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Effect of preparation conditions on the properties of bioactive glasses for testing SBF

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Abstract A simulated body fluid (SBF) with ion concentrations similar to body fluid, proposed by Kokubo et al., is widely used to evaluate bone-bonding potential through the formation of an apatite layer. To be confident of the evaluation of the potential for the apatite formation in SBF, standard substrates are required. Although Na_2O–CaO–SiO_2 glasses have been focused upon as candidate standard substrates, it has not been clarified whether the preparation conditions of the glasses affect their apatite formation potential in SBF. In this study, Na_2O–CaO–SiO_2 glasses were prepared by a conventional melting–quenching method with different melting periods and annealing processes to examine their properties, including apatite formation in SBF. The Na_2O–CaO–SiO_2 glasses show reproducible apatite-forming ability when prepared using moderate melting and annealing processes, and can be useful substrates to test the reproducibility of SBF.

1 Introduction

Generally, artificial materials implanted into bone defects are encapsulated by fibrous tissue and thus are isolated from the body. This is a normal response of the body. However, so-called bioactive ceramics, including bioactive glasses in the system Na_2O–CaO–SiO_2–P_2O_5 [1, 2], sintered hydroxyapatite (HA) ceramics [3], glass–ceramic A–W [4] and so on, directly bond to living bone. Bioactive ceramics show their biological affinity to living bone through the formation of a bone-like apatite layer after implantation in a bony defect [5, 6]. The potential for formation of this apatite layer on the implanted substrate in the body environment is essential for the direct bonding to living bone. To estimate the potential for apatite layer formation on a ceramic material in in vitro testing, Kokubo and his colleagues developed a simulated body fluid similar with regard to inorganic ions to human blood plasma [7–10]. This simulated body fluid proposed by Kokubo et al. is often abbreviated as SBF. Materials that form apatite in SBF are expected to form apatite in the body and bond to living bone; therefore, SBF has been widely used to estimate the in vivo bone bioactivity of various types of materials.

SBF is a solution with inorganic ion concentrations similar to those of human blood plasma, but which does not contain any cell or protein. The ion concentrations of SBF and human blood plasma are given in Table 1. The pH of SBF is typically adjusted to 7.25–7.40 at 36.5°C. The potential for formation of an apatite layer in SBF relates well with the bone-bonding ability of the material in bony defects. Fujibayashi et al. [11] implanted irregular and angular particles 100–300 μm in size of glasses with different compositions from the Na_2O–CaO–SiO_2 system of glasses into holes in rabbit tibiae. The depth of bone growth from the periphery to the interior of the holes at 3 and 6 weeks after implantation increased with the increasing apatite-forming ability of the glasses, as estimated in SBF. These results support that some glasses in the Na_2O–CaO–SiO_2 system show bone-bonding ability, and that the

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Table 1 Ion concentrations and pH of simulated body fluid and human blood plasma

Ion	Concentration (mM)	
	Simulated fluid	Blood plasma
Na^+	142.0	142.0
K^+	5.0	5.0
Mg^{2+}	1.5	1.5
Ca^{2+}	2.5	2.5
Cl^-	147.8	103.0
HCO_3^-	4.2	4.2
HPO_4^{2-}	1.0	1.0
SO_4^{2-}	0.5	0.5
pH	7.40	7.20–7.40

mM = mol m^{-3}

degree of apatite formation ability in SBF does relate to the bone-bonding potential of the examined materials in vivo. As for the reproducibility of the SBF in the evaluation of the apatite formation, Takadama et al. [12] conducted round-robin tests of SBF and hydroxyapatite formation on Na_2O–CaO–SiO_2 glasses. They outlined a detailed procedure for the preparation of SBF so as to achieve high reproducibility and evaluated Na_2O–CaO–SiO_2 glasses as standard substrates because the composition of glasses in this system can be continuously varied. However, they did not describe the details of the procedures used to prepare the Na_2O–CaO–SiO_2 glasses used for evaluation. The properties of these glasses are generally dependent on preparation conditions such as melting period, annealing temperature and annealing period, which have effects on the thermal properties of the obtained glasses and hence apatite-forming ability in SBF. Therefore, to establish a well-characterized standard substrate for SBF evaluation, the effects of the preparation conditions on the properties of these glasses should be clarified. If the reproducibility of the apatite-forming ability of the Na_2O–CaO–SiO_2 glasses can be shown, this shows that the Na_2O–CaO–SiO_2 glasses are suitable as standard substrates for checking SBF. In this study, we prepared glasses in the Na_2O–CaO–SiO_2 system by conventional melt–quenching techniques and monitored thermal histories so as to be confident of the standard substrates. In addition to apatite formation after exposure to SBF, in order to examine the homogeneity of and differences in the glasses prepared with different thermal histories, their thermal behaviors were characterized by differential thermal analysis (DTA). The effects of storage conditions of the specimens were also examined as the surfaces of glasses often react with surrounding water in the atmosphere.

2 Materials and methods

2.1 Preparation of glasses

Glasses with three different starting compositions—$25Na_2O\cdot 25CaO\cdot 50SiO_2$ (mol%) (50S) $22.5Na_2O\cdot 22.5CaO\cdot 55SiO_2$ (mol%) (55S), and $17.5Na_2O\cdot 17.5CaO\cdot 65SiO_2$ (mol%) (65S)—were selected for examination according to the report by Fujibayashi et al. [11]. These glasses were prepared by a conventional melting–quenching method under differing conditions, as given in Table 2. Extra pure sodium carbonate (Na_2CO_3, Nacalai Tesque Inc., Kyoto, Japan), calcium carbonate ($CaCO_3$, Nacalai Tesque Inc.,

Table 2 Conditions for preparation of the glasses

Notation	Composition	Melting		Annealing	
		Temperature (°C)	Period (h)	Temperature (°C)	Period (h)
50S_NA	50S	1450	2	–	–
50S_540_0.5h_M_1h_A	50S	1450	0.5	540	1
50S_540_2h_M_1h_A	50S	1450	2	540	1
50S_540_2h_M_2h_A	50S	1450	2	540	2
50S_540_2h_M_8h_A	50S	1450	2	540	8
50S_540_8h_M_1h_A	50S	1450	8	540	1
50S_560_2h_M_1h_A	50S	1450	2	560	1
50S_580_2h_M_1h_A	50S	1450	2	580	1
55S_NA	55S	1450	2	–	–
55S_580_2h_M_1h_A	55S	1450	2	580	1
55S_580_2h_M_8h_A	55S	1450	2	580	8
65S_NA	65S	1450	2	–	–
65S_590_2h_M_1h_A	65S	1450	2	590	1
65S_590_2h_M_8h_A	65S	1450	2	590	8

–: not conducted

Japan) and silicon oxide (SiO_2, Nacalai Tesque Inc., Japan) were mixed well using an automatic mortar. The mixture was put into a platinum crucible without a cover and placed into a $MoSi_2$ electronic furnace (SB-2025D, Motoyama Co., Ltd., Japan) preheated to 1450°C. After the defined period at 1450°C, the melt was poured onto a stainless plate and pressed with a second stainless steel plate to quench. The obtained glass plates were put into another electric furnace (KDF-S70G, Denken Co., Ltd., Japan) and kept at the defined annealing temperature as given in Table 2. After the defined period, the power to the furnace was turned off and the glass plate was cooled in the furnace.

2.2 Examination of thermal properties

In order to determine the glass transition temperature, glasses 50S_NA and 55S_NA were pulverized and analyzed by differential thermal analysis (DTA, TG-DTA2020S, MAC Science Co., Ltd., Japan) at a heating rate of 5°C min^{-1}. For glass 65S_NA, the pulverized glass was put into a platinum pan and melted again at 1450°C for 10 min, quenched and analyzed in order to enhance the DTA analysis signal. The crystalline phase of the samples after heating was examined by X-ray diffraction (XRD, RINT2200V/PC-LR, Rigaku Co., Japan).

To analyze the crystallization behaviors of 50S and 55S glasses, these glasses were analyzed by DTA using specimens of about 10 mg and greater than 1.0 mm in diameter. Specimens were put into a platinum pan and heated at various heating rates. The crystallization temperature was determined from the DTA curve. The modified Kissinger plot given in Eq. 1 was adopted to determine the activation energy for the crystal growth of the glass [13].

$$\ln\left(\frac{\phi^n}{T_p^2}\right) = -\frac{mE}{RT_p} + \text{const.} \tag{1}$$

where ϕ is the heating rate (K/min), T_p the peak temperature of crystallization (K), E the activation energy for the crystal growth, R the gas constant, n and m are the parameters of crystallization mechanism.

It has been reported that bulk nucleation is dominant when the glass specimen size is larger than 0.5 mm. Therefore, the values of $m = 3$ and $n = 4$, which are those for bulk nucleation [14], were used in this equation.

2.3 Soaking in SBF

Specimens 10 mm × 10 mm × 2 mm were cut from the glass plates and polished with #400 abrasive paper. In order to examine the apatite-forming ability of the glasses, the specimens were soaked in 20 ml of SBF, at ion concentrations as given in Table 1, at pH 7.40 and 36.5°C. The surface structures of the glasses were examined by thin-film X-ray diffraction (TF-XRD, RINT2200V/PC-LR, Rigaku Co., Japan) before and after soaking in SBF.

2.4 Examination of storage condition

The effects on the glasses of the storage conditions were examined. Specimens 10 mm × 10 mm × 2 mm were cut from the glass plates and polished with #400 abrasive paper. The specimens were kept in a polypropylene container at high humidity (more than 90%), atmospheric humidity (about 30%) or low humidity (about 10%) at 36.5°C. High and low humidity conditions were maintained in the presence of water or silica gel, respectively.

3 Results

3.1 Thermal properties

Figure 1 shows the DTA curves of 50S_NA, 55S_NA and 65S_NA at a heating rate of 5°C/min. The glass transition and crystallization temperatures were determined from the DTA curves. The glass transition temperature of the glasses increased with increasing SiO_2 content. The annealing temperature was decided from the glass transition temperatures.

The powder XRD patterns of the as-prepared 50S_NA, 55S_NA and 65S_NA glasses are shown in Fig. 2. All of the glasses in this study gave only halo patterns as as-prepared glasses without annealing, which indicates that these glasses were amorphous. In order to confirm the crystallization after heating above the crystallization temperatures expected by DTA curves, 50S_NA, 55S_NA and 65S_NA glasses after heating at 750, 850 and 950°C were examined by powder XRD. Figure 3 shows the powder XRD patterns of the 50S_NA, 55S_NA and 65S_NA glasses after heating at 750, 850 and 950°C, respectively. The crystalline phases that appeared after the heat treatments were dependent only on the compositions of the glasses and were independent of the preparation conditions.

The peak temperatures of crystallization of the glasses determined from DTA analysis at different heating rates are summarized in Table 3. From these results, modified Kissinger plots were constructed and the activation energies for the crystal growth of the glasses were determined. The activation energy values are shown in Fig. 4. The activation energies of the glasses depended on the melting and annealing conditions. For the 50S glass (50S_540_Ah$_M$_1h$_A$), the activation energy for crystal growth became constant when the melting period was 2 h or more, and was low when the melting period was 0.5 h. The annealing temperatures did not have a significant effect on the

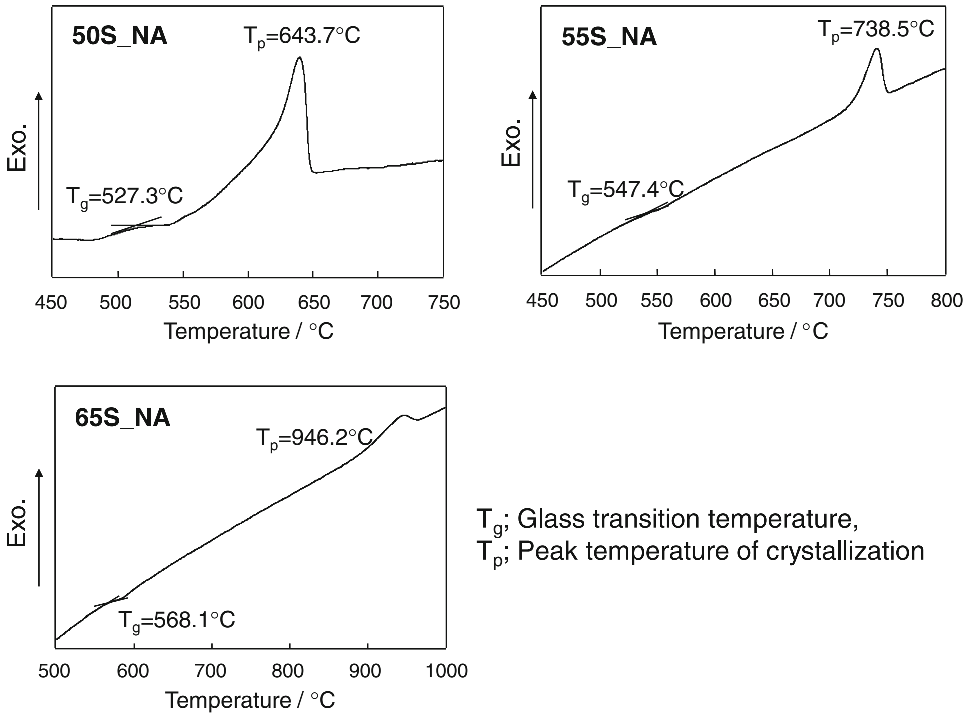

Fig. 1 DTA curves of 50S_NA, 55S_NA and 65S_NA heated at a rate of 5°C min^{-1}

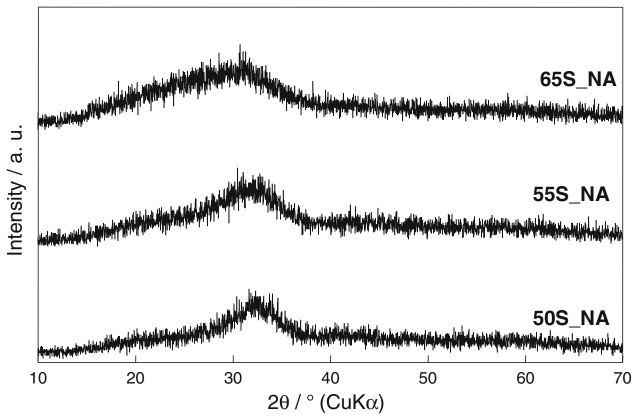

Fig. 2 Powder XRD patterns of 50S_NA, 55S_NA and 65S_NA

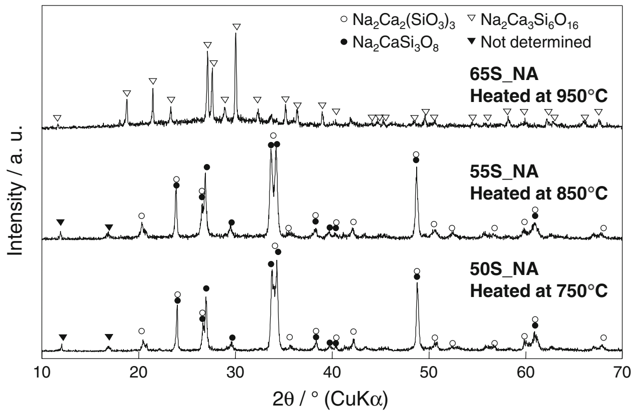

Fig. 3 Powder XRD patterns of 50S_NA, 55S_NA and 65S_NA heated at a rate of 5°C min^{-1} up to 750, 850 and 950°C, respectively

Table 3 Peak temperature of crystallization of Na_2O–CaO–SiO_2 glasses

Sample	Peak temperature of crystallization (K) Heating rate (K min^{-1})				
	1	2	5	10	20
50S_NA	883.4	896.5	917.2	930.8	955.5
50S_540_0.5h_M_1h_A	885.8	899.8	915.5	934.1	952.3
50S_540_2h_M_1h_A	884.8	894.4	909.4	922.8	938.1
50S_540_2h_M_2h_A	877.1	890.0	901.3	915.9	927.8
50S_540_2h_M_8h_A	873.7	882.0	893.9	909.9	922.6
50S_540_8h_M_1h_A	879.9	894.4	908.5	917.8	935.9
50S_560_2h_M_1h_A	880.4	890.6	906.9	920.6	932.4
50S_580_2h_M_1h_A	876.4	887.4	902.9	913.2	925.6
55S_NA	963.1	984.7	1012	1040	1075
55S_580_2h_M_1h_A	960.7	976.6	1000	1021	1041
55S_580_2h_M_8h_A	955.8	970.3	989.1	1007	1025

activation energy of 50S glasses (50S_B_2h_M_1h_A) in the examined temperature range from 540 to 580°C. For both 50S glasses (50S_540_2h_M_Ch_A) and 55S glasses (55S_580_2h_M_Ch_A), the activation energies were increased by the annealing process and the values became constant when the annealing period was 1 h or more.

3.2 Apatite-forming ability

Figure 5 shows the TF-XRD patterns of the surfaces of the 50S glasses with different melting periods (50S_540_

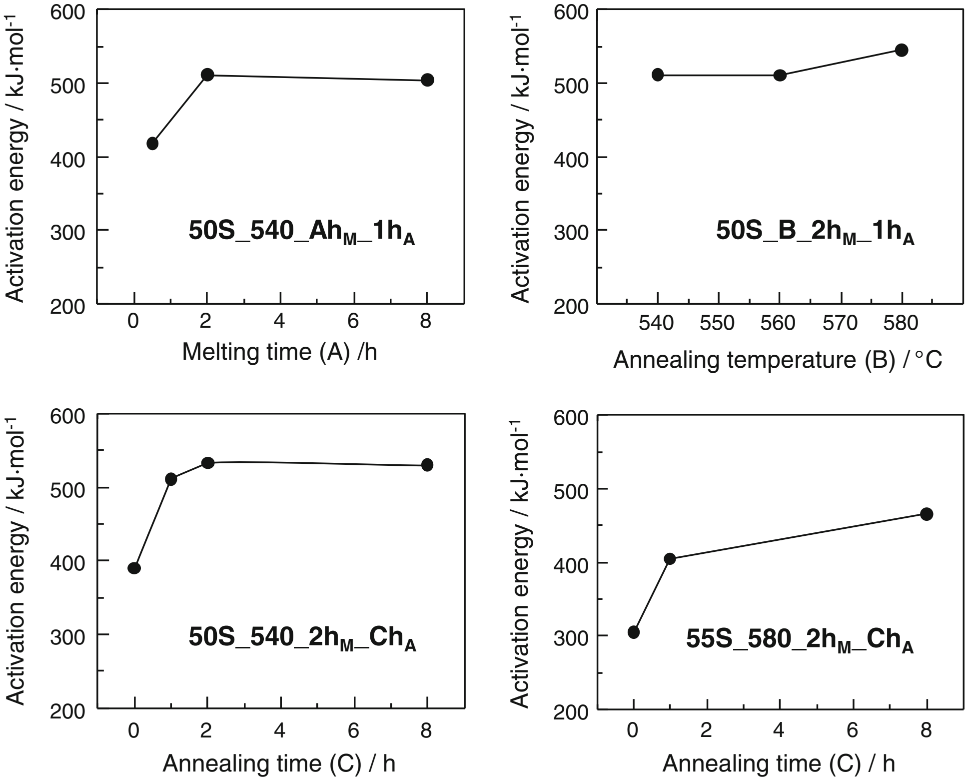

Fig. 4 Activation energy of crystal growth of Na_2O–CaO–SiO_2 glasses

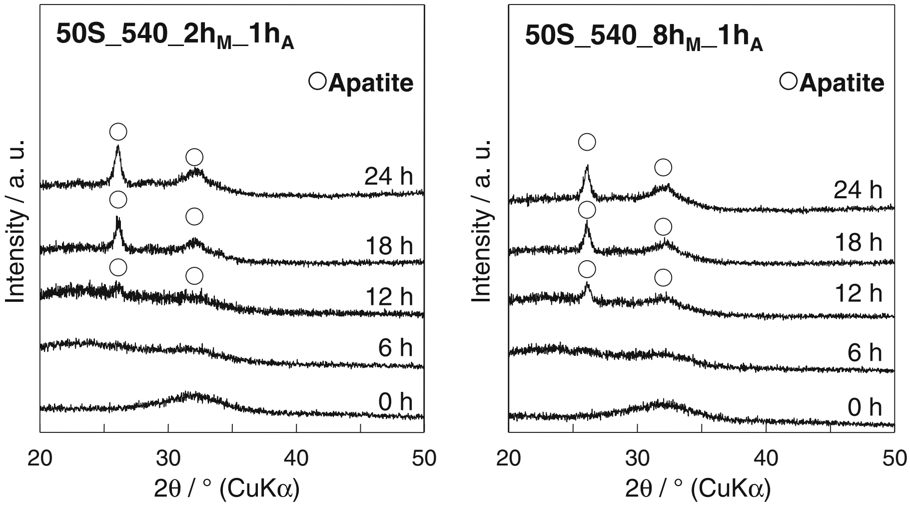

Fig. 5 TF-XRD patterns of the surfaces of 50S glasses with different melting periods after soaking in SBF for various periods. (0 h; before soaking)

2h$_M$_1h$_A$ and 50S_540_8h$_M$_1h$_A$) before and after soaking in SBF for various periods. Figures 6, 7 and 8 show the TF-XRD patterns of the surfaces of the 50S (50S_540_2h$_M$_1h$_A$, 50S_540_2h$_M$_2h$_A$ and 50S_540_2h$_M$_8h$_A$), 55S (55S_580_2h$_M$_1h$_A$ and 55S_580_2h$_M$_8h$_A$) and 65S glasses (65S_590_2h$_M$_1h$_A$ and 65S_590_2h$_M$_8h$_A$) with different annealing periods before and after soaking in SBF for various periods. The results of apatite formation as determined by TF-XRD are summarized in Table 4. The induction periods for apatite formation were 12, 24 and 72 h for 50S, 55S and 65S, respectively. The induction periods were dependent only on the glass composition, not on the melting and annealing conditions.

3.3 Storage condition

Figure 9 shows the TF-XRD patterns of the 50S_540_2h$_M$_1h$_A$, 55S_580_2h$_M$_1h$_A$ and 65S_590_2h$_M$_1h$_A$ glasses after storage under various conditions for 24 h. When the glasses were kept under high humidity, calcite ($CaCO_3$) peaks were detected on the surfaces of all glasses. Other undetermined peaks were also detected. The peak

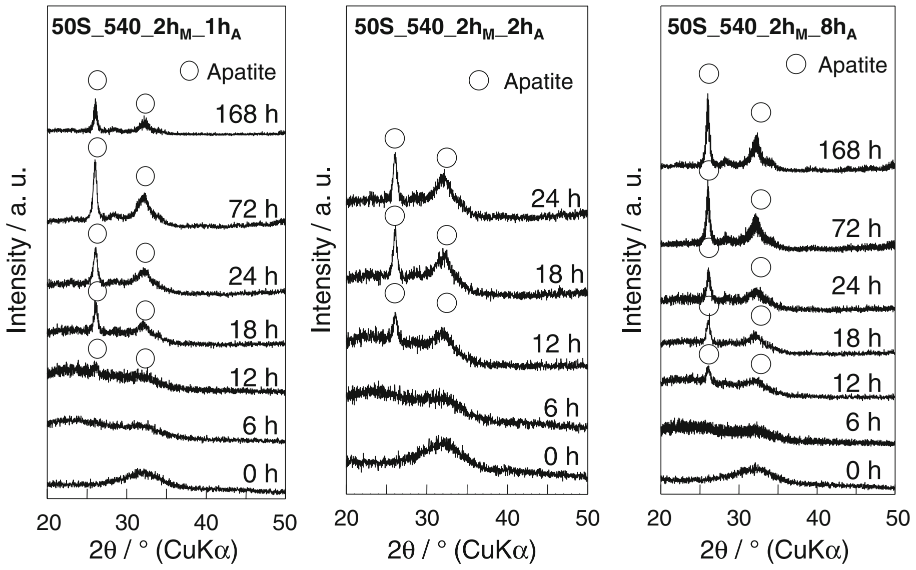

Fig. 6 TF-XRD patterns of the surfaces of 50S glasses with different annealing periods after soaking in SBF for various periods. (0 h; before soaking)

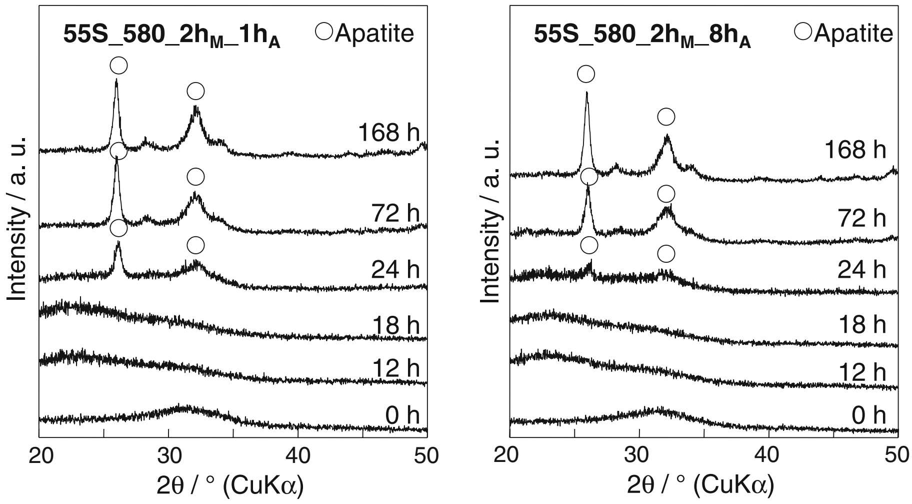

Fig. 7 TF-XRD patterns of the surfaces of 55S glasses with different annealing periods after soaking in SBF for various periods (0 h; before soaking)

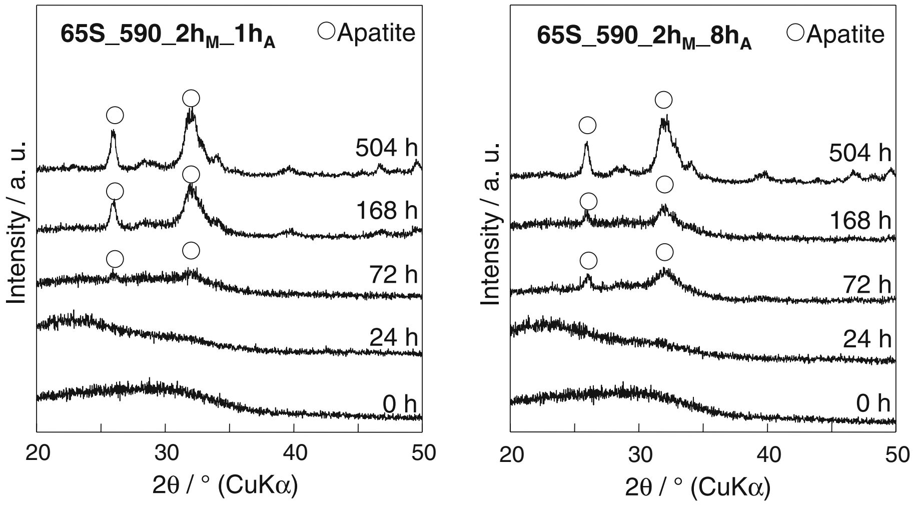

Fig. 8 TF-XRD patterns of the surfaces of 65S glasses with different annealing periods after soaking in SBF for various periods (0 h; before soaking)

Table 4 Apatite formation of Na_2O–CaO–SiO_2 glasses in SBF

Sample	Apatite formation (h)						
	6	12	18	24	72	168	504
50S_540_2h$_M$_1h$_A$	–	○	○	○	○	○	*
50S_540_2h$_M$_2h$_A$	–	○	○	○	*	*	*
50S_540_2h$_M$_8h$_A$	–	○	○	○	○	○	*
50S_540_8h$_M$_1h$_A$	–	○	○	○	*	*	*
55S_580_2h$_M$_1h$_A$	*	–	–	○	○	○	*
55S_580_2h$_M$_8h$_A$	*	–	–	○	○	○	*
65S_590_2h$_M$_1h$_A$	*	*	*	–	○	○	○
65S_590_2h$_M$_8h$_A$	*	*	*	–	○	○	○

○: Apatite formation, –: no apatite formation, *: not examined

intensities decreased with decreasing SiO_2 content in the glasses. These results indicate that the glasses were reacting with CO_2 in the high humidity conditions.

4 Discussion

The crystallization behaviors of the glasses depended on the melting and annealing conditions. When the melting period for the 50S glass was 0.5 h, the activation energy was smaller than that for 2 and 8 h melting periods. This might be due to the inhomogeneity of the glass. As the raw materials of the glasses used in this study were powders, the initial melting stage is a solid reaction and it takes period to form a homogeneous melt. These results indicate that a short melting period should not be assumed adequate to obtain homogeneous glass, and inhomogeneity may affect the apatite-forming ability. Therefore, melting periods of at least 2 h are recommended.

The activation energy for crystal growth was increased by about 100 kJ/mol by the annealing. This increase would be due to the removal of residual strain during the annealing process as it is thought that residual strain in the glasses decreases the activation energy for crystal growth. The annealing process is essential for glass preparation so as to remove residual stress induced during the quenching process, as residual stress induces destruction of the glass. Although glass made without annealing could not be cut or polished because the residual stress led to easy induction of fractures, glass that received annealing treatment for 1 h could be processed to give specimens of defined size and surfaces. Moreover, for both 50S and 55S glasses, the activation energies for the glasses that received annealing treatment for 1 h were almost the same as those annealed for 8 h. These results indicate that 1 h of annealing at a moderate temperature is enough to remove residual stress.

The induction periods for apatite formation were 12, 24 and 72 h for 50S, 55S and 65S glasses, respectively. Takadama et al. [12] reported induction periods of apatite formation on 50S and 55S glasses, and their results were consistent with the present results. The apatite-forming ability of the glasses was dependent on their composition, regardless of the annealing conditions used in this study. Fujibayashi et al. [11] reported that Na_2O–CaO–SiO_2 glasses showed bone-bonding ability, and their bone-bonding ability was related to the degree of apatite formation ability in SBF. These Na_2O–CaO–SiO_2 glasses can act not only as indicators of the bone-bonding ability of materials but also as materials that can be used to test the reproducibility of SBF.

As we were not able to examine the apatite-forming ability of the glasses without the annealing due to their low processability, related to the presence of residual stress, we

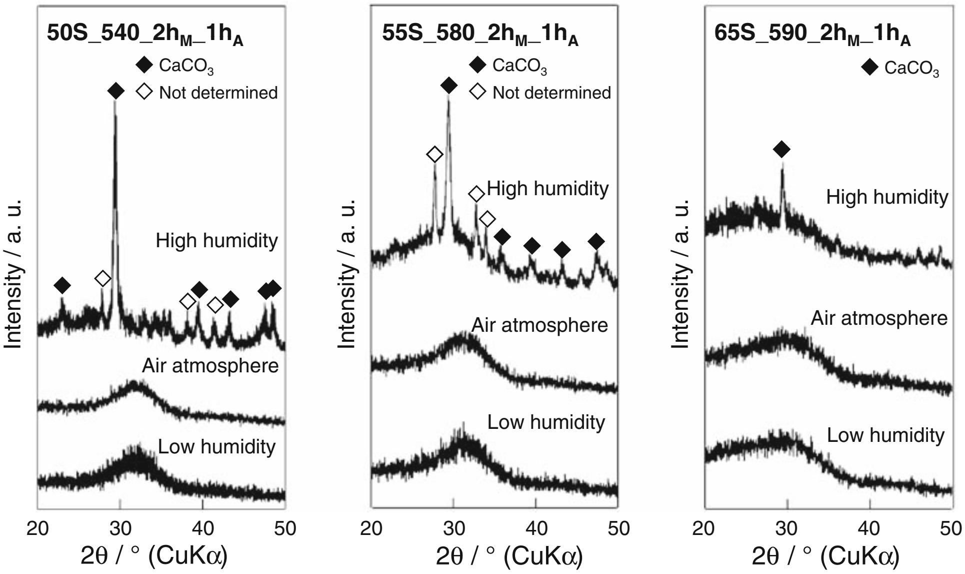

Fig. 9 TF-XRD patterns of 50S_540_2h$_M$_1h$_A$, 55S_580_2h$_M$_1h$_A$ and 65S_590_2h$_M$_1h$_A$ kept under various conditions for 24 h

were not able to test the possibility that glasses made without annealing show different apatite-forming ability from those that were annealed. However, the purpose of this study was to examine the effects of the preparation conditions of standard substrates, and standard substrates must be processable so as to control their size and surfaces. Therefore, unannealed glasses cannot be used as standard substrates.

This study also showed that storage conditions are also important in the preparation of standard glasses. When the glasses were kept under high humidity conditions for only 24 h, calcite had precipitated on all the glasses. The amount of precipitate increased with decreasing SiO_2 content in the glass. The glasses with high apatite-forming ability easily reacted with CO_2 in the atmosphere in the presence of water. The chemical durability of the silicate glasses generally decreased with increasing network modifier Na_2O and CaO, and decreasing network former, SiO_2. Even under normal atmospheric conditions, the precipitation of calcite is assumed to occur when the storage period becomes long. These glasses cannot be used as standard substrates after the precipitation of calcite because their reaction in SBF would be changed. Therefore, the glasses must be kept under low humidity conditions to prevent this surface reaction.

5 Summary

Preparation conditions, such as the melting and annealing periods, had effects on the thermal properties of the obtained glasses. However, the apatite-forming ability of the glasses in SBF depended only on their composition, and decreased with increasing SiO_2 content. These results indicate that Na_2O–CaO–SiO_2 glasses show reproducible apatite-forming ability when they are prepared under moderate processes, and can be useful substrates to test the reproducibility of SBF. This study also revealed that storage conditions are very important and that humidity must be avoided.

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