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Preparation of bioactive spherical particles in the CaO–SiO_2 system through sol–gel processing under coexistence of poly(ethylene glycol)

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Abstract

Glasses in CaO–SiO_2 system provide bioactive materials that achieve direct bonding to living bone. In the development of bioactive organic–inorganic composites, CaO–SiO_2 particles with controlled size and morphology are expected to be useful as inorganic fillers. Our previous study showed that sol–gel processing of tetraethoxysilane (TEOS) and calcium nitrate tetrahydrate (CNT, $Ca(NO_3)_2\cdot 4H_2O$) in the presence of poly(ethylene glycol) (PEG) can produce bioactive CaO–SiO_2 spherical particles. However, the detailed conditions for preparing the particles with controlled morphology have not been revealed. In the present study, we investigated relationships between starting composition, specifically the weight ratios of TEOS, CNT and PEG, and morphology of the obtained gels. Aggregation of spherical particles was obtained when PEG was added, and the size of particles decreased from micro- to nano-sized with increasing amounts of PEG. Within 1 day of soaking in a simulated body fluid (SBF) a bone-like apatite layer was formed on the surface of all gels starting from the molar ratio of CNT/TEOS = 3/7 (which is assumed to be $30CaO\cdot 70SiO_2$), followed by heat treatment at 600 °C.

Keywords: Sol–gel processes; Microstructure-final; CaO–SiO_2; Biomedical application

1. Introduction

Since the development of Bioglass®,[1] some types of bioactive ceramics including glasses[1,2] and glass–ceramics[3,4] have been developed for biomedical applications such as artificial bone substitutes. However, the application of bioactive ceramics is still limited because bioactive ceramics are harder and more brittle than general bone. One of the most important uses of bioactive ceramics is as a filler for bioactive composites, because the mechanical properties of natural bone result from the composite structure of bone matrix.[5]

Bioactive ceramics bond directly to living bone, i.e., show osteoconduction, through a bone-like apatite layer formed on their surfaces after implantation in bony defects. Ohtsuki et al.[6] reported that glasses in the CaO–SiO_2 binary system were important in formation of the apatite layer in the body environment. Therefore, CaO–SiO_2 particles with controlled size and morphology are expected to be useful as inorganic fillers in novel bioactive composites for repairing bone. Martínez et al.[7] reported that CaO–SiO_2 glasses prepared by sol–gel methods from tetraethoxysilane (TEOS) and calcium nitrate tetrahydrate (CNT, $Ca(NO_3)_2\cdot 4H_2O$) formed the bone-like apatite layer in simulated body fluid (SBF) proposed by Kokubo and coworkers.[8,9] On the other hand, the size and morphology of silica gels prepared by hydrolysis of TEOS in the presence of water-soluble polymer were identified as interconnected porous body or spherical particles, dependent on the starting compositions, by Nakanishi and Soga.[10] Based on these findings, our previous study showed that sol–gel processing of TEOS and CNT in the presence of poly(ethylene glycol) (PEG) can produce bioactive CaO–SiO_2 particles.[11] However, the relationships between the starting compositions and size and morphology of the obtained particles have not been investigated systematically in the TEOS–CNT–PEG system. In this study,

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we investigated the compositional dependence of morphology of CaO–SiO_2 particles prepared through sol–gel processing in the TEOS–CNT–PEG system. Potential for bone-like apatite formation on the prepared particles after exposure to SBF was also examined to estimate their osteoconductivity.

2. Experimental

2.1. Preparation of gels

Tetraethoxysilane (TEOS, $Si(C_2H_5O)_4$, Nacalai Tesque Inc., Kyoto, Japan), calcium nitrate tetrahydrate (CNT, $Ca(NO_3)_2{\cdot}4H_2O$, Nacalai Tesque Inc., Kyoto, Japan), and poly(ethylene glycol) (PEG, Aldrich, Milwaukee, USA) with molecular weight of 10,000 were used as starting compounds, and concentrated nitric acid (60% HNO_3, Nacalai Tesque Inc., Kyoto, Japan) was used as a catalyst for hydrolysis of TEOS.

The starting compositions for preparation of the gels are summarized in Table 1. We focused on two series of compositions: one contained different ratios of CNT/TEOS and a constant molar ratio of PEG/TEOS = 0.002, denoted as P2(*x*C*y*S); the other contained different amounts of PEG with a constant molar ratio of CNT/TEOS = 3/7 (0.43), denoted as P*z*(3C7S). These gels were prepared by the following procedure. PEG and CNT were first dissolved in distilled water, and then the nitric acid was added. TEOS was then added to the aqueous solution under vigorous stirring. After stirring for 20 min, the resulting solution was transferred into a square polystyrene case with its top tightly sealed and then kept at 40 °C for 1 day for gelation and ageing. The obtained wet gel was immersed in distilled water for 3 h to remove PEG. The distilled water was renewed every 1 h. The obtained wet gel was dried at 40 °C for 7 days and then heated at 600 °C for 2 h.

2.2. Characterization

The gelation time was determined simply by tilting the container when the fluidity of the solution was lost or when the solution became turbid. Thermogravimetric and differential thermal analysis (TG–DTA: TG–DTA2020S, MAC Science Co., Ltd., Yokohama, Japan) were carried out in air after drying for 7 days, using alumina (α-Al_2O_3) as the standard material. In the TG–DTA analysis, samples of 9.0–10.0 mg were heated at 5 °C/min up to 600 °C. To determine the calcium contents of the dried gels, elemental characterization was carried out by energy dispersive spectroscopy (EDS: EX-54140, JEOL Ltd., Japan), after the samples were fired at 600 °C for 2 h. The prepared gels were characterized by Fourier transform infrared spectroscopy (FT-IR: Spectrum GX, PerkinElmer Ltd., England). For FT-IR measurement, the prepared gels were pulverized and mixed homogeneously with powdered KBr at a sample: KBr mass ratio of 1:100. The morphology of the prepared gels was observed under a scanning electron microscope (FE-SEM: S-4800N, Hitachi Ltd., Japan, and SEM: JSM5600, JEOL Ltd., Japan). Specimens were coated with gold prior to FE-SEM and SEM observation.

Table 1
Starting compositions (molar ratio) of the solutions for preparation of the gels

Code	Composition (molar ratio)				
	PEG	CNT	TEOS	H_2O	HNO_3
P2(*x*C*y*S)	0.002	0.00–0.43	1	14.7	0.25
P*z*(3C7S)	0–0.006	0.43	1	14.7	0.25

PEG: poly(ethylene glycol) (molecular weight = 10,000); CNT: calcium nitrate tetrahydrate; TEOS: tetraethoxysilane. *x*: 10 × (CNT/CNT + TEOS); *y*: 10 × (TEOS/CNT + TEOS); *z*: 1000 × PEG.

2.3. In vitro test of bone-bonding property

Previous reports revealed that the potential bone-bonding properties of ceramics could be evaluated by apatite formation on the ceramic surface in a SBF (Na^+ 142.0, K^+ 5.0, Mg^{2+} 1.5, Ca^{2+} 2.5, Cl^- 147.8, $HCO_3{}^-$ 4.2, $HPO_4{}^{2-}$ 1.0, $SO_4{}^{2-}$ 0.5 mmol/L(mM)) proposed by Kokubo and coworkers.[8,9] SBF is an acellular solution that has a similar composition of inorganic ions to that of human blood plasma. SBF was prepared with pH 7.25 at 36.5 °C described in previous reports.[8,9] Some of the prepared P*z*(3C7S) gels, which have starting compositions of $30CaO{\cdot}70SiO_2$ in molar ratio, were ground to form a powdered sample and compressed to form pellets with diameter 15 mm and height 2 mm. The specimens were then soaked in 30 mL of SBF. After incubation at 36.5 °C for 1 day, the specimens were taken from the SBF, gently washed with ultrapure water, and dried at room temperature. The surface structures of the specimens were analysed by thin-film X-ray diffraction (TF-XRD: RINT2200V/PC-LR, Rigaku Co., Japan). In the TF-XRD analysis, the surfaces of the specimens were fixed at an angle of 1° against the direction of the incident beam. The surface morphologies of the specimens were observed under FE-SEM.

3. Results

Fig. 1 shows the gelation time of solutions with the starting composition given in Table 1. All of the prepared solutions were transparent and without any precipitation after stirring for 20 min. The gelation time of the solution decreased with increasing ratios of CNT/TEOS when PEG was kept at the constant concentration. When the CNT/TEOS molar ratio was constant at 3/7, the gelation time of the solutions was reduced for the solution containing molar ratios 0.001 and 0.002 of PEG while it increased for the solution containing molar ratios 0.004 and 0.006 of PEG. After gelation, syneresis was observed in all of the gels during the ageing time. The amount of liquid by syneresis seemed to decrease with increasing amounts of PEG in P*z*(3C7S) samples.

Fig. 2 shows the appearance of gels prepared from the P*z*(3C7S) solutions after ageing at 40 °C for 1 day. Crack-free monoliths were obtained after gelation and ageing. The gel prepared from the solution free from PEG (PEG/TEOS = 0.000) was transparent. Gels prepared from the solutions containing PEG were opaque (PEG/TEOS = 0.002) or translucent (PEG/TEOS = 0.004 and 0.006). No serious fracture of the wet gel monoliths was observed on immersion in distilled water and

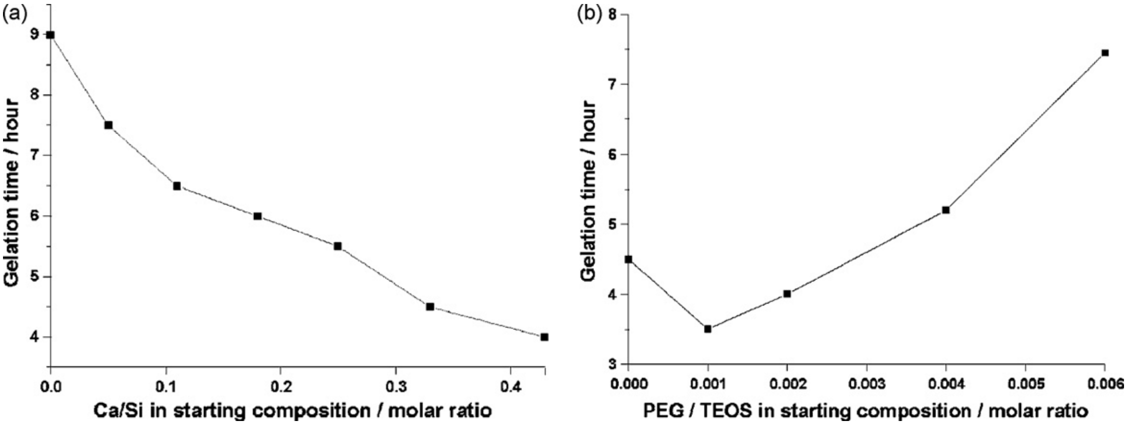

Fig. 1. The gelation time of the examined solutions. (a) P2(*x*C*y*S) samples prepared at various Ca/Si ratios under coexistence with PEG/TEOS at 0.002. (b) P*z*(3C7S) samples prepared at Ca/Si = 3/7 under coexistence with PEG at various ratios of PEG/TEOS.

the transparency of the gels gradually decreased during immersion. All of the gels prepared from P2(*x*C*y*S) solutions were white and opaque.

Fig. 3 shows results of EDS analysis of Ca/Si molar ratios in gels prepared from the P2(*x*C*y*S) solutions, after heat treatment at 600 °C for 2 h. The calcium content incorporated into the gel increased with increasing amounts of CNT in the starting composition. Some calcium salts might remain in the solvent and hence were not incorporated in the gel structure. Furthermore, some of the calcium ions in the gel were removed during the washing process after syneresis. These processes might result in gels having lower calcium contents than the starting compositions.

Fig. 4 shows SEM photographs of the prepared P2(*x*C*y*S) gels after heat treatment at 600 °C for 2 h. Interconnected pores were observed in the gels prepared with starting compositions low in calcium nitrate content (0.00 < Ca/Si < 0.25). Aggregation of spherical particles was observed with starting compositions of Ca/Si at 0.33 and 0.43. The domain size of gels prepared with starting compositions of Ca/Si in the range 0.00–0.43 increased with increasing Ca/Si ratios. This means that the domain size of the polymerized silicate phase increased with increasing calcium content. It is clear that the morphology of the gels was significantly affected by the calcium content of the starting compositions. Fig. 5 shows FE-SEM photographs of the P*z*(3C7S) gels after heat treatment at 600 °C for 2 h. No pores

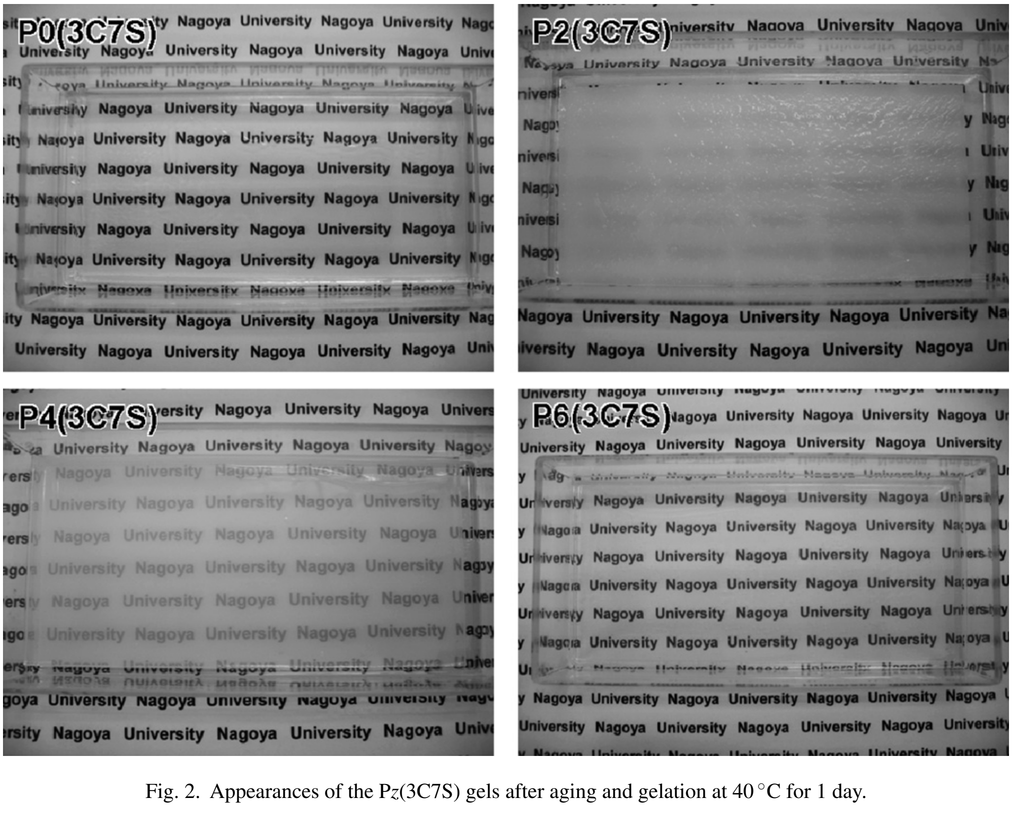

Fig. 2. Appearances of the P*z*(3C7S) gels after aging and gelation at 40 °C for 1 day.

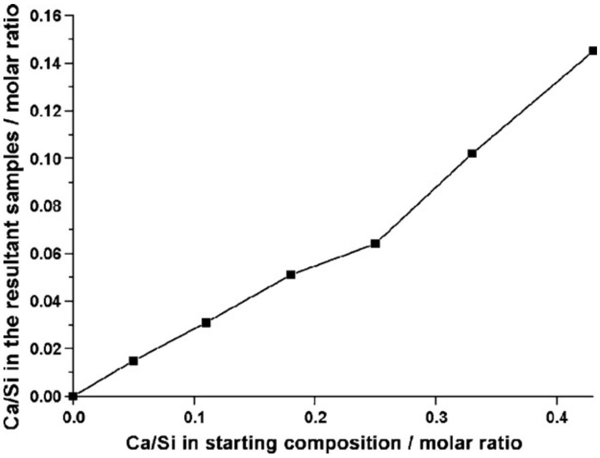

Fig. 3. Ca/Si molar ratios of the P2(*x*C*y*S) samples (after heating at 600 °C for 2 h), which were synthesized from various Ca/Si ratios ranging from 0/10 to 3/7.

were observed on the P0(3C7S) gel that was prepared from the composition free from PEG. Aggregation of spherical particles was observed in the gels prepared from compositions ranging from $P = 0.001$ to $P = 0.006$. Aggregation of nano-sized particles was observed at high magnification (Fig. 5) when the gels were prepared at compositions with $P > 0.004$.

Fig. 6 summarizes the relationship between the morphology of the resultant gels and starting compositions in the TEOS–CNT–PEG system. The higher concentrations of PEG resulted in nano-sized particles of CaO–SiO_2 gel, whereas micro-sized particles or interconnected pores were formed with lower concentrations of PEG. The morphology of the prepared ceramic particles is governed not only by the TEOS/CNT molar ratio, but also by the PEG concentration during the sol–gel process.

Fig. 7 shows FT-IR spectra of the P*z*(3C7S) samples after heating at 600 °C for 2 h. There was no remarkable difference among spectra of the gels prepared with various PEG amounts. The IR peaks at 460 cm^{-1} and 800 cm^{-1} were ascribed to the Si–O–Si bending and stretching modes, respectively. Peaks assigned to silanol groups and/or Si–O–Ca bonds appeared at 950 cm^{-1} while the symmetric stretching vibration of Si–O–Si bonds appeared at 1080 cm^{-1} and vibration of H_2O appeared at 1630 cm^{-1}. No peaks assigned to the ionic nitrate or PEG were observed, indicating complete removal of these components during the heat treatment. To examine the process of removal of the PEG and nitrate components and formation of the samples, two of the P*x*(3C7S) gel samples with starting compositions of $P = 0.000$ and $P = 0.002$ were analysed by TG–DTA (Fig. 8). It can be seen from Fig. 8 that endothermic peaks accompanied by a remarkable decrease in mass are observed around 80 °C in both the samples prepared without PEG (PEG/TEOS = 0.000) and with PEG (PEG/TEOS = 0.002). The losses of mass are attributed to evaporation of alcohols and water from the gels. In the case of the sample prepared with PEG (PEG/TEOS = 0.002) an exothermic peak at around 250 °C, accompanied by a slight decrease in mass, was also observed. This decrease is attributed to the decomposition and removal of PEG and/or nitrate from the sample. Previous reports showed that removal of nitrate appears as an endothermic peak at about 550 °C.[12] The exothermic peak was assigned to the decomposition of PEG.

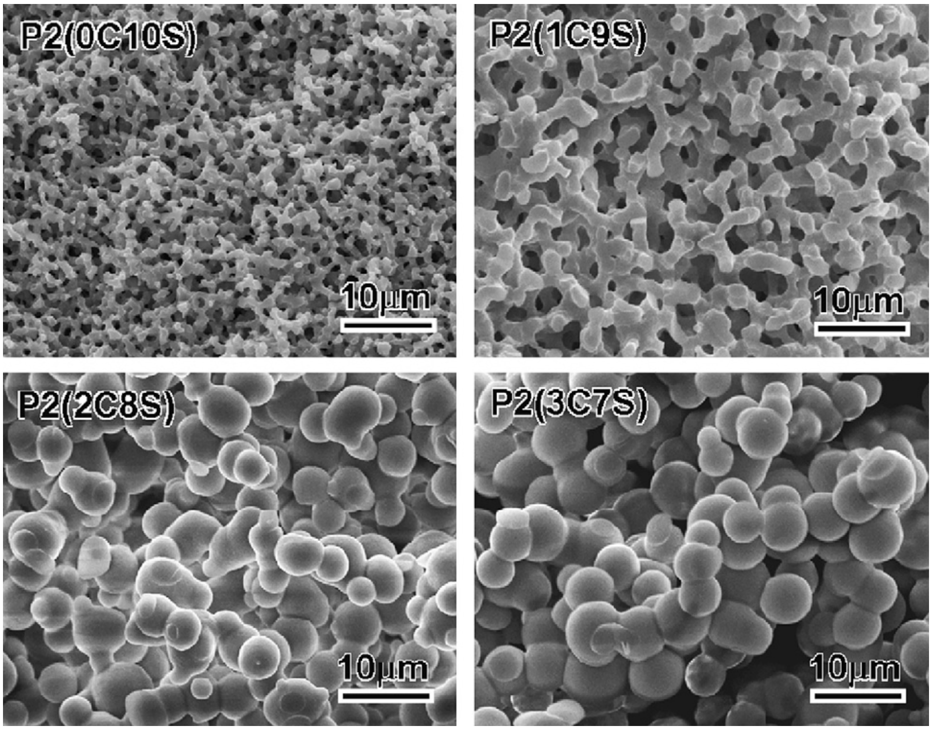

Fig. 4. SEM photographs of the P2(*x*C*y*S) samples heated at 600 °C for 2 h.

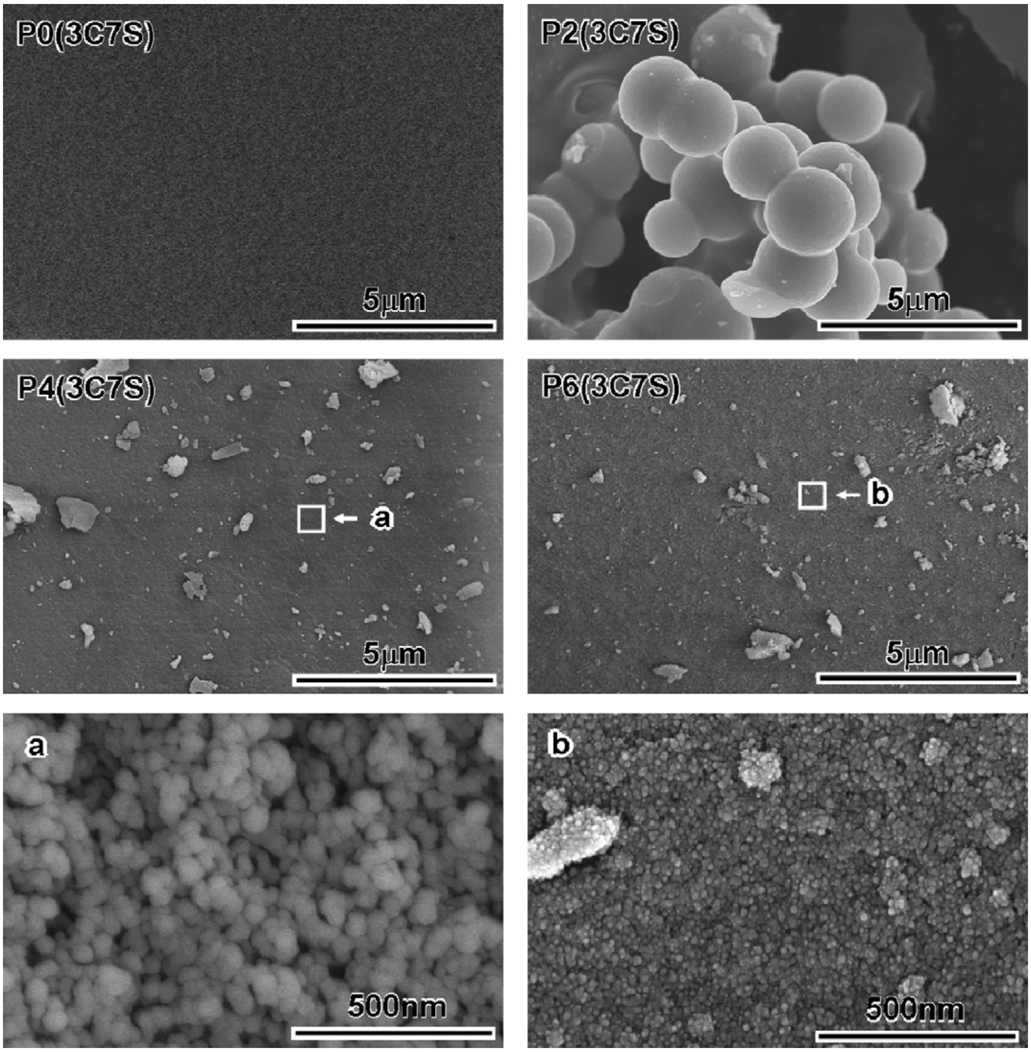

Fig. 5. SEM photographs of the P*z*(3C7S) samples heated at 600 °C for 2 h. (a) and (b) are observed at high magnification of P4(3C7S) and P6(3C7S), respectively.

Fig. 9 shows TF-XRD patterns of the P*z*(3C7S) samples, previously heated at 600 °C, before and after soaking in SBF for 1 day. Samples were typically amorphous before soaking. Peaks around 26° and 32° are assigned to 002 and envelop of 211, 112, and 300 peaks of hydroxyapatite (JSPDS Card # 72-1243). These broad peaks were detected in all examined samples within 1 day after soaking in SBF. Fig. 10 shows FE-SEM photographs of the P*z*(3C7S) samples heated at 600 °C, before and after soaking in SBF for 1 day. All the particles were compressed to a compacted specimen for soaking in SBF. The P2(3C7S) specimen was prepared from microspheres, however, the microspheres were broken by pressing. Deposition of fine particles was observed on all the examined samples irrespective of the amounts of PEG. The morphology of the particles deposited on the specimens after soaking in SBF is similar to that of particles deposited on bioactive glasses and glass–ceramics.[6,13,14] From the results of the TF-XRD and SEM observations, we confirmed that the samples prepared at the composition of P*z*(3C7S) have potential to deposit bone-like apatite on their surfaces after exposure to body fluid, irrespective of the amounts of PEG added during the sol–gel processing.

4. Discussion

It is clear from the results on the formation of gels in this experiment that the rate of gel formation increases with increasing Ca/Si ratios in the starting solution while it is decreased with high amounts of PEG in the solution. The reactions forming a silica phase through sol–gel processing are generally described as follows[15]:

$$\equiv\text{Si–OR} + \text{H}_2\text{O} \rightarrow \equiv\text{Si–OH} + \text{ROH} \quad (1)$$

$$\equiv\text{Si–OH} + \text{HO–Si}\equiv \rightarrow \equiv\text{Si–O–Si} + \text{H}_2\text{O} \quad (2)$$

where R is an alkyl group, C_xH_{2x+1}. The hydrolysis replaces alkoxide groups (OR) with hydroxyl groups (OH) within several minutes of mixing the starting constituents. Addition of $Ca(NO_3)_2$ may therefore accelerate the condensation reactions involving silanol groups, which produce siloxane bonds (Si–O–Si) plus the by-products alcohol (ROH) or water. Calcium ion (Ca^{2+}) acts as a network modifier in calcium silicate

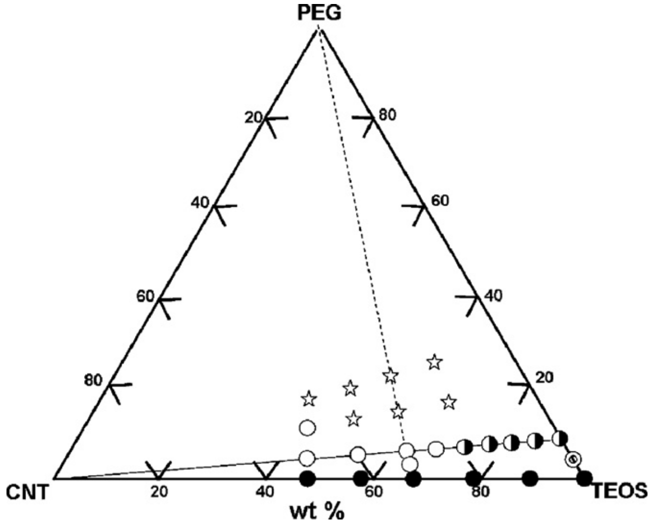

Fig. 6. Relation between the morphology of the resultant gels and starting composition of the solution in the system TEOS(tetraethoxysilane)–CNT(calcium nitrate tetrahydrate)–PEG(polyethylene glycol). (●) non-porous bulk, ◎: isolated macropores, (◐) interconnected pores, (○) micro-particle aggregation, (☆) nano-particle aggregation. Broken line indicates P*z*(3C7S) compositions with varied amounts of PEG, while solid line indicates the P2(*x*C*y*S) compositions with varied amounts of calcium nitrates.

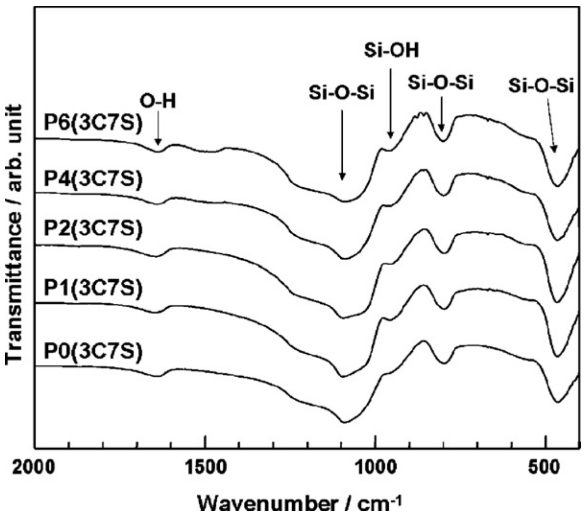

Fig. 7. FT-IR spectra of the P*z*(3C7S) samples after heating at 600 °C for 2 h.

glass. The introduction of calcium ions is expressed[12] by

$$\mathrm{Ca^{2+} + 2[{\equiv}Si\text{–}OH] \rightarrow {\equiv}Si\text{–}O\cdots Ca\cdots O\text{–}Si{\equiv} + 2H^{+}} \qquad (3)$$

One calcium ion will be present for each pair of non-bridging oxygen atoms in the silica network. This reaction may occur more rapidly than the polycondensation of silanol groups given as Eq. (2). On the other hand, the addition of PEG at a PEG/TEOS ratio of 0.002 or more retarded the gelation. This is due to the interfering effect of PEG molecules during polycondensation, because PEG could not react with hydrated silica and calcium but leads to phase separation and the formation of a porous structure or particles during polycondensation.

The amount of calcium incorporated in the resultant gels is dependent on the starting composition. Some part of the calcium added in the solution is incorporated into the gels, as shown in Fig. 3. During polycondensation and ageing, calcium ions are released from the gel. Shrinkage due to syneresis makes supernatant in the case and calcium ions are released from the gel into the supernatant. Furthermore, calcium ions were removed from the gels during leaching of PEG with distilled water.

The morphology of the resultant gel was determined by sol–gel processing as porous samples with interconnected pores, and micrometre- and nanometre-sized particles to nano-sized particles, according to the amounts of PEG in the solution. This trend of morphology in the resultant gels is quite similar to that seen in the binary TEOS–poly(ethylene oxide) system without calcium salts. Nakanishi et al.[16] explained that the effect of the polymer originates in domain formation, which occurs under phase separation, during gelation. According to the research by Nakanishi et al., systems containing at least one kind of water-soluble polymer can be estimated by the thermodynamic treatment known as the Flory–Huggins formulation.[17] The Gibbs free energy change of mixing for binary system can be expressed as

$$\Delta G \propto RT\left[\left(\frac{\phi_1}{P_1}\right)\ln\phi_1 + \left(\frac{\phi_2}{P_2}\right)\ln\phi_2 + \chi_{12}\phi_1\phi_2\right] \qquad (4)$$

Here, ϕ_i and P_i ($i = 1, 2$) denote the volume fraction and the degree of polymerization of each component, respectively, while χ_{12} is the interaction parameter. The first two terms are determined from the volume fraction of components and ΔG

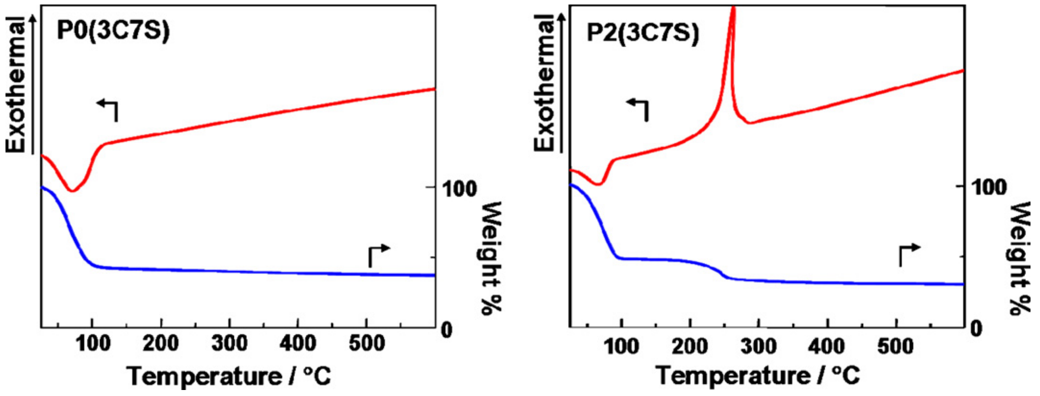

Fig. 8. TG–DTA results of the P*z*(3C7S) gels dried at 40 °C for 7 days.

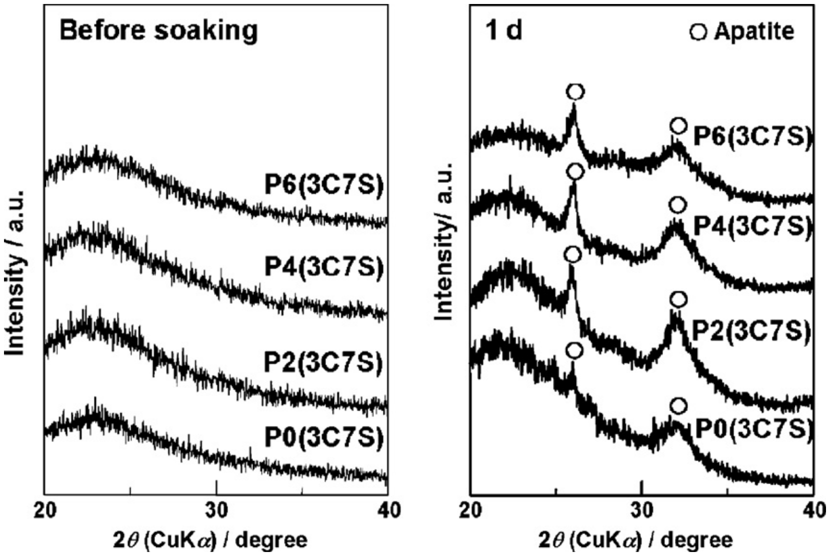

Fig. 9. TF-XRD patterns of the Pz(3C7S) samples prepared with different PEG amounts and heated at 600 °C, before and after soaking in SBF for 1 day.

of a system is changed by the increase or decrease of the last term. When the value of ΔG turns from negative to positive, the driving force of phase separation is generated. Nakanishi et al. explained that the driving force for phase separation is polymerization of silica. Therefore, the volume fractions of polymer and CaO–SiO_2 gel phase determine the size of the domain formed by phase separation. If the volume fractions of the two phases are nearly equal during phase separation, the gel phase is changed to a porous body.[16] In addition, in the present study, calcium ion affects the morphology of the gel. Gelation was accelerated by addition of the calcium salt. Acceleration of the CaO–SiO_2 gel formation reaction may increase the driving force for phase separation. The faster gelation by addition of calcium salt also leads silica gel to fast domain formation. From these consideration, an increase in calcium content in the solution results in precipitation of droplets of CaO–SiO_2 during the gelation process. In solutions with PEG/TEOS of 0.002 the droplets appeared to have a diameter of 5 μm (see Fig. 4). Increasing the PEG concentration in the solution allowed nano-sized droplets to form. When the ratio of PEG/TEOS was more than 0.004, particles of 50 nm diameter were formed (see Fig. 5). Consequently, the formation of non-bridging oxygen by the addition of calcium ion and the domain formation by PEG led to form nanometre-sized particles in the solutions containing large amounts of ion (Ca/Si = 2/8 or higher) in the presence of PEG at concentrations of 0.004 or higher.

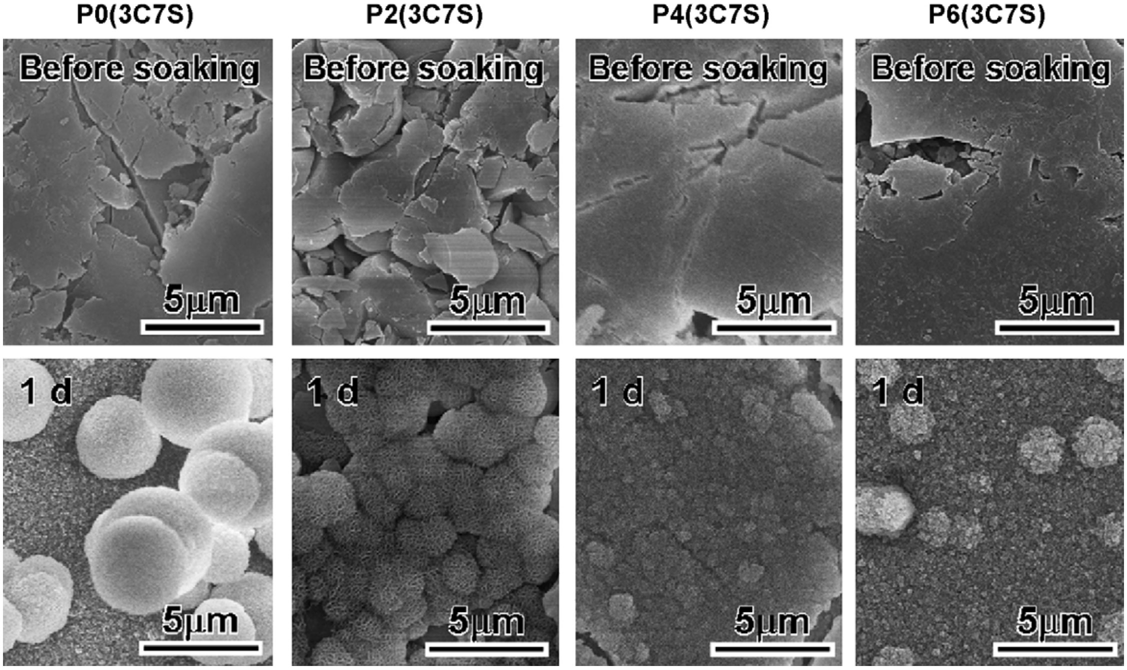

Fig. 10. FE-SEM photographs of the Pz(3C7S) samples prepared with PEG and heated at 600°C, before and after soaking in SBF for 1 day.

Calcium-incorporated silica gels prepared in the present study show high potential for bone-like apatite formation in SBF. Bioactivity of ceramics is evaluated by the formation of a bone-like apatite layer in SBF proposed by Kokubo and coworkers.[8,9] Cho et al.[18] reported that apatite formation on silica gel is dependent on the kind of water-soluble polymer used to produce phase separation in the sol–gel processing. This means that the potential induction of apatite nucleation on the surface of silica hydrogels was significantly affected by the surface morphology and microstructures determined by the sol–gel processing. In the present results, it is clear that incorporation of calcium ions into the prepared silica gel structure may help bone-like apatite formation in SBF, and results in the bone-like apatite formation irrespective of the morphology of the silica gels. The formation of bone-like apatite on the silica gels was enhanced by addition of calcium ions in the silica gel when the gels had micrometre-sized or nanometre-sized particles. These results show that the morphology and size of the $CaO–SiO_2$ gel can be controlled by addition of PEG to result in production of suitable particles with bone-bonding ability for the fabrication of bioactive organic–inorganic composites.

5. Conclusion

Bioactive particles of the $CaO–SiO_2$ binary system were prepared by a sol–gel method to fabricate bioactive organic–inorganic composites. Spherical particles could be obtained by addition of PEG to the $CaO–SiO_2$ binary system under acidic conditions. The gels were obtained either as an interconnected porous structure or as an aggregation of spherical particles according to the calcium content. The particle or domain size decreased with increasing amounts of PEG. After heating at 600 °C, gels formed bone-like apatite on their surfaces within 1 day after soaking in a simulated body fluid. Consequently, particles in the $CaO–SiO_2$ system are expected to be useful as bioactive fillers in the production of organic–inorganic composites for bone repair.

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