

Apatite-forming ability of vinylphosphonic acid-based copolymer in simulated body fluid: effects of phosphate group content

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6 2 **simulated body fluid: effects of phosphate group content**
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3 1 **Abstract**

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5 2 Phosphate groups on materials surfaces are known to contribute to apatite formation
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8 3 upon exposure of the materials in simulated body fluid (SBF) and improved affinity of
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11 4 the materials for osteoblast-like cells. Typically, polymers containing phosphate groups
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14 5 are organic matrices consisting of apatite–polymer composites prepared by biomimetic
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17 6 process using SBF. Ca^{2+} incorporation into the polymer accelerates apatite formation in
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21 7 SBF owing because of increase in the supersaturation degree, with respect to apatite in
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24 8 SBF, owing to Ca^{2+} release from the polymer. However, the effects of phosphate content
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27 9 on the Ca^{2+} release and apatite-forming abilities of copolymers in SBF are rather elusive.
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30 10 In this study, a phosphate-containing copolymer prepared from vinylphosphonic acid
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33 11 (VPA), 2-hydroxyethyl methacrylate (HEMA), and triethylene glycol dimethacrylate
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36 12 (TEGDMA) was examined. The release of Ca^{2+} in Tris-NaCl buffer and SBF increased
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40 13 as the additive amount of VPA increased. However, apatite formation was suppressed as
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43 14 the phosphate groups content increased despite the enhanced release of Ca^{2+} from the
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46 15 polymer. This phenomenon was reflected by changes in the surface zeta potential. Thus,
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49 16 it was concluded that the apatite-forming ability of VPA-HEMA-TGEDMA- CaCl_2
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52 17 copolymer was governed by surface state rather than Ca^{2+} release in SBF.
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3 **1. Introduction**
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7 2 Bone-bonding bioactive ceramics, such as Bioglass [1], glass-ceramics A-W
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9 3 [2], and sintered hydroxyapatite (HAp) [3], have been clinically employed as bone
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11 4 substitutes for repairing severe bone defects induced by accident or disease. When
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13 5 artificial materials are implanted in the affected bone area, the fibrous tissue
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15 6 encapsulates and isolates the materials surrounding the living bone. In contrast,
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17 7 bioactive ceramics can bond to living bone directly owing to their ability to form a
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19 8 bone-like apatite layer on their surface. However, bioactive ceramics have some
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21 9 drawbacks e.g., they cannot deform easily to fit into the defect area or they exert stress
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23 10 shielding effects after implantation. Such issues are due to the brittleness and high
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25 11 Young's modulus of ceramics.
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38 12 As a result, organic–inorganic composites, for bone substitutes, have been
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41 13 examined to improve the mechanical properties of bioactive ceramics. The biomimetic
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44 14 process using simulated body fluid (SBF) is one of the methods commonly employed
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47 15 for preparing apatite–organic polymer composites. Such composites are expected to
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51 16 show mechanical properties similar to that of living bone as well as bioactivity. In this
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54 17 process, functional groups that can induce heterogeneous nucleation of apatite such as –
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57 18 COOH [4], –SO₃H [5], –PO₃H₂ [4], Si–OH [6], Ti–OH [7], or Ta–OH [8] are introduced
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3 1 into the organic matrix to obtain the composite.
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6 2 The heterogeneous nucleation of apatite is promoted by the release of chemical
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9 3 species, thereby increasing the supersaturation degree with respect to apatite [9]. For
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12 4 example, to release Ca^{2+} from the polymers, calcium salt [5, 10] is added or treatment
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15 5 with aqueous solutions of calcium salts [11–14] is performed. In the case of CaCl_2
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18 6 treatment, polymers that feature excellent swelling properties in aqueous solution are
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22 7 used, thus facilitating the release of Ca^{2+} to SBF [12].
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25 8 Phosphate groups are effective for not only apatite formation, but also activity
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28 9 of osteoblast-like cells [15–16]. The cell adhesion and growth was increased as
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32 10 phosphate content increases. The incorporation of phosphate groups into the polymer is
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35 11 expected to afford various composites with high biological compatibility.
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38 12 In our previous research, a phosphate-containing copolymer was prepared from
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41 13 vinylphosphonic acid (VPA) and triethylene glycol dimethacrylate (TEGDMA) through
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44 14 radical polymerization [17]. Although the low added amount of sodium *p*-toluene
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47 15 sulfonate (*p*-TSS), as a polymerization accelerator, inhibited degradation of the polymer,
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51 16 apatite was not formed in SBF irrespective of the composition employed. This
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54 17 phenomenon suggests that the presence of phosphate groups is insufficient to induce
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57 18 apatite formation.
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3 1 Conversely, incorporating Ca^{2+} could be effective for improving the
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6 2 apatite-forming ability of the copolymer. Moreover, the phosphate groups in the
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9 3 polymer are expected to influence the adsorption and release of Ca^{2+} because phosphate
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12 4 is hydrophilic and can readily instigate ion–ion interactions with Ca^{2+} [18].
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16 5 Furthermore, phosphate content is also expected to affect heterogeneous
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19 6 nucleation of apatite on the copolymer in SBF due to the above mentioned ion-ion
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22 7 interaction. Increase in carboxyl group content promotes the heterogeneous nucleation
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25 8 thorough interaction with Ca^{2+} [4]. Several researchers investigated apatite-forming
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28 9 ability of the synthetic polymer [19] or natural polymer [20] containing phosphonic acid
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31 10 through the phosphorylation process. However, these reports had no discussion
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34 11 regarding the effects of phosphate group content on surface condition and its apatite
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37 12 formation behavior in SBF. These points are important to obtain the apatite-phosphate
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40 13 polymer composites through the biomimetic process using SBF.
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44 14 In this study, VPA-based copolymers having different phosphate contents were
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47 15 prepared by addition of 2-hydroxyethyl methacrylate (HEMA) and TEGDMA. Apatite
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50 16 formation on the copolymers in SBF was investigated and discussed in terms of Ca^{2+}
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53 17 release, ionic interaction between the phosphate group and Ca^{2+} , and variation in the
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56 18 surface zeta potential.
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2. Materials and methods

2.1. Preparation of VPA-HEMA-TEGDMA copolymers

Table 1 lists the amounts of monomers used for the preparation of the copolymers. The amount of the monomers totaled to 10 g. The specimens are denoted as xV_yH_zT , where x , y , and z refer to the amounts (mol%) of VPA (V), HEMA (H), and TEGDMA (T), respectively. Monomers VPA (95%, Tokyo Chemical Industry Co., Ltd., Tokyo, Japan), HEMA (95%, Wako Pure Chemical Industries, Ltd., Osaka, Japan), and TEGDMA (90%, Wako Pure Chemical Industries, Ltd.) were mixed. Then, 0.5 wt.% *p*-TSS (98%, Tokyo Chemical Industry Co., Ltd.) and 2 wt.% *N,N'*-dimethyl-*p*-toluidine (97%, Wako Pure Chemical Industries, Ltd.) were added to the combined monomers. Subsequently, (\pm)-camphorquinone (97%, Wako Pure Chemical Industries, Ltd.) was added at a concentration of 1 mol% relative to the total molar amount of monomers; the mixture was stirred in the dark for 1 h.

Then, the 1.1-g mixture was poured into polypropylene cups and irradiated under blue light (460 nm) for 1 h to polymerize the monomers. The obtained copolymer specimens were dried at 60°C for 1 day, and subsequently cut (10 mm \times 10 mm \times 1 mm) and polished with waterproof abrasive paper (SiC, #1000). The specimens were

1 then soaked in ultra pure water for 1 day at room temperature to remove unreacted
2 reagents. Subsequently, the copolymer specimens were soaked in 30 mL of 1 kmol·m⁻³
3 calcium chloride solution at 36.5°C for 1 day.

4 5 **2.2. Soaking of specimens in SBF and Tris-NaCl buffer solutions**

6 The copolymers specimens were soaked in 30 cm³ SBF at 36.5°C for various
7 times up to 5 days. SBF (Na⁺ 142.0, K⁺ 5.0, Mg²⁺ 1.5, Ca²⁺ 2.5, Cl⁻ 147.8, HCO₃⁻ 4.2,
8 HPO₄²⁻ 1.0, SO₄²⁻ 0.5 mol·m⁻³) was prepared by adding NaCl, NaHCO₃, KCl,
9 K₂HPO₄·3H₂O, MgCl₂·6H₂O, CaCl₂, and Na₂SO₄ (Nacalai Tesque, Inc., Kyoto, Japan)
10 to ultra pure water in this order [6]. The pH of the resulting solution was adjusted to
11 7.40 by addition of tris(hydroxymethyl)aminomethane (Nacalai Tesque, Inc.) and an
12 appropriate volume of 1 kmol m⁻³ HCl solution.

13 Also, the specimens were soaked in 30 cm³ Tris-NaCl buffer at 36.5°C for 1
14 day to measure the amount of Ca²⁺ released from the copolymer specimens. Tris-NaCl
15 buffer (142 mol·m⁻³ NaCl and 50 mol·m⁻³ tris(hydroxymethyl)aminomethane) was
16 prepared by sequential addition of NaCl and tris(hydroxymethyl)aminomethane to ultra
17 pure water. Then, an appropriate volume of 1 kmol·m⁻³ HCl solution was added to the
18 solution to adjust the pH to 7.40.

2.3. Characterization

Following soaking of the copolymer specimens in CaCl₂ solution, the specimens were analyzed by wavelength-dispersive X-ray fluorescence spectroscopy (ZSX101e, Rigaku Co., Tokyo, Japan) to determine the Ca content. The surface of the copolymer specimens soaked in SBF for various periods was analyzed with thin-film X-ray diffraction (TF-XRD; MXP3V, Mac Science, Co., Yokohama, Japan), scanning electron microscopy (SEM) using an S-3500N scanning electron microscope (Hitachi Co., Tokyo, Japan) equipped with an energy-dispersive X-ray (EDX) analysis system (EMAX Energy, Horiba Ltd., Kyoto, Japan), and Fourier transform infrared (FT-IR; FT/IR-6100, JASCO Co., Tokyo, Japan) spectroscopy using an attenuated total reflectance method. In the TF-XRD analysis, the angle of the X-ray (Cu K α) was fixed at 1° relative to the surface of the sample. For the SEM-EDX analysis, the surfaces of the samples were coated with carbon using a carbon coater (CADE, Meiwafofos Co., Ltd., Osaka, Japan). For the FT-IR analysis, a diamond prism was used to record the FT-IR spectra at a resolution of 1 cm⁻¹.

The concentrations of Ca in the Tris-NaCl buffer and P and Ca in SBF after soaking the copolymer specimens were measured using inductively coupled plasma

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3 1 optical emission spectrometry (Optima 4300DV CYCLON, PerkinElmer Inc., London,
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6 2 UK). The pH of the SBF solution following soaking of the different specimens was
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9 3 determined using a pH meter (F-23IIC, Horiba Ltd.).
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12 4 The surface zeta potential of the copolymer specimens in SBF was measured
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15 5 using a zeta potential analyzer (Otsuka Electronics Co., Osaka, Japan) connected to
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18 6 box-like quartz cell. After the copolymer specimens were soaked in SBF for various
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21 7 periods, the surface of the specimens was washed with ultra pure water. The washed
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24 8 specimen was introduced into the quartz cell. Then, fresh SBF and polyethylene latex
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27 9 particles (Otsuka Electronics Co.) were injected into the cell. To measure the surface
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30 10 zeta potential, the electrophoretic mobility of the particles was measured using the laser
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33 11 Doppler method.
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43 **3. Results**

44 14 Figure 1a shows the content of Ca in the specimens prepared with varying
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47 15 amounts of VPA after soaking in CaCl₂ solution. The content increased as the
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50 16 VPA/HEMA ratio increased. The Ca concentration in Tris-NaCl buffer after soaking the
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53 17 specimens for 1 day is shown in Fig. 1b. The concentration increased with increasing
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56 18 VPA/HEMA contents.
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3 1 Figure 2 shows SEM images of the specimens after soaking in SBF for various
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6 2 periods. The deposition was observed on the surface of 01V94H05T after soaking SBF
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9 3 within 1 day. The morphology of deposition consisted of flake-like particles. On the
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12 4 other hand, deposition was not formed on the 10V85H05T and 40V85H05T within 5
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15 5 days.

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19 6 Figure 3 shows the TF-XRD patterns of the specimens after soaking in SBF for
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22 7 various periods. After soaking for 1 day, 01V94H05T displayed two broad peaks at 2θ
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25 8 26° and 32° , which were assigned to apatite (JCPDS #09-0432). In contrast, these peaks
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28 9 were not observed in 10V85H05T and 40V55H05T regardless of the soaking time.

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32 10 Figure 4 shows the variations in the concentration of P and Ca in SBF and
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35 11 solution pH after soaking the specimens for various periods. As observed in Fig. 3a, for
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38 12 01V94H05T, the concentration of P decreased with increasing soaking times. In contrast,
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41 13 the concentration of P remained rather constant after soaking 10V85H05T or
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44 14 40V55H05T. Conversely, for all three specimens, the concentration of Ca initially
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47 15 increased and then decreased slightly with increasing soaking times (Fig. 4c, d). The
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50 16 concentration of Ca increased in the order of $01V94H05T < 10V85H05T < 40V55H05T$.
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53 17 The solution pH, after soaking, decreased monotonically for 40V55H05T, whereas that
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56 18 of the remaining specimens initially increased slightly and subsequently remained

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3 1 unchanged (Fig. 4b).
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6 2 Figure 5 shows the changes in the molar ratio of Ca/P and content of P on the
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9 3 surfaces of 01V94H05T and 10V85H05T, and SEM images of 01V94H05T after
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12 4 soaking in SBF for various periods analyzed by SEM-EDX. The Ca/P ratio for both
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15 5 specimens decreased in the first 3 h of soaking and then increased. The Ca/P ratio of
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18 6 01V94H05T was higher than that of 10V85H05T irrespective of soaking time. The P
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22 7 content of 01V94H05T increased after 6 h of soaking, whereas that of 10V85H05T
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25 8 remained constant at all soaking times studied. Deposition was first observed after 12 h
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28 9 on the surface of 01V94H05T in SBF.
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32 10 Figure 6 shows the changes in the zeta potential of 01V94H05T and
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35 11 10V85H05T. The potential of 01V94H05T changed from negative to positive after
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38 12 soaking in SBF for 9 h. In contrast, the potential of 10V85H05T only increased slightly
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41 13 from the negative value to attain a zero value after 6 h of soaking.
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44 14 Figure 7 shows the FT-IR spectra of 01V94H05T and 10V85H05T after
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47 15 soaking in SBF for various periods. The peak at 900 cm^{-1} , which was attributed to C–C
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50 16 stretching vibrations of HEMA, was observed for 01V94H05T after soaking in SBF for
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53 17 0–9 h [21-22]. The peak disappeared after 12 h of soaking owing to the formation of a
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57 18 deposition layer on the specimen. In contrast, 10V85H05 displayed a peak at 889 cm^{-1} ,
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1 which was attributed to P–O bond in the $\text{–P–O}^{\text{–}}\cdots\text{Ca}^{2+}$ complex, as well as the peak
2 corresponding to C–C stretching at all soaking times investigated [17, 19].

3 4 **4. Discussion**

5 The amount of Ca incorporated into the prepared copolymer and released into
6 Tris-NaCl increased with increasing contents of the phosphate group (Fig. 1). The
7 swelling property of the copolymer is affected by not only the cross-link density, but
8 also the charge of the functional groups [16, 23]. The repulsion induced by ionic groups
9 with the same charge acts as a driving force for swelling. The increase in the phosphate
10 group content enhances swelling, therefore higher contents of phosphate would promote
11 the adsorption of Ca^{2+} onto the copolymer upon CaCl_2 treatment and release of Ca^{2+} into
12 the solution.

13 Apatite formation on the copolymer was rather suppressed upon increases in
14 the phosphate content despite the enhanced release of Ca^{2+} from the copolymer. To
15 further understand this phenomenon, the supersaturation degree with respect to apatite
16 in SBF was calculated. Figure 8 shows changes in the relative supersaturation degree, σ ,
17 of the copolymers in SBF, calculated using Equation (1) [24]:

$$\sigma = \frac{IP_{HAp}^{1/\nu} - Ksp_{HAp}^{1/\nu}}{Ksp_{HAp}^{1/\nu}}, \quad (1)$$

where IP_{HAp} , Ksp_{HAp} , and ν are the ionic activity products of HAp, solubility product of HAp (5.5×10^{-118}), and the number of ions in an HAp molecule (18), respectively. The

IP_{HAp} was estimated according to Equation (2):

$$IP_{HAp} = (\gamma_{Ca^{2+}})^{10} (\gamma_{PO_4^{3-}})^6 (\gamma_{OH^-})^2 [Ca^{2+}]^{10} [PO_4^{3-}]^6 [OH^-]^2. \quad (2)$$

The values of $\gamma_{Ca^{2+}}$, $\gamma_{PO_4^{3-}}$, and γ_{OH^-} are respectively 0.36, 0.06, and 0.72 at physiological ionic strength ($\mu = 0.16$) [25]. For all the specimens, the degree of supersaturation increased slightly and subsequently decreased. The degree of supersaturation increased in the order of 40V55H05T \approx 01V94H05T < 10V85H05T. However, apatite was only observed on 01V94H05T (Fig. 2 and 3). These results suggest that apatite formation of the copolymer was governed by surface chemical state rather than increase in supersaturation degree owing to Ca^{2+} release.

The difference in the surface state of the specimens having various contents of phosphate in SBF can be interpreted as follows. The zeta potential of 01V94H05T increased more significantly than that of 10V85H05T after 6 h of soaking in SBF (Fig. 6). As reported, the potential of soft solids, such as a gel or a polymer, is governed by not only the charge on the outermost surface, but also the charge inside the solid, unlike that of hard solids such as metal oxides [26–27]. Therefore, Ca^{2+} would accumulate near

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3 1 the surface of the soft specimens after soaking in SBF. Furthermore, the complex $-P-$
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6 2 $O^- \cdots Ca^{2+}$ formed on 10V85H05T only upon soaking in SBF (Fig. 7). Based on the
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9 3 result, it is assumed that the amount of free Ca^{2+} is larger than that of Ca^{2+} tightly bound
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12 4 to phosphate groups on the surface and/or inside 01V94H05T and that the negative
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15 5 charge on 10V85H05T is neutralized in SBF upon tight binding with Ca^{2+} . The free
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18 6 Ca^{2+} would readily bond with phosphate ions in SBF for conversion into apatite. The
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22 7 decrease in the zeta potential of 01V94H05T after 9 h of soaking supports this
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25 8 assumption. Conversely, further ion adsorption to induce apatite nucleation did not
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28 9 occur on 10V85H05T.

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32 10 The surface potential has been previously reported as a contributing factor to
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35 11 the formation of apatite on various substrates in SBF [28]. For example, the zeta
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38 12 potential of high-molecular-weight polyethylene containing $-SO_3H$ groups and Ca^{2+}
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41 13 becomes positive upon soaking in SBF and subsequently adsorbs PO_4^{3-} to induce
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44 14 apatite nucleation [29]. In contrast, the polymer modified with $-SO_3H$ only did not form
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47 15 apatite in SBF. Accordingly, as observed, the surface potential of 10V85H05T was
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51 16 insufficiently positive to adsorb PO_4^{3-} and therefore it did not form apatite.

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54 17 The results in this study showed that materials with larger amounts of
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57 18 phosphate group inhibit apatite formation in SBF. However, as reported, phosphate
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1 groups in the self-assembled monolayer on gold can interact with PO_4^{3-} after binding
2 with Ca^{2+} in SBF, subsequently instigating apatite formation [4]. This suggests that the
3 binding state of the phosphate groups with Ca^{2+} is different in the present results.
4 Specifically, the acidity of the phosphate-containing compounds is different owing to
5 their different chemical structure [30]. The effects of chemical structure and space
6 distribution of phosphate groups on the binding state and apatite formation on
7 phosphate-containing polymers deserve further investigation in future work.

8

9 **5. Conclusion**

10 The effect of the amount of phosphate groups on the apatite-forming ability of
11 VPA-HEMA-TEGDMA treated with CaCl_2 solution was investigated in SBF. Increasing
12 the content of VPA enhanced the release of Ca^{2+} from copolymer. However, apatite
13 formation was only induced on the copolymer prepared with 1 mol% of VPA (lowest
14 amount studied). Higher VPA content rather inhibited the apatite formation because
15 PO_4^{3-} could not react with free Ca^{2+} on the surface due to the increase in amount of Ca^{2+}
16 tightly binding with phosphate group. It was found that phosphate groups in VPA
17 produce the unsuitable surface condition for heterogeneous nucleation of the apatite.
18 The future works are required to precisely investigate the effects of detailed chemical

1 structure and binding state with Ca^{2+} of the phosphate groups on apatite formation.

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3 **References**

4 [1] Hench LL. Bioceramics. J Am Ceram Soc. 1998;81:1705-8.

5 [2] Kokubo T, Kim HM, Kawashita M. Novel bioactive materials with different
6 mechanical properties. Biomaterials. 2003;24:2161-2175.

7 [3] Jarcho M, Bolen CH, Thomas MB, Bobick J, Kay JF, Doremus RH. Hydroxyapatite
8 synthesis and characterization in dense polycrystalline forms. J Mater Sci, 1976;11:
9 2027–2035.

10 [4] Tanahashi M, Matsuda T. Surface functional group dependence on apatite formation
11 on self-assembled monolayers in a simulated body fluid. J Biomed Mater Res
12 1997;34:305-315.

13 [5] Kawai T, Ohtsuki C, Kamitakahara M, Miyazaki T, Tanihara M, Sakaguchi Y,
14 Konagaya S. Coating of an appetite layer on polyamide films containing sulfonic groups
15 by a biomimetic process. Biomaterials 2004;25:4529-4534.

16 [6] Cho SB, Nakanishi K, Kokubo T, Soga N, Kamamura T, Kitsugi T, Yamamuro T.
17 Dependence of apatite formation on silica gel on its Structure: effect of heat treatment. J
18 Am Ceram Soc 1995;78:1769-1774.

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1 [7] Uchida M, Kim HM, Fujibayashi S, Nakamura T. Structural dependence of apatite
2 formation on titania gels in a simulated body fluid. J Biomed Mater Res A,
3 2003;64A:164-170.

4 [8] Miyazaki T, Kim HM, Kokubo T, Kato H, Nakamura T. Induction and acceleration
5 of bonelike apatite formation on tantalum oxide gel in simulated body fluid. J Sol-Gel
6 Sci Tech 2001;21:83-88.

7 [9] Ohtsuki C, Kokubo T, Yamamuro T, Mechanism of apatite formation on CaO—SiO₂
8 —P₂O₅ glass in a simulated body fluid. J Non-Cryst Solids, 1992;143:84-92.

9 [10] Miyazaki T, Ohtsuki C, Akioka Y, Tanihara M, Nakao J, Sakaguchi Y, Konagaya S.
10 Apatite deposition on polyamide films containing carboxyl group in a biomimetic
11 solution. J Mater Sci Mater Med. 2003;14:569-74.

12 [11] Takeuchi A, Ohtsuki C, Miyazaki T, Tanaka H, Yamazaki M, Tanihara M.
13 Deposition of bone-like apatite on silk fiber in a solution that mimics extracellular fluid.
14 J Biomed Mater Res A, 2003;65A:283-189.

15 [12] Nakata R, Miyazaki T, Morita Y, Ishida E, Iwatsuki R, Ohtsuki C. Apatite
16 formation abilities of various carrageenan gels in simulated body environment. J Ceram
17 Soc Japan 2010;118:487-490.

18 [13] Kawashita M, Nakao M, Minoda M, Kim HM, Beppu T, Miyamoto T, Kokubo T,

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1 Nakamura T. Apatite-forming ability of carboxyl group-containing polymer gels in a
2 simulated body fluid. *Biomaterials* 2003;24:2477-2484.

3 [14] Leonor IB, Kim HM, Balas F, Kawashita M, Reis RL, Kokubo T, Nakamura T,
4 Functionalization of different polymers with sulfonic groups as a way to coat them with
5 a biomimetic apatite layer. *J Mater Sci Mater Med* 2007;18:1923-1930.

6 [15] Gemeinhart RA, Bare CM, Haasch RT, Gemeinhart EJ. Osteoblast-like cell
7 attachment to and calcification of novel phosphate-containing polymeric substrate. *J*
8 *Biomed Mater Res A* 2006;78A:433-440.

9 [16] Tan J, Gemeinhart RA, Ma M, Saltzman WM, Improved cell adhesion and
10 proliferation on synthetic acid-containing hydrogel. *Biomaterials* 2005;26:3663-3671.

11 [17] Hamai R, Shirosaki Y, Miyazaki T. Biomineralization behavior of a
12 vinylphosphonic acid-based copolymer added with polymerization accelerator in
13 simulated body fluid. *J Asian Ceram Soc* 2015;3:407-411.

14 [18] Ellis J, Anstice M, Wilson AD. The glass polyphosphonate cement: A novel
15 glass-ionomer cement based on poly(vinyl phosphonic acid). *Clin Mater*
16 *1991;7:341-346.*

17 [19] Jin S, Gonsalves E. Functionalized copolymers and their composites with
18 polylactide and hydroxyapatite. *J Mater Sci Mater Med* 1999;10:363-368.

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1 [20] Yin YJ, Luo XY, Cui JF, Wang CY, Guo XM, Yao KD. A study on
2 biomineralization Behavior of N-methylene phosphochitosan scaffolds. *Macromol*
3 *Biosci* 2004;4:971-977.

4 [21] Ferreira L, Vidal MM, Gil MH. Evaluation of poly(2-hydroxyethyl methacrylate)
5 gels as drug delivery systems at different pH values. *Int J Pharm* 2000;194:169–180.

6 [22] Faria MDG, Dias JJCT, Fausto R. Conformation stability for methyl acrylate: a
7 vibrational spectroscopic ab initio MO study. *Vib. Spectr* 1991;2:43-60.

8 [23] Garrett Q, Laycock B, Garrett RW. Hydrogel lens monomer constituents modulate
9 protein sorption. *Invest Ophthalmol Vis Sci* 2000;41:1687-1695.

10 [24] Hata K, Kokubo T, Nakamura T, Yamamuro T. Growth of a Bonelike Apatite Layer
11 on a Substrate by a Biomimetic Process. *J Am Ceram Soc* 1995;78:1049-1053.

12 [25] Neuman W, Neuman M. *The chemical dynamics of bone mineral*. Chicago:
13 University of Chicago Press; 1958.

14 [26] Ohshima H, Makino K, Kondo T. Interfacial electric phenomena and Donnan
15 potential in membranes. *Membrane* 1987;12:425-430.

16 [27] Makino K, Ohshima H, Kondo T. Surface potential an ion-penetrable charged
17 membrane. *J Theor Biol* 1987;125:367-368.

18 [28] Kim HM, Himeno T, Kawashita M, Lee JH, Kokubo T, Nakamura T. Surface

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1 potential change in bioactive titanium metal during the process of apatite formation in
2 simulated body fluid. J Biomed Mater Res A, 2003;67A:1305-1309.
3 [29] Leonor IB, Kim HM, Balas F, Kawashita M, Reis RL, Kokubo T, Nakamura T.
4 Surface potential change in bioactive polymer during the process of biomimetic apatite
5 formation in a simulated body fluid. J Mater Chem 2007;17:4057-4063.
6 [30] MoszernM, Salz U, Zimmermann J. Chemical aspects of self-etching enamel-
7 dentin adhesives: A systematic review. Dent Mater 2005;21:895-910.

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1 **Figure and table captions**

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3 **Fig. 1** (a) Ca content in the copolymer specimens after soaking in $1 \text{ kmol m}^{-3} \text{ CaCl}_2$
4 solution and (b) Ca concentration in Tris-NaCl buffer following soaking of the different
5 copolymer specimens ($N = 3$).

6 **Fig. 2** SEM images of the specimens after soaking in SBF for various periods.

7 **Fig. 3** TF-XRD patterns of the copolymer specimens following soaking in SBF for
8 various periods

9 **Fig. 4** Changes in (a) P concentration, (b) pH, and (c, d) Ca concentration in SBF
10 following soaking of the different copolymer specimens ($N = 3$).

11 **Fig. 5** Changes in the (a) molar ratio of Ca/P and (b) abundance of P on the surface of
12 01V94H05T and 10V85H05T following soaking in SBF

13 **Fig. 6** Changes in the surface zeta potentials of 01V94H05T and 10V85H05T following
14 soaking in SBF ($N = 3$).

15 **Fig. 7** FT-IR spectra of 01V94H05T and 10V85H05T following soaking in SBF for
16 various periods

17 **Fig. 8** Changes in the relative supersaturation degree in SBF following soaking of the
18 different copolymer specimens

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1 **Table 1** Composition of the monomers employed during synthesis of the copolymer
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6 2 specimens
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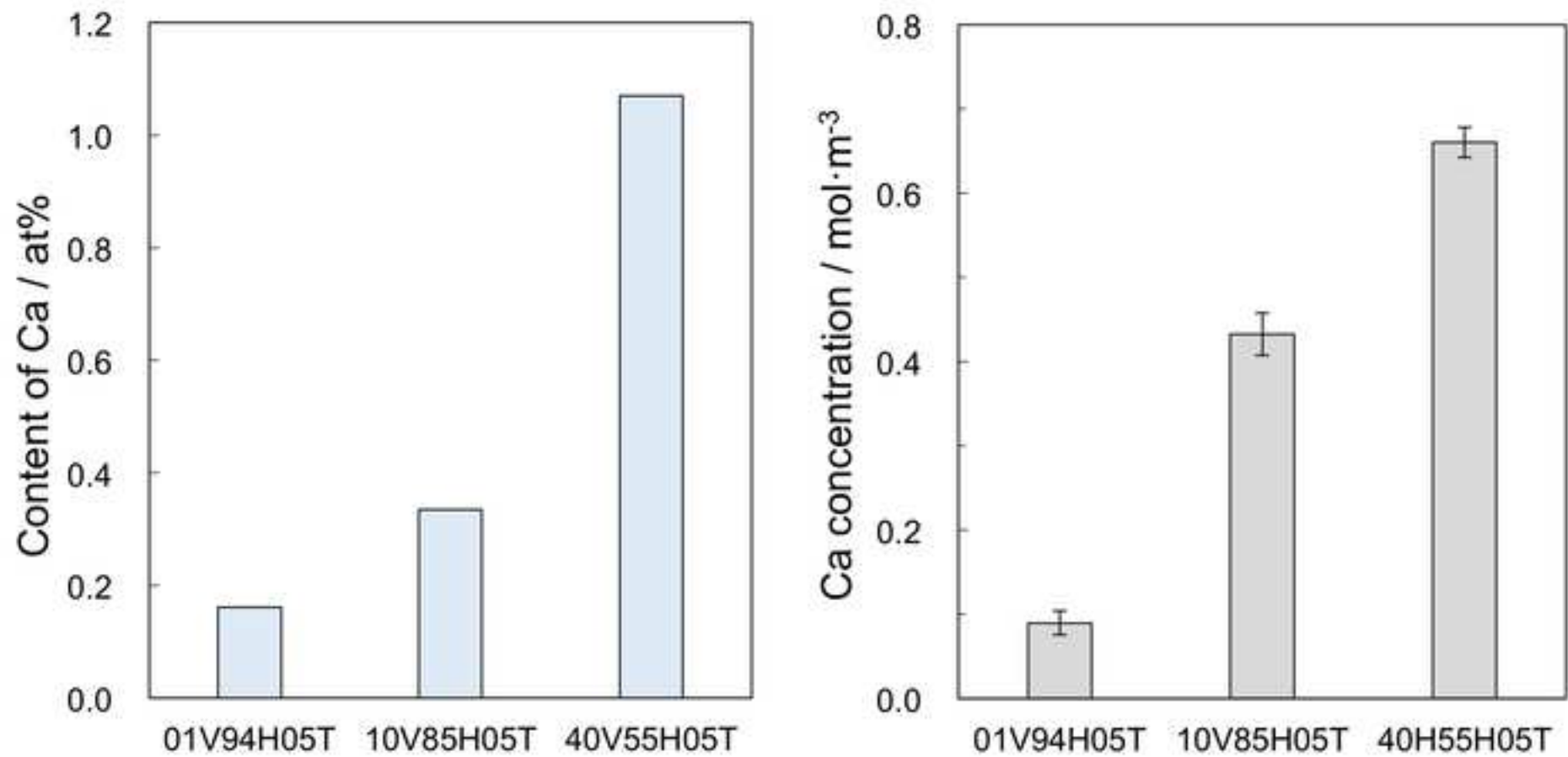


Fig. 1

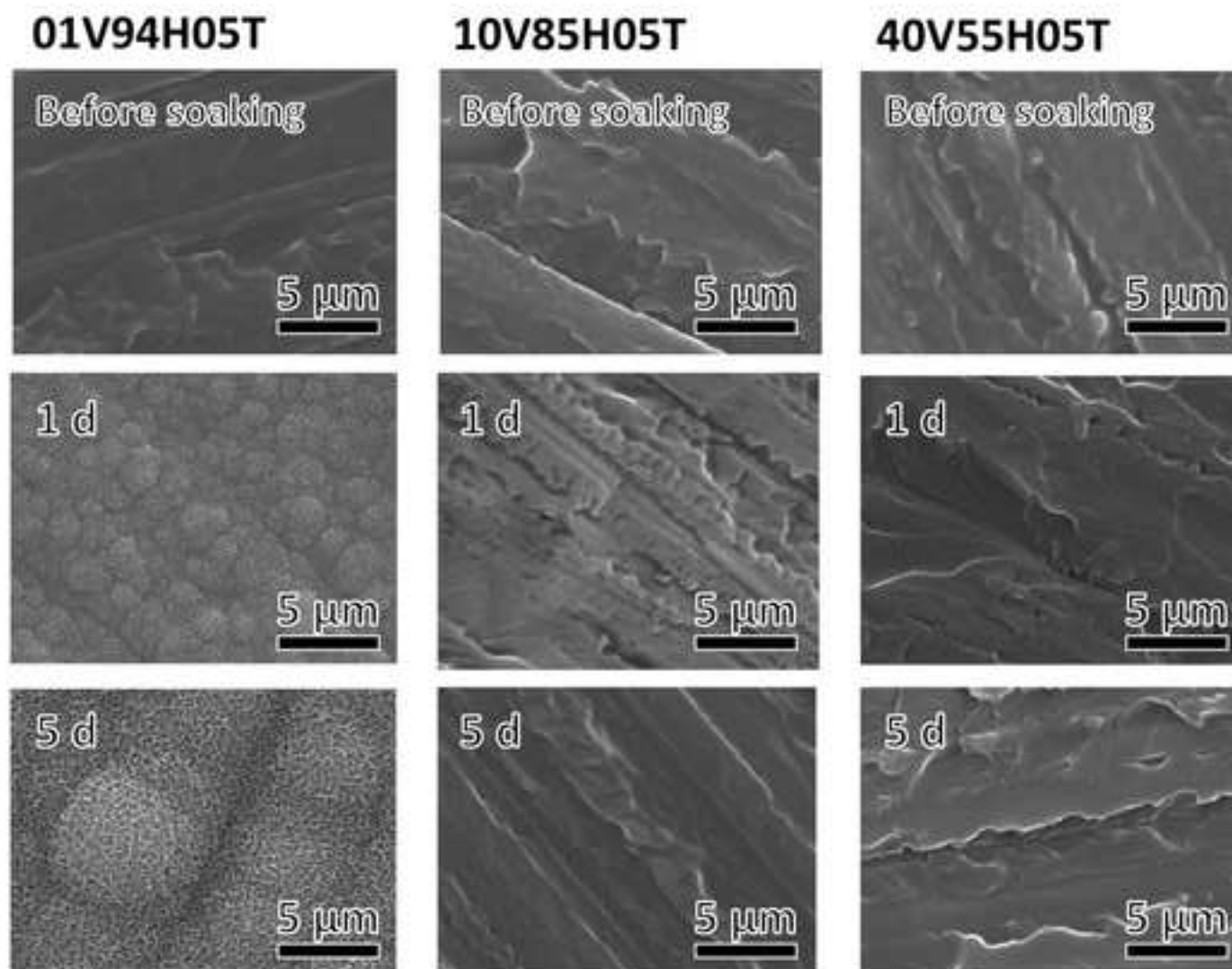


Fig. 2

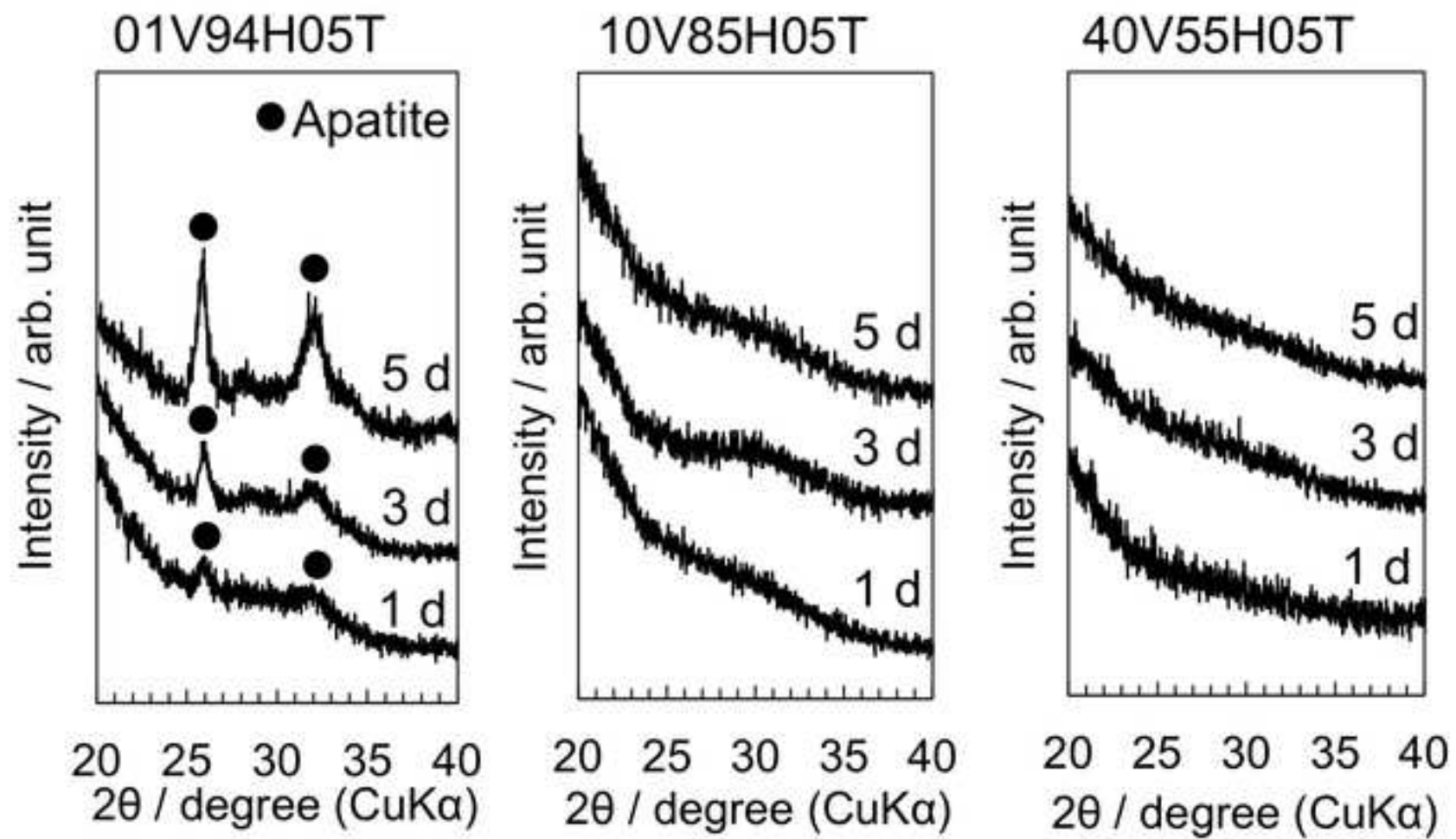


Fig. 3

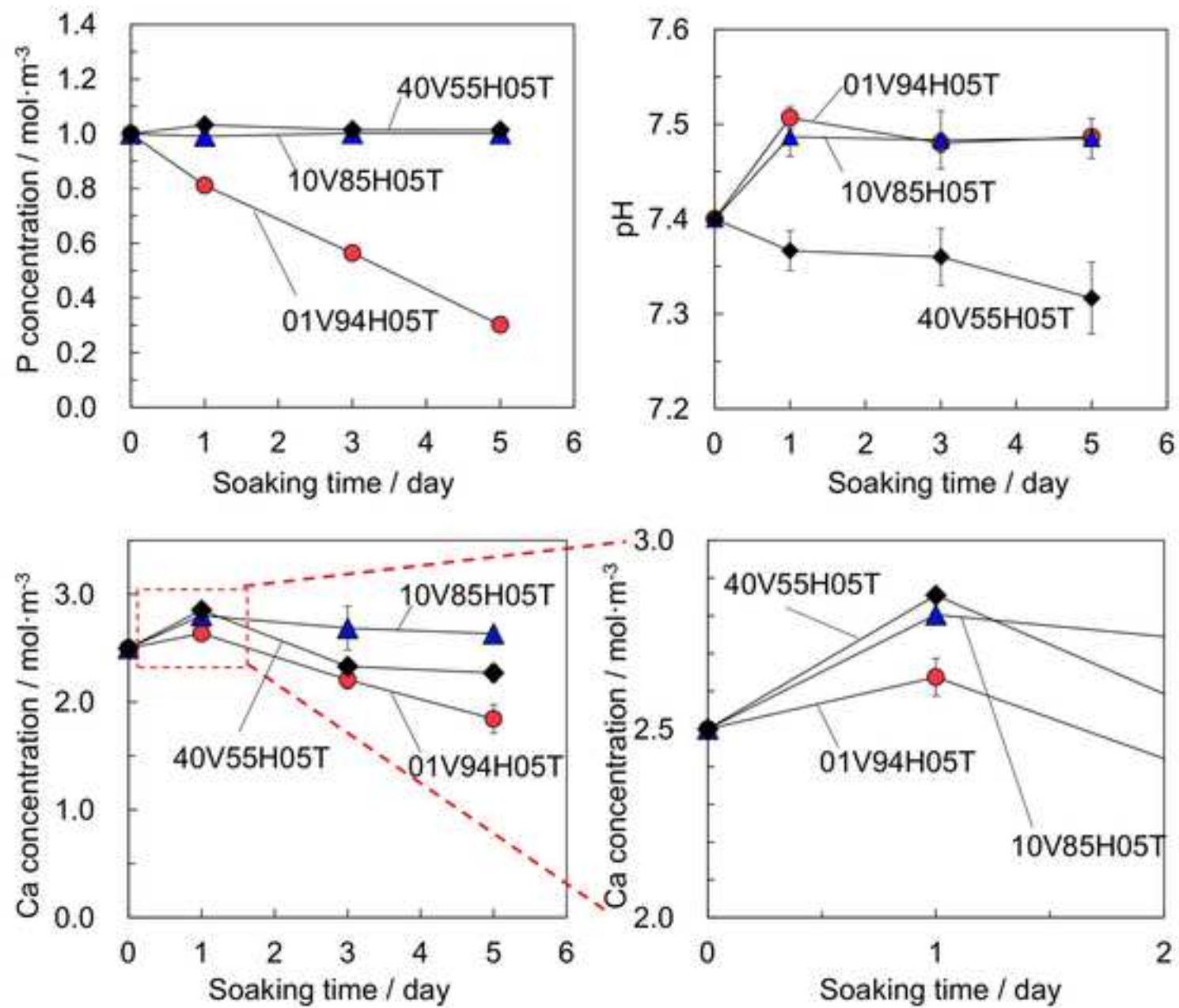


Fig. 4

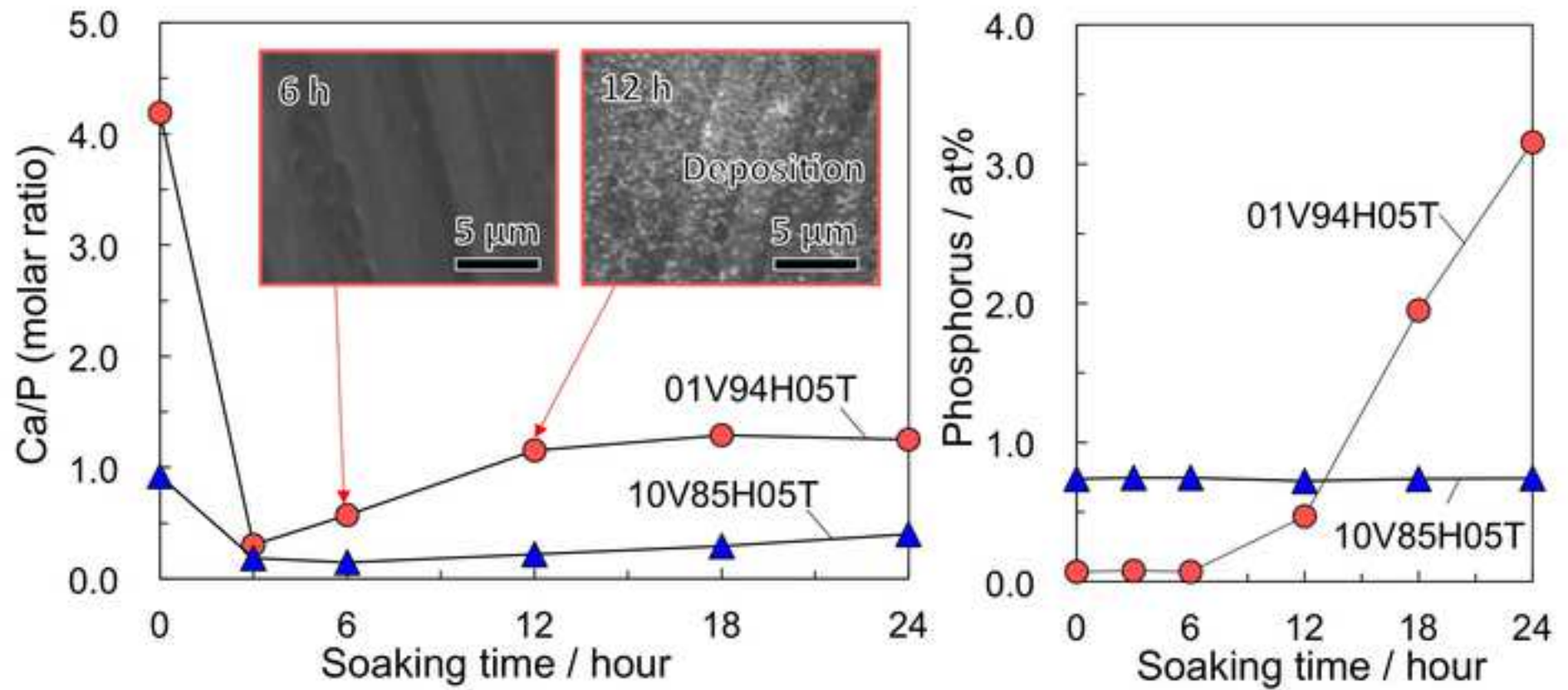


Fig. 5

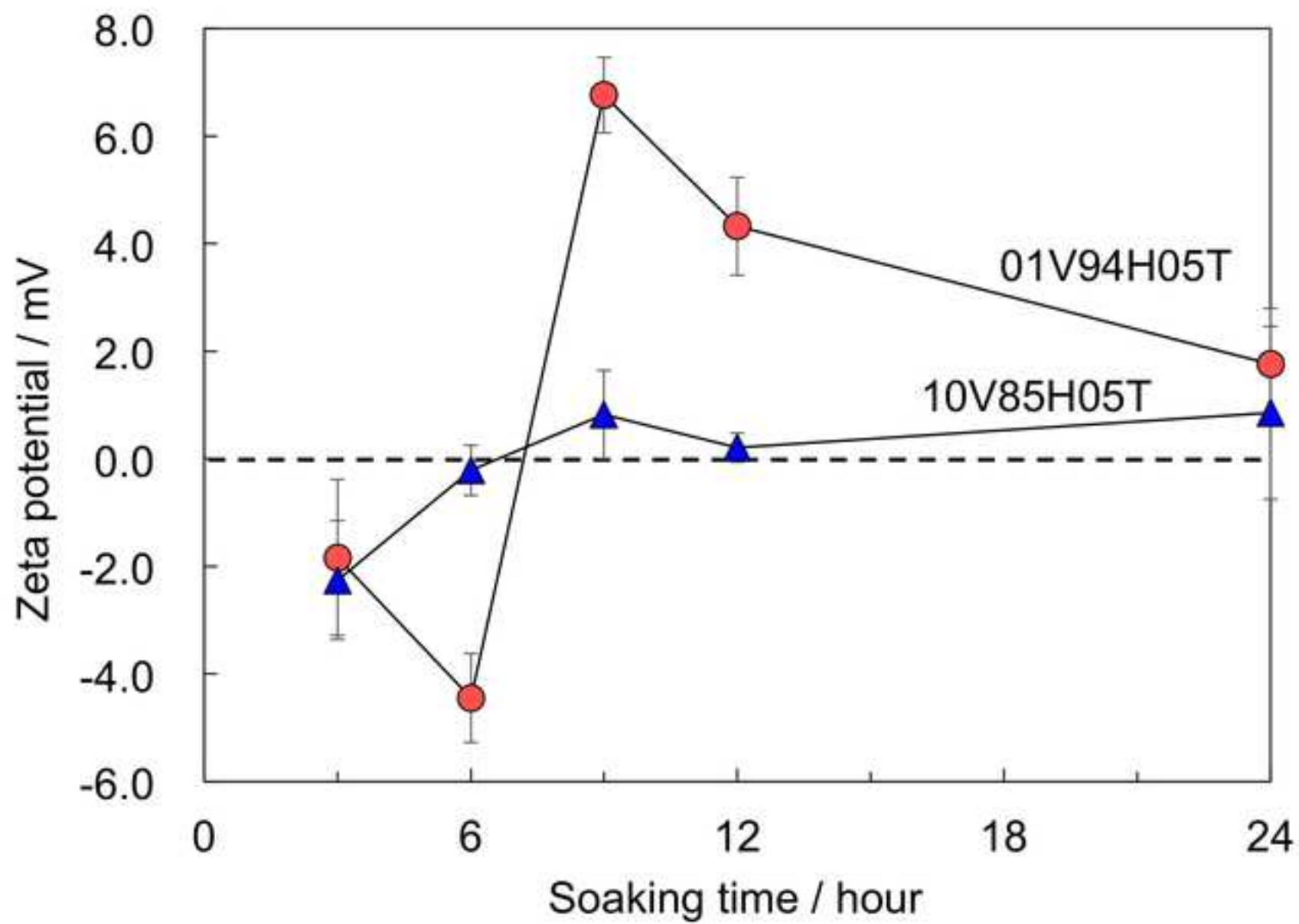


Fig. 6

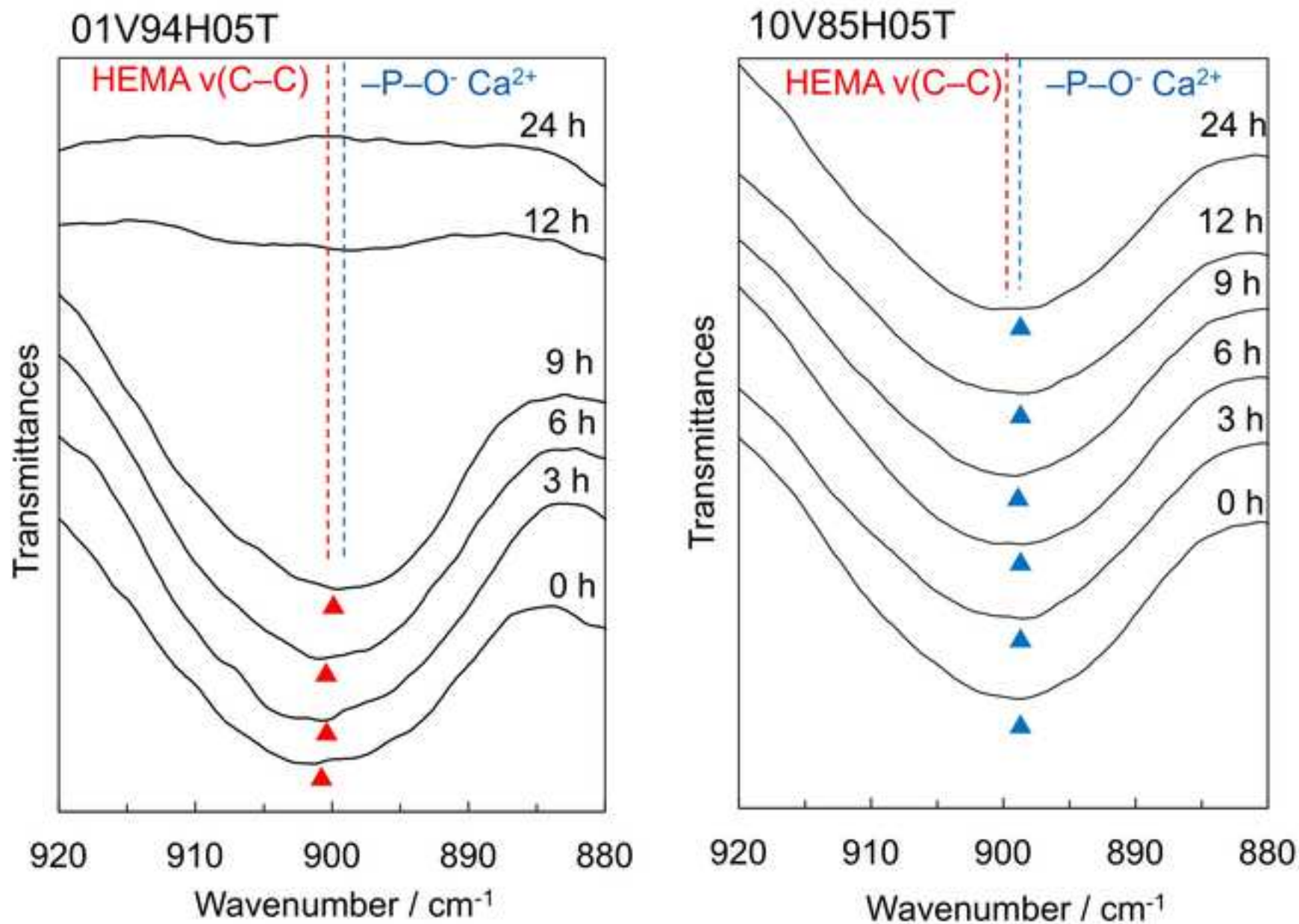


Fig. 7

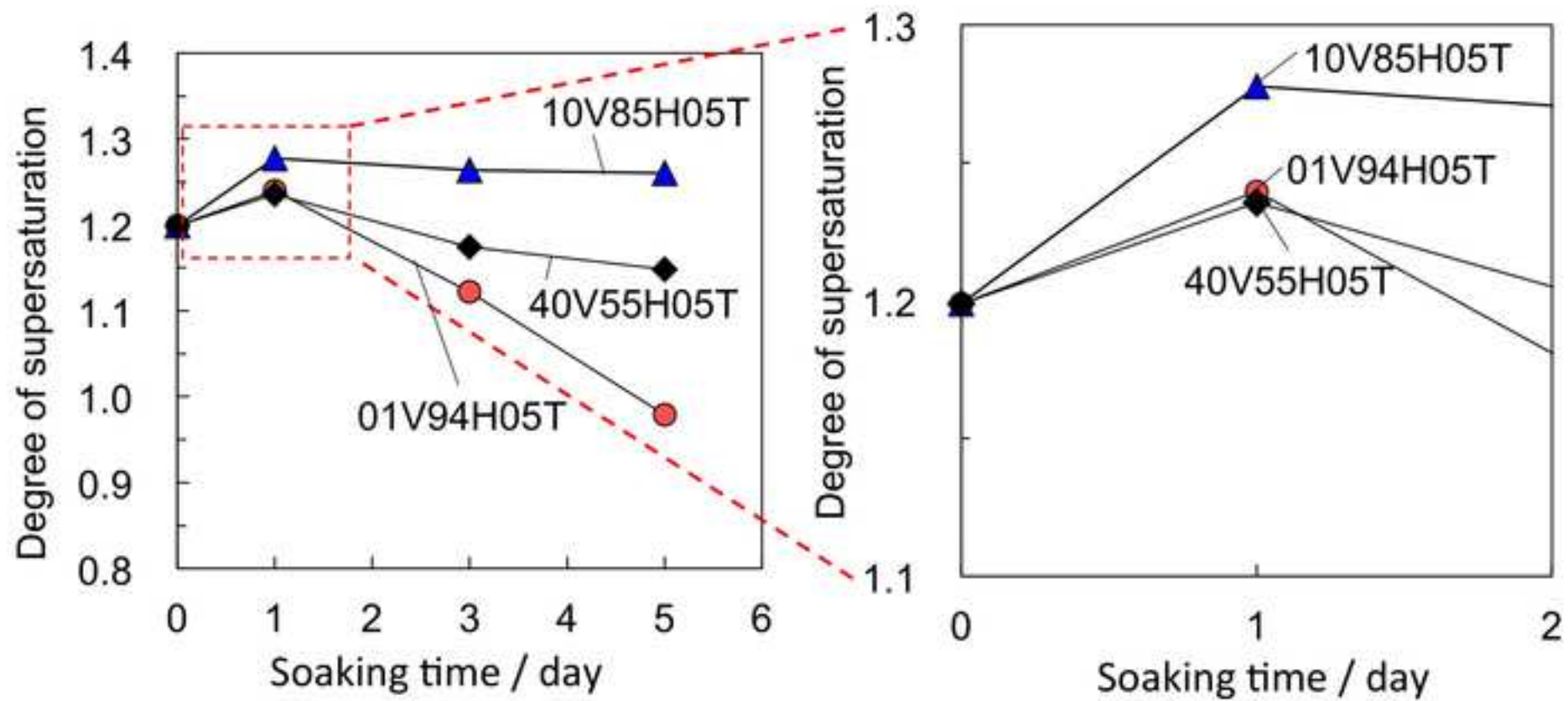


Fig. 8

Table 1

Specimen	VPA /mol%	HEMA / mol%	TEGDMA / mol%
40V55H05T	40	55	5
10V85H05T	10	85	5
01V94H05T	1	94	5