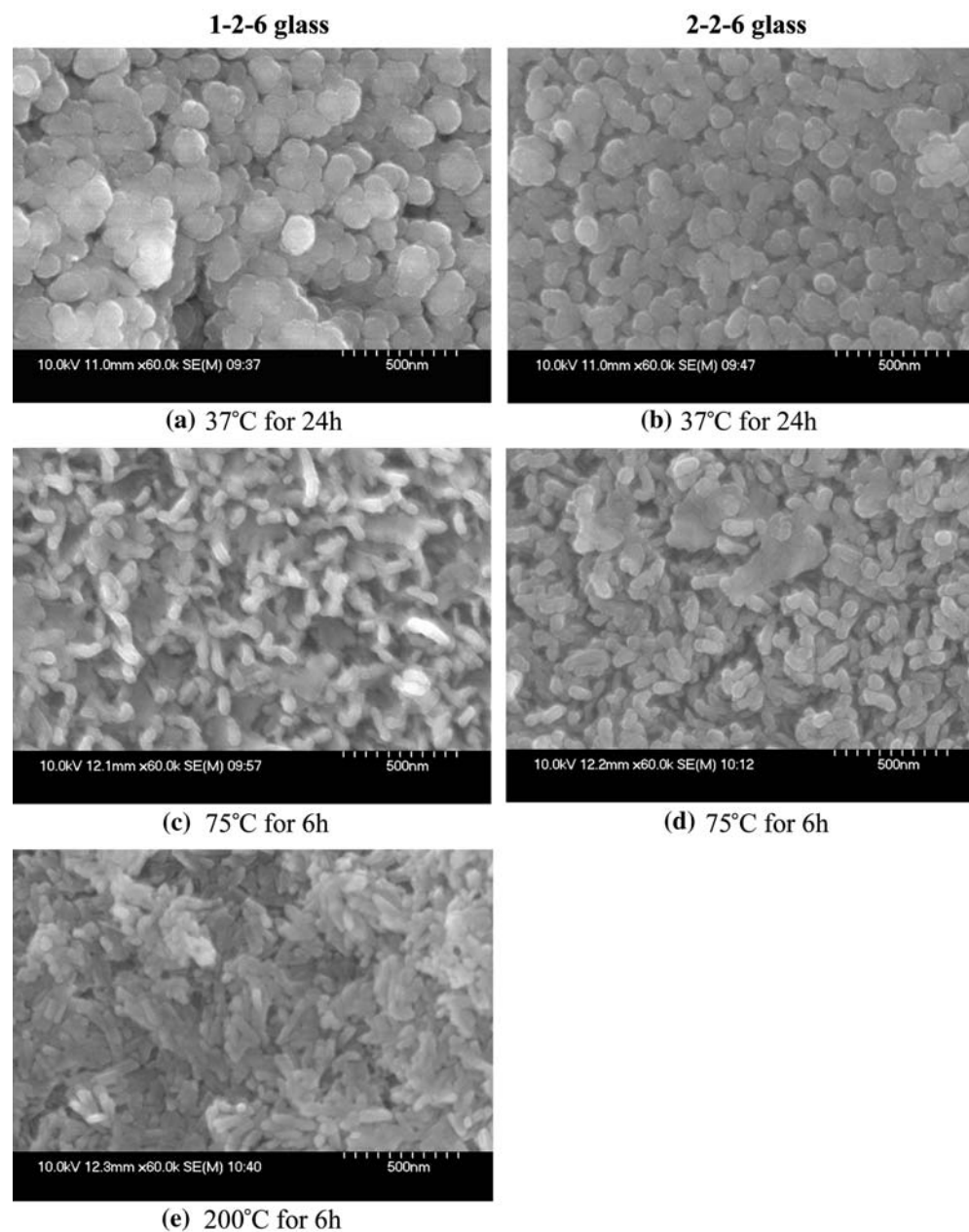


Fig. 6 XRD patterns for 2-2-6 glass particles (125–180 μm) reacted in a 0.25 M K_2HPO_4 solution (pH 9.0) at the temperatures and for the times shown. The XRD pattern for a commercial HA (Sigma Co., Cat. # H-0252) is shown by the upper line in the figure

Fig. 7 Appearance of the material on the surface of 1-2-6 and 2-2-6 glass particles (125–180 μm) after reaction in a 0.25 M K_2HPO_4 solution (pH 9.0) at the temperatures and for the times shown



became more compact. The grain shape changed from spheroid-like at 37 °C to cylindrical/needle-like at 75 °C with a decrease in grain size for both 1-2-6 and 2-2-6 glasses. After 6 h at 200 °C, the HA grains grew into continuous, plate-like clusters for 1-2-6 glass. The SEM images show that grain shape can be controlled by altering the reaction temperature.

Effect of carbonate

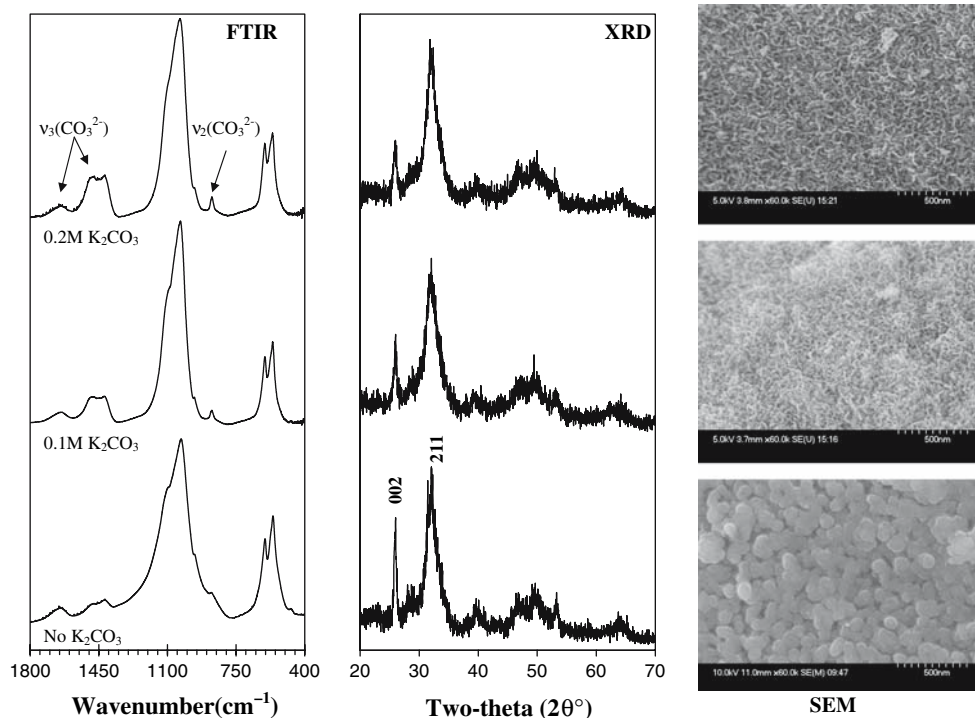
Since biological apatite is always carbonated, an attempt was made to see if carbonated HA (CHA) [5, 13, 14] could be made by adding CO_3^{2-} ions to the phosphate solution.

FT-IR spectra, XRD patterns and SEM images of the external surface for 2-2-6 irregular glass particles reacted in a 0.25 M K_2HPO_4 solution containing no, 0.1 M or 0.2 M K_2CO_3 at 37 °C for 24 h are shown in Fig. 8.

The carbonate ions (CO_3^{2-}) occupy two different sites in carbonated apatite [4]. The peaks in the region of 1,650 and 1,400 cm^{-1} in the FT-IR spectra are due to the ν_3 vibrational mode of carbonate ions and the peak at 873 cm^{-1} is due to the ν_2 vibrational mode.

As shown in the FT-IR spectra in Fig. 8, the intensity of the peaks corresponding to the carbonate ions and the intensity ratio of the carbonate/phosphate peak increased with increasing carbonate concentration in the phosphate

Fig. 8 FT-IR spectra, XRD patterns, and SEM images of the appearance of the surface of the 2-2-6 glass (125–180 μm) after reaction at 37 °C for 24 h in a 0.25 M K_2HPO_4 solution containing varying amounts of K_2CO_3 as shown



solution. This trend confirms the existence of carbonate ions in the HA. It is also noticed that phosphate ion ν_3 splitting became less obvious with increasing carbonate concentration, which is interpreted as a decrease in crystallinity.

The XRD patterns in Fig. 8 did not change as the carbonate concentration increased and apatite was the only phase detected, demonstrating no apparent change in the crystallographic space group of the material occurred upon carbonate incorporation [5]. However, a slight peak broadening was observed as carbonate concentration increased. This may indicate that CHA has a smaller crystal size and lower crystallinity compared with HA, which agrees with the FT-IR spectra. Carbonate substitution may also change the lattice parameters. A contraction in a -axis and expansion in c -axis were reported as a result of carbonate inclusion in the apatite structure by Nelson [5], but the contraction or expansion was not sufficient to cause a measurable change in peak position in the XRD patterns.

The SEM images in Fig. 8 of the carbonated reaction products on the particle surface show that the HA grains were predominantly needle-like and smaller compared with a spheroid-like shape for the HA formed in the solution with no carbonate. Carbonate addition also can control the grain shape.

Chemical analysis, calcium/phosphate ratio of reacted glass particles

The Ca and P elemental analysis of reacted particles was performed by EDS. The molar ratio of Ca/P was calculated

using their average atomic contents from a scanned area. Table 4 lists the Ca/P ratio of particles reacted at the temperatures, for the times and in the solutions shown.

The transformed HA was calcium-deficient in all cases and the Ca/P ratio varied from 1.22 to 1.50 compared to the stoichiometric value of 1.67. The carbonated HA had a higher Ca/P ratio (1.50) than that formed without carbonate (1.22). This indicated that the carbonate substitution was mainly a B type reaction—carbonate ions substituted for phosphate ions in the lattice which would decrease the phosphate ion content, thus increasing the Ca/P ratio [15].

The reaction products formed in a 0.25 M K_2HPO_4 and a 0.25 M K_2HPO_4 + 0.1 M K_2CO_3 solution at 37 °C for 24 h were analyzed by XPS. Table 5 lists the O, Ca, C, P atomic content before and after sputtering for 2 min. The reaction product made in the solution with 0.1 M K_2CO_3 had a carbon content about 10% higher in both cases and confirmed the existence of carbon on the surface. The carbon content of the reaction product with 0.1 M K_2CO_3 after sputtering for 2 min was 18%, which is higher than

Table 4 The Ca/P ratio from EDS analysis of irregular glass particles that reacted in a 0.25 M phosphate solution at the temperatures and for the times shown

Glass	37 °C (24 h)	75 °C (6 h)	200 °C (6 h)	37 °C (24 h), 0.1 M K_2CO_3
1-2-6	1.27 ± 0.10	1.36 ± 0.11	1.30 ± 0.10	—
2-2-6	1.22 ± 0.09	1.40 ± 0.11	1.42 ± 0.11	1.50 ± 0.12

Table 5 XPS elemental analysis (atomic percent) of the surface of 2-2-6 glass particles reacted in a 0.25 M K_2HPO_4 and a 0.25 M $K_2HPO_4 + 0.1$ M K_2CO_3 solution at 37 °C for 24 h (%)

Element		O	Ca	C	P
Sputter time(0 s)	No K_2CO_3	57	15	14	14
	0.1 M K_2CO_3	53	13	24	10
Sputter time(120 s)	No K_2CO_3	62	16	8	14
	0.1 M K_2CO_3	55	15	18	12

Experimental error is 1–5%

expected since the carbon content reported [5, 13] for CHA made by aqueous syntheses is about 5%. The residue carbonate on the surface and the highly surface sensitive nature of XPS may be the reason for this.

Reaction of microspheres

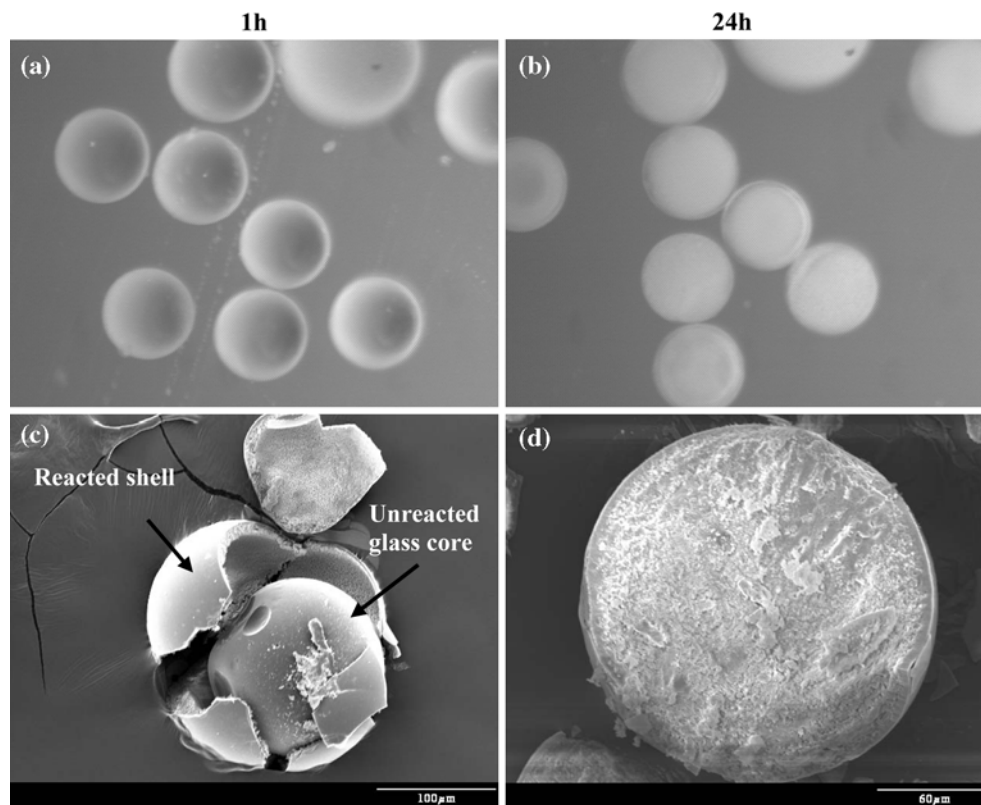
Because of their spherical shape, microspheres have natural advantages for visual observation. Two RTVM images for 2-2-6 glass microspheres (45–90 μm) reacted in a 0.25 M K_2HPO_4 solution (pH 9.0) at 37 °C for 1 and 24 h are shown in Fig. 9(a) and (b). As indicated by the RTVM images, there was no change in the diameter or shape of the microspheres after reaction, which is similar to the

behavior of lithium dysprosium borate glasses studied by Conzone [16].

In Fig. 9(c) and (d), the reacted microspheres (125–180 μm) were intentionally broken to reveal the cross section (interior). The 1 h image shows a relatively thin (about 20 μm) reacted outer layer shell surrounding a core that is most likely unreacted glass. The unreacted glass core has its initial spherical shape. The cross section of microspheres reacted for 24 h showed that it did not contain an unreacted core, and appeared fully reacted. The reaction started from the outer surface and progressed concentrically inward to the very center of the spheres.

The SEM image of the reaction products on the external surface of the 2-2-6 glass microspheres reacted in a 0.25 M K_2HPO_4 solution (pH 9.0) for 24 h is shown in Fig. 10. The surface grains were mainly rod-like, whereas the grains on the surface of the irregular glass particles (before spheridization) reacted under the same conditions were spheroid-like, see Fig. 7(b). The appearance of the external surface of the irregular 2-2-6 glass particles reacted at 75 °C for 6 h in Fig. 7(d) shows similar grain shape to Fig. 10, but with a slightly larger grain size. The spheridization (heat treatment) effect on the shape of the transformed HA particles is similar to an increase in reaction temperature.

Fig. 9 Real time video microscopy images of 2-2-6 glass microspheres (45–90 μm) reacted at 37 °C in a 0.25 M K_2HPO_4 solution (pH 9.0) for 1 h (a) and 24 h (b) and SEM images of the cross section of microspheres reacted for 1 h (c) and 24 h (d)



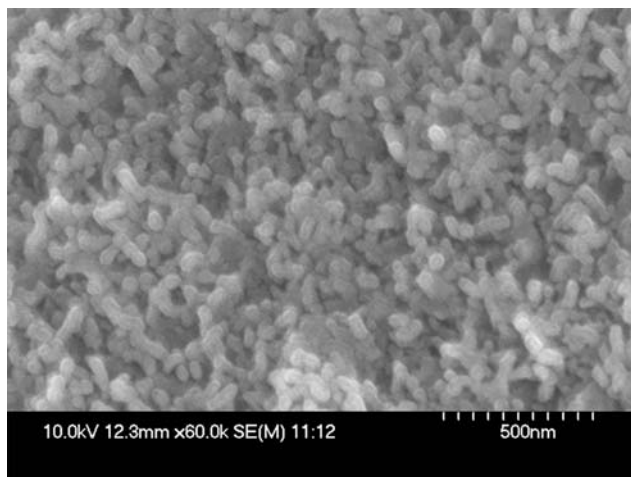


Fig. 10 The appearance of the external surface of the 2-2-6 glass microspheres reacted in a 0.25 M K_2HPO_4 solution at pH 9.0 for 24 h at 37 °C

Discussion

Reaction process

The reaction that sodium calcium borate glasses undergo in a 0.25 M K_2HPO_4 solution is essentially a dissolution and precipitation process. Sodium calcium borate glasses dissolve in the phosphate solution and Na^+ , Ca^{2+} , and B^{3+} in the glass are released to the solution. At the same time, as soon as the Ca^{2+} goes into solution, it reacts immediately with PO_4^{3-} to form the most chemically insoluble calcium phosphate, which in this case is a poorly crystalline HA.

After the first layer of HA deposits on the glass surface, the solution can still pass freely through the porous reacted layer and continues to dissolve the underlying glass until the glass is fully reacted or transformed. Basically, the borate glass acts as a soluble matrix containing calcium, that releases Na^+ and B^{3+} to the solution while at the same time the PO_4^{3-} and OH^- in the solution react with calcium. Without changing geometric size and shape, the material changes from the starting glass into crystalline HA.

When silica-based bioactive glasses dissolve in aqueous solution, alkali ions at the glass surface are exchanged for hydrogen ions in the surrounding solution first, leaving an alkali-depleted silica gel layer on the glass surface. The subsequent ion exchange and solution diffusion are controlled by this layer, which is poorly soluble and which can act as a protective layer. The dissolution of this silica gel layer controls the overall dissolution rate of the glass. Due to the dealcalization, the protective layer has a higher silica content and a lower alkali content [17].

However, when glasses such as the sodium calcium borate glasses used in the present study dissolve, the entire glass dissolves uniformly. Borate glasses react faster than

silicate glasses since no protective gel layer is formed. The dissolution rate of the glass is controlled by bulk dissolution instead of dissolution of a single species within the glass. Unlike most silicate glasses, borate glass undergoes congruent dissolution with an equivalent loss of B, Na and Ca.

The calcium content of the glass and the solution temperature are major factors affecting the reaction rate of sodium calcium borate glasses. An increase in calcium content reduces the glass solubility (reduces its reaction rate in aqueous solution) whereas an increase in temperature increases the reaction rate. Since the phosphate ion participates in the reaction, its concentration also affects the reaction rate.

Ca/P ratio

The HA transformed from sodium calcium borate glass is non-stoichiometric and has a lower Ca/P ratio than stoichiometric HA. Calcium-deficiency is characteristic of aqueous synthesis of HA. Most such studies have quoted a Ca/P ratio in a range of 1.5–1.67 [12, 18–21], but a Ca/P ratio as low as 1.2 has also been reported [22]. On the other hand, some evidence exists that HA precipitated from carbonate rich solution has Ca/P molar ratios above 1.67 [2, 14, 21], and as high as 2.2 [15, 23]. The Ca/P ratio can be affected by crystallinity [11], ion substitution [14], solution pH value [14] and original Ca/P ratio in solution [23].

As a matter of fact, the Ca/P ratio in human bone varies with age and the location of the bony site. Naturally occurring bone mineral is made of nanometer sized, poorly crystalline calcium phosphate with an apatitic structure. It is the Ca/P ratio in conjunction with nanocrystalline size and the poor crystallinity nature that yields the specific solubility properties of bone mineral. Because bone tissues undergo constant tissue repair regulated by mineral-resorbing cells (osteoclasts) and mineral-producing cells (osteoblasts), solubility behavior of minerals is important in maintaining a delicate metabolic balance between these cell activities [19]. Thus, it is desirable to find a way of controlling the Ca/P ratio, crystal size and crystallinity of synthesized HA to have better compatibility with human body environment.

Conclusion

The reaction of sodium calcium borate glass in a phosphate solution to form HA is essentially a process of dissolution of the glass followed by precipitation of HA. In general, the reaction product is initially an amorphous calcium phosphate, which gradually changes into a poorly crystalline

and calcium-deficient HA, which is very similar to the HA in human bone in terms of crystallinity and crystal size.

The reaction rate can be controlled by the reaction temperature, calcium content in glass and phosphate concentration in solution. The crystallinity, grain shape and crystal size of HA can be controlled by altering reaction temperature and carbonate concentration.

Carbonated HA can be easily made by adding carbonate ions to the phosphate solution. Carbonated HA more closely resembles the composition of natural bone. It is also possible to form other ion substituted apatites, for example, fluoroapatite, by adding appropriate ions to the solution.

The transformation process does not change the original glass shape and size, which makes it advantageous for certain applications where pre-controlled size and shape are desirable.

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References

1. L. L. HENCH and J. WILSON, *An Introduction to Bioceramics*, World Scientific, Singapore (1993)
2. N. PATEL, S. M. BEST, I. R. GIBSON, S. KE, K. A. HING and W. BONFIELD, *Key Eng. Mater.* **191–195** (2001) 7
3. L. L. HENCH, *J. Am. Ceram. Soc.* **74** (1991) 1487
4. I. REHMAN and W. BONFIELD, *J. Mater. Sci. Mater. Med.* **8** (1997) 1
5. D. G. A. NELSON and J. D. B. FEATHERSTONE, *Calcif. Tissue Int.* **34** (1982) 69
6. R. E. WUTHIER, G. S. RICE, J. E. B. WALLACE, R. L. WEAVER, R. Z. LEGEROS and E. D. EANES, *Calcif. Tissue Int.* **37** (1985) 401
7. K. A. HING, S. M. BEST and W. BONFIELD, *J. Mater. Sci. Mater. Med.* **10** (1999) 135
8. A. H. VERHOEF and H. W. D. HARTOG, *J. Non-Cryst. Solids* **182** (1995) 221
9. L. GUO, M. HUANG, Y. LENG, J. E. DAVES and X. ZHANG, *Key Eng. Mater.* **192–195** (2001) 187
10. C. DU, F. Z. CUI, K. D. GROOT and P. LAYROLLE, *Key Eng. Mater.* **218–220** (2002) 39
11. I. I. BARBA, A. J. SALINAS and M. V. REGI, *J. Biomed. Mater. Res.* **51** (2000) 191
12. M.T. PHAM, W. MATZ, H. REUTHER, E. RICHTER, G. STEINER and S. OSWALD, *J. Biomed. Mater. Res.* **59** (2002) 254
13. J. BARRALET, S. BEST and W. BONFIELD, *J. Biomed. Mater. Res.* **41** (1998) 79
14. I.R. GIBSON and W. BONFIELD, *J. Biomed. Mater. Res.* **59** (2002) 697
15. J.E. BARRALET, S. ALDRED, A. J. WRIGHT and A. G. A. COOMBES, *J. Biomed. Mater. Res.* **60** (2002) 360
16. S. D. CONZONE, R. F. BROWN, D. E. DAY and G. J. EHRHARDT, *J. Biomed. Mater. Res.* **60** (2002) 260
17. D. E. Clark, C. G. Pantano and L. L. Hench, *Corrosion of Glass*, Books for Industry and The Glass Industry (1979)
18. A. YASUKAWA, T. MATSUURA, M. NAKAJIMA, K. KANDORI and T. ISHIKAWA, *Mater. Res. Bull.* **34** (1999) 589
19. Lee et al., US patent 6,117,456, September 12, 2000
20. P. W. BROWN and R. I. MARTIN, *J. Phys. Chem. B.* **103** (1999) 1671
21. B. J. MEENAN, A. BOYD, E. LOVE and M. AKAY, *Key Eng. Mater.* **192–195** (2001) 15
22. Z.H. CHENG, A. YASUKAWA, K. KANDORI and T. ISHIKAWA, *Langmuir* **14** (1998) 6681
23. D. J. GREENFIELD and E. D. EANES, *Calcif. Tissue Res.* **9** (1972) 152