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1 **Bioactive Carbon–PEEK Composites Prepared by Chemical Surface Treatment**

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3 Toshiki Miyazaki¹, Chisato Matsunami¹ and Yuki Shirosaki²

4

5 ¹Graduate School of Life Science and Systems Engineering, Kyushu Institute of

6 Technology, Japan

7 ²Frontier Research Academy for Young Researchers, Kyushu Institute of Technology,

8 Japan

9

10

11 **Corresponding author:**

12 Toshiki Miyazaki

13 Graduate School of Life Science and Systems Engineering, Kyushu Institute of

14 Technology, 2-4, Hibikino, Wakamatsu-ku, Kitakyushu 808-0196, Japan

15 Tel/Fax: +81-93-695-6025

16 E-mail: tmiya@life.kyutech.ac.jp

17

1 **Abstract**

2 Polyetheretherketone (PEEK) has attracted much attention as an artificial intervertebral
3 spacer for spinal reconstruction. Furthermore, PEEK plastic reinforced with carbon fiber
4 has twice the bending strength of pure PEEK. However, the PEEK-based materials do
5 not show ability for direct bone bonding, i.e., bioactivity. Although several trials have
6 been conducted for enabling PEEK with bioactivity, few studies have reported on
7 bioactive surface modification of carbon–PEEK composites. In the present study, we
8 attempted the preparation of bioactive carbon-PEEK composites by chemical treatments
9 with H₂SO₄ and CaCl₂. Bioactivity was evaluated by *in vitro* apatite formation in
10 simulated body fluid (SBF). The apatite formation on the carbon–PEEK composite was
11 compared with that of pure PEEK. Both pure PEEK and carbon-PEEK composite
12 formed the apatite in SBF when they were treated with H₂SO₄ and CaCl₂; the latter
13 showed higher apatite-forming ability than the former. It is conjectured that many
14 functional groups able to induce the apatite nucleation, such as sulfo and carboxyl
15 groups, are incorporated into the dispersed carbon phase in the carbon–PEEK
16 composites.

17

18 **Keywords**

19 Polyetheretherketone (PEEK), carbon–PEEK composite, Apatite, Bioactivity, Simulated
20 body fluid (SBF), Surface modification

21

1 **1 Introduction**

2 Spinal diseases such as disk herniation, spinal stenosis and compression fracture
3 cause neuropathic pain [1,2]. In severe cases, implantation of artificial intervertebral
4 spacers is needed. Titanium and its alloys, carbon and polyetheretherketone (PEEK) are
5 used for this purpose. In particular, PEEK has attracted much attention, as it reduces
6 stress shielding by having a lower elastic modulus than metallic biomaterials [3]. PEEK
7 plastic reinforced with carbon fiber has a bending strength twice as high as pure PEEK
8 [4] and is expected to become a novel biomaterial for spinal reconstruction. Although
9 elastic modulus of carbon-PEEK is higher than that of pure PEEK, it is analogous to
10 that of human cortical bone with carbon content less than 30%. Nonetheless, neither
11 PEEK nor PEEK reinforced with carbon fiber show ability for direct bone bonding.
12 Thus, autograft implantation around the spinal devices is needed for bone fixation in
13 bone tissue. Additional surgery is needed to extract the autograft, which subjects the
14 patient to severe physical strain. Therefore, tight bone integration of PEEK medical
15 devices is necessary for long-term stability *in vivo* and improvement of patients' quality
16 of life.

1 It is reported that an essential requirement for artificial materials to exhibit
2 bioactivity is the formation of bone-like apatite on their surfaces in the body
3 environment through chemical reaction with body fluid [5]. The apatite formation can
4 be reproduced in simulated body fluid (SBF) with inorganic ion concentrations similar
5 to human extracellular fluid [6]. The development of bioactive PEEK has been
6 attempted by several techniques [7]. For example, sulfuric acid treatment [8], sol-gel
7 titania coating [9], PEEK–hydroxyapatite composite [10-12] and composite of PEEK
8 and sol-gel-derived CaO-SiO₂ powder [13] have been proposed. Main technique of
9 bioactive PEEK preparation is mixing PEEK with bioactive ceramic particles. However,
10 only a part of the added ceramics is exposed to body fluid to contribute to their
11 bioactivity. So large amount of the ceramic addition is needed for achieving bone
12 bonding. The composite with high ceramic content is liable to be brittle. Ha *et al.* also
13 prepared a bioactive carbon–PEEK composite by Ti coating via vacuum plasma
14 spraying and subsequent NaOH treatment [14]. In this case, a sodium titanate layer
15 formed by the NaOH treatment contributed to the apatite formation. However, no other
16 studies have been proposed on the bioactivity of the carbon–PEEK composite. In

1 particular, using a surface modification to provide the composite itself with
2 apatite-forming ability has not been suggested.

3 In the present study, we carried out chemical treatments of the carbon-PEEK
4 composite with sulfuric acid and calcium salt solution. Subsequently, *in vitro*
5 apatite-forming ability was evaluated in SBF. The effect of carbon incorporation into
6 PEEK plastics on the apatite formation is discussed. It is assumed that functional group
7 able to induce the apatite formation well incorporated on the composites, since carbon
8 has higher reactivity than PEEK. Actually, surface modification of the carbon by
9 carboxyl group [15] and sulfo group [16] has been reported.

10

11 **2 Materials and Methods**

12 Disks of the pure PEEK (Victrex plc, UK) and carbon-PEEK composite
13 containing 30% by mass of carbon fiber (diameter of 10 μ m, thickness of 1.2 mm;
14 Sumitomo Chemical Co., Japan), and rectangular pure carbon substrates (10 \times 10 \times 1
15 mm; Nilaco Co., Japan) were soaked in 5 mL of concentrated sulfuric acid (97 wt%) at
16 20 °C for 10 min, and then soaked in ultrapure water for 10 min and dried at 60 °C for

1 30 min. After the treatment, they were soaked in 30 mL of 1 M-CaCl₂ solution at
2 36.5 °C for 24 hr. SBF (Na⁺ 142.0, K⁺ 5.0, Mg²⁺ 1.5, Ca²⁺ 2.5, Cl⁻ 147.8, HCO₃⁻ 4.2,
3 HPO₄²⁻ 1.0, SO₄²⁻ 0.5 mM) was prepared by the sequential addition of NaCl, NaHCO₃,
4 KCl, K₂HPO₄·3H₂O, MgCl₂·6H₂O, CaCl₂ and Na₂SO₄ (Nacalai Tesque Inc., Kyoto,
5 Japan) to ultra-pure water according to the previous literature [6]. The pH of the
6 resulting solution was adjusted to 7.4 by the addition of
7 tris(hydroxymethyl)aminomethane (Nacalai Tesque Inc.) and the appropriate volume of
8 a 1 M-HCl solution. The samples were then soaked in 30 mL of SBF at 36.5 °C for 14
9 days.

10 The chemical structures of the samples were analyzed by Fourier transform
11 infrared spectroscopy (FT-IR; FT/IR-6100, JASCO Co., Tokyo, Japan) using an
12 attenuated total reflectance method. The scan range for the FT-IR analysis was set from
13 400 to 4000 cm⁻¹ at a resolution of 4 cm⁻¹. The surface morphology and elemental
14 composition were characterized by energy dispersive X-ray (EDX) analysis using an
15 EMAX Energy system (Horiba Ltd., Japan) equipped with a scanning electron
16 microscope (SEM; S-3500N; Hitachi Co., Japan).

1 The crystalline structure of the samples was characterized by thin-film X-ray
2 diffraction (TF-XRD; MXP3V, Mac Science, Co., Yokohama, Japan). Monochromated
3 Cu-K α radiation was used for the TF-XRD analysis, which was fixed at 1° against the
4 surface of each specimen with a scan rate of 0.02°·sec⁻¹. The coverage ratio of the
5 apatite precipitate formed on the substrates in SBF was determined from SEM
6 photographs using ImageJ software. The amount of Ca²⁺ incorporated into the
7 substrates by CaCl₂ treatment was determined by soaking in ultrapure water at room
8 temperature for 24 hr and measuring the Ca²⁺ concentration of the water by ion/pH
9 meter (F-23IIC, Horiba Ltd.). Contact angle of the specimens before and after H₂SO₄
10 treatment was measured by a contact angle meter (DMe-200, Kyowa Interface Science
11 Co., Ltd., Saitama, Japan). One sample was used for one condition except measurement
12 of contact angle and coverage ratio of the apatite.

13

14 **3 Results and Discussion**

15 Figure 1 shows water contact angle of the substrates before and after H₂SO₄
16 treatment. The contact angle was significantly decreased for all the substrates after the

1 treatment. This means that H_2SO_4 treatment is quite effective for making PEEK and
2 carbon more hydrophilic.

3 Figure 2 shows EDX spectra of the surfaces of pure PEEK and carbon-PEEK
4 composites subjected to H_2SO_4 treatment. Peaks of S are observed for both specimens.
5 This suggests that the sulfo group is incorporated into both the substrates.

6 Figure 3 shows SEM photographs of the surfaces of pure PEEK and
7 carbon-PEEK composites subjected to H_2SO_4 and $CaCl_2$ treatments. Needle-like
8 particles were partially observed on the treated surfaces. The present results indicate
9 that calcium sulfate may be precipitated on the treated specimens, since the typical
10 calcium sulfate crystal takes needle-like structure.

11 The coverage ratios of the apatite particle on pure PEEK and carbon-PEEK
12 calculated from SEM photographs were $6.59 \pm 7.33\%$ and $64.6 \pm 14.0\%$, respectively
13 ($N=10$). The amount of Ca^{2+} incorporated into pure PEEK and carbon-PEEK by $CaCl_2$
14 treatment was 2.1 ± 0.9 and 3.6 ± 0.3 $\mu\text{mol}/\text{substrate}$, respectively. This means that
15 carbon-PEEK has significantly higher ability of the apatite formation in SBF than pure
16 PEEK even after the same chemical treatments.

1 Figure 4 shows TF-XRD patterns of the surfaces of pure PEEK and carbon-PEEK
2 composites subjected to H₂SO₄ and CaCl₂ treatments. Peaks assigned to CaSO₄·2H₂O
3 (JCPDS#33-0311) were observed for both substrates after the treatment. The peaks at
4 20.7, 23.4, 29.1, 31.1, 33.3° were due to $\bar{1}\bar{2}\bar{1}$, 040, $\bar{1}\bar{4}\bar{1}$, 121 and 051 diffraction of
5 CaSO₄·2H₂O, respectively [17]. This indicates that the deposited needle-like particles
6 are CaSO₄·2H₂O.

7 Figure 5 shows SEM photographs of the surfaces of pure PEEK and
8 carbon-PEEK composites subjected to H₂SO₄ and CaCl₂ treatments, which were soaked
9 in SBF for 14 days. Spherical particles were partially observed on pure PEEK, while the
10 surface of carbon-PEEK substrates were almost covered with fine particles. Ca/P molar
11 ratio of the deposits was 1.42 for both the specimens. This result coincides with
12 characteristic of bone mineral and bone-like apatite with Ca deficiency.

13 Figure 6 shows TF-XRD patterns of the surfaces of pure PEEK and carbon-PEEK
14 composites subjected to H₂SO₄ and CaCl₂ treatments, which were soaked in SBF for 14
15 days. Peaks assigned to low-crystalline apatite (JCPDS #09-0432) were observed at 26
16 and 32°. The peak at 26° is due to 002 diffraction of apatite while a broader peak at

1 about 32° is an envelope of 211, 112 and 300 diffractions of apatite [16]. Peak intensity
2 of the carbon-PEEK composite was higher than that of pure PEEK. These results also
3 support higher apatite-forming ability of the carbon-PEEK composites.

4 Figure 7 shows FT-IR ATR spectra of the surfaces of pure carbon substrates
5 subjected to H_2SO_4 treatment. Peaks assigned to $\text{O}=\text{S}=\text{O}$ were observed at 1050 and
6 1200 cm^{-1} , while that assigned to $\text{C}=\text{O}$ was observed at 1650 cm^{-1} [16]. This means that
7 sulfo group can be incorporated even on pure carbon by H_2SO_4 treatment.

8 Figure 8 shows SEM photographs of the surfaces of pure carbon substrates
9 subjected to H_2SO_4 and CaCl_2 treatment, which were soaked in SBF for 14 days.
10 Deposits were observed on the specimens after soaking in SBF. They were confirmed to
11 contain Ca and P by EDX. This indicates that H_2SO_4 -treated pure carbon has ability to
12 form a calcium phosphate in SBF, although it was not confirmed with the apatite.

13 The hydrophilic sulfo group was incorporated into both the pure PEEK and
14 carbon-PEEK composites by H_2SO_4 treatment. This is supported by the significant
15 improvement of hydrophilicity shown in Fig. 1. Judging from the result that the
16 incorporated sulfo group was converted to calcium sulfate dihydrate by the subsequent

1 CaCl₂ treatment, it was eliminated from the PEEK chain. The sulfonation reaction of the
2 aromatic ring is represented as follows [18]:

3



5

6 On the other hand, desulfonation occurs as a reverse reaction in aqueous conditions. The
7 eliminated sulfo group may react with Ca²⁺ to form the calcium sulfate dihydrate, which
8 has the lowest solubility in water among various calcium sulfates [19]. The release of
9 the calcium sulfate would induce the apatite formation by increasing the supersaturation
10 degree of the surrounding SBF with respect to the apatite.

11 The present results indicate that both pure PEEK and carbon-PEEK composites can
12 form the apatite in SBF when they are treated with H₂SO₄ and CaCl₂ solutions. The
13 carbon-PEEK composites showed significantly higher ability for apatite formation than
14 pure PEEK (Figs. 5 and 6). It is assumed that many functional groups able to induce
15 apatite nucleation are incorporated into the dispersed carbon phase in the carbon-PEEK
16 composites. This assumption is supported by the results that even pure carbon can be

1 incorporated into the sulfo group and carboxyl group by H₂SO₄ treatment (Fig. 6), and
 2 that the treated carbon forms calcium phosphate in SBF (Figs. 7 and 8). Both the sulfo
 3 group and the carboxyl group have the ability for apatite formation in the body
 4 environment [20–22]. Furthermore, carbon-PEEK composites contained larger amounts
 5 of Ca²⁺ than the pure PEEK by CaCl₂ treatment. Actually, Ca concentration of SBF was
 6 2.7 and 3.7 mM after soaking of pure PEEK and carbon-PEEK, respectively, for 7 days
 7 (Original SBF: 2.5 mM). The ionic activity product (*IP*) of the hydroxyapatite was
 8 estimated according to equation (2) and using Ca²⁺ and PO₄³⁻ concentrations, and pH of
 9 SBF.

$$10 \quad IP = (g_{Ca^{2+}})^{10} (g_{PO_4^{3-}})^6 (g_{OH^-})^2 [Ca^{2+}]^{10} [PO_4^{3-}]^6 [OH^-]^2 \quad (2)$$

11 The ionic activity coefficient value of $\gamma_{Ca^{2+}}$, $\gamma_{PO_4^{3-}}$ and g_{OH^-} are supposed to be
 12 0.36, 0.06 and 0.72 at physiological ionic strength ($\mu=0.16$) [23]. The pH of SBF after
 13 soaking of both the specimens for 7 days was almost the same (7.58). Judging from the
 14 result that the carbon-PEEK released larger amount of Ca²⁺ than pure PEEK, the
 15 composite would provide higher degree of supersaturation with respect to the

1 hydroxyapatite. Overall, these factors would contribute to superior apatite formation on
2 the carbon-PEEK composites.

3 Zhao *et al.* reported that apatite covered almost the entire surface of the specimen
4 after soaking in SBF for 28 days [8]. On the other hand, most of the surface treated with
5 carbon-PEEK composite was covered with the apatite within 14 days. This suggests
6 that surface modification in the present study is effective for acceleration of the apatite
7 formation. However, most of clinically used bioactive materials form the apatite within
8 1 week [24]. Therefore further acceleration in the apatite formation is needed for
9 medical application in future work. Moreover, adhesion of the apatite layer on the
10 substrates was not so high. This would be attributed to desulfonation from PEEK.
11 Therefore improvement of this point is also needed to proceed to further assessment of
12 biological compatibility such as cell culture.

13

14 **4 Conclusions**

1 Carbon-PEEK composites were found to show bioactivity by surface chemical
2 modification using H₂SO₄ and CaCl₂ solutions. Their bioactivity was significantly
3 higher than that of pure PEEK. It was assumed that many functional groups able to
4 induce apatite nucleation are incorporated into the dispersed carbon phase in the
5 composites. These composites are expected to become novel biomaterials for spinal
6 reconstruction for their bioactivity and high mechanical strength. Furthermore, the
7 present modification process would be effective for the development of bioactive
8 carbon-based materials such as graphene.

9

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- 24

1 **Figure captions**

2

3 **Fig. 1.** Water contact angle of the substrates before and after H₂SO₄ treatment (N=3).

4 **Fig. 2.** EDX spectra of the surfaces of pure PEEK and carbon-PEEK composites
5 subjected to H₂SO₄ treatment.

6 **Fig. 3.** SEM photographs of the surfaces of pure PEEK and carbon-PEEK composites
7 subjected to H₂SO₄ and CaCl₂ treatments.

8 **Fig. 4.** TF-XRD patterns on the surfaces of pure PEEK and carbon-PEEK composites
9 subjected to H₂SO₄ and CaCl₂ treatments.

10 **Fig. 5.** SEM photographs of the surfaces of pure PEEK and carbon-PEEK composites
11 subjected to H₂SO₄ and CaCl₂ treatments, which were soaked in SBF for 14 days.

12 **Fig. 6.** TF-XRD patterns of the surfaces of pure PEEK and carbon-PEEK composites
13 subjected to H₂SO₄ and CaCl₂ treatments, which were soaked in SBF for 14 days.

14 **Fig. 7.** FT-IR ATR spectra of the surfaces of pure carbon substrates subjected to
15 H₂SO₄ treatment.

16 **Fig. 8.** SEM photograph of the surfaces of pure carbon substrates subjected to H₂SO₄
17 and CaCl₂ treatments, which were soaked in SBF for 14 days.

Figure

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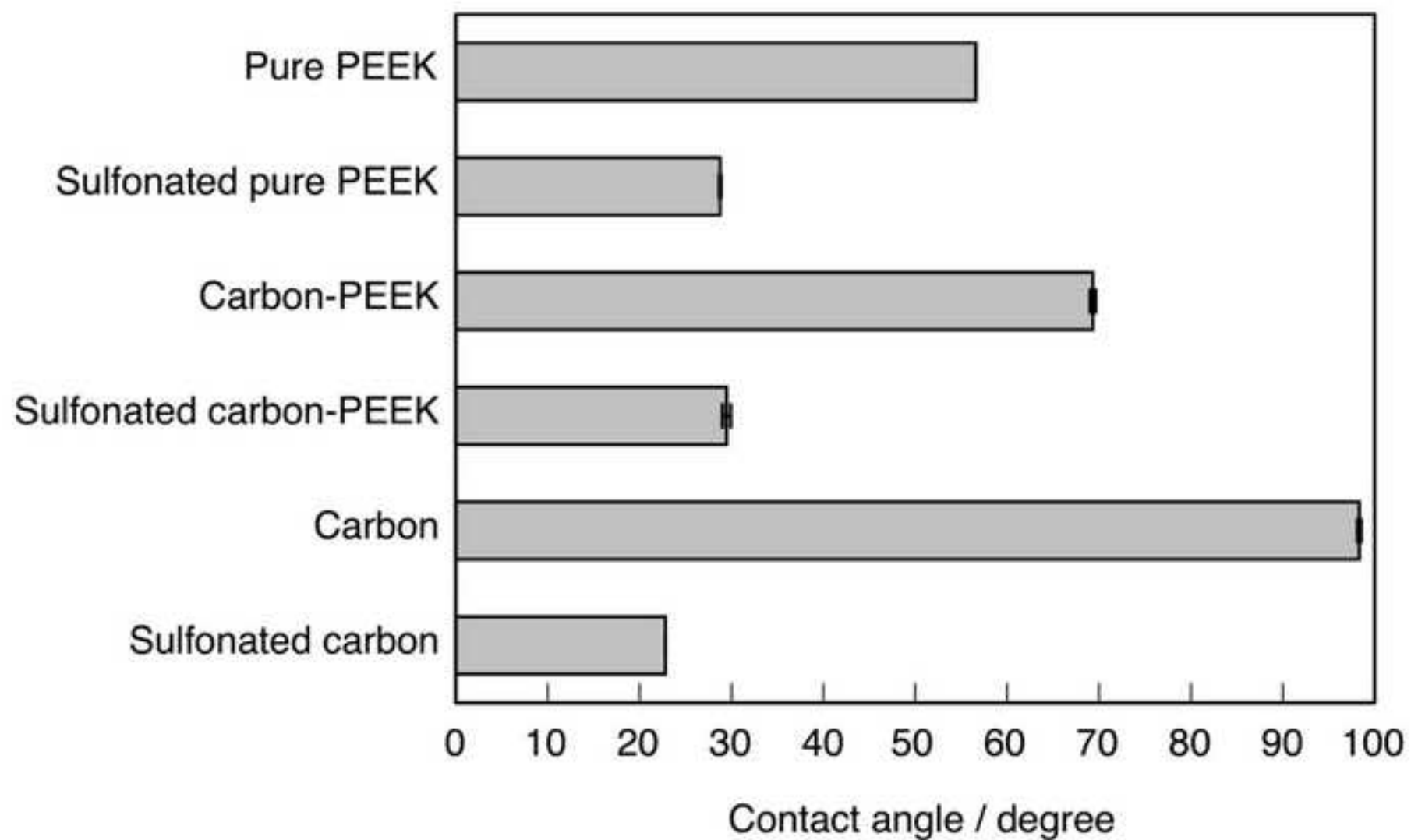


Fig. 1

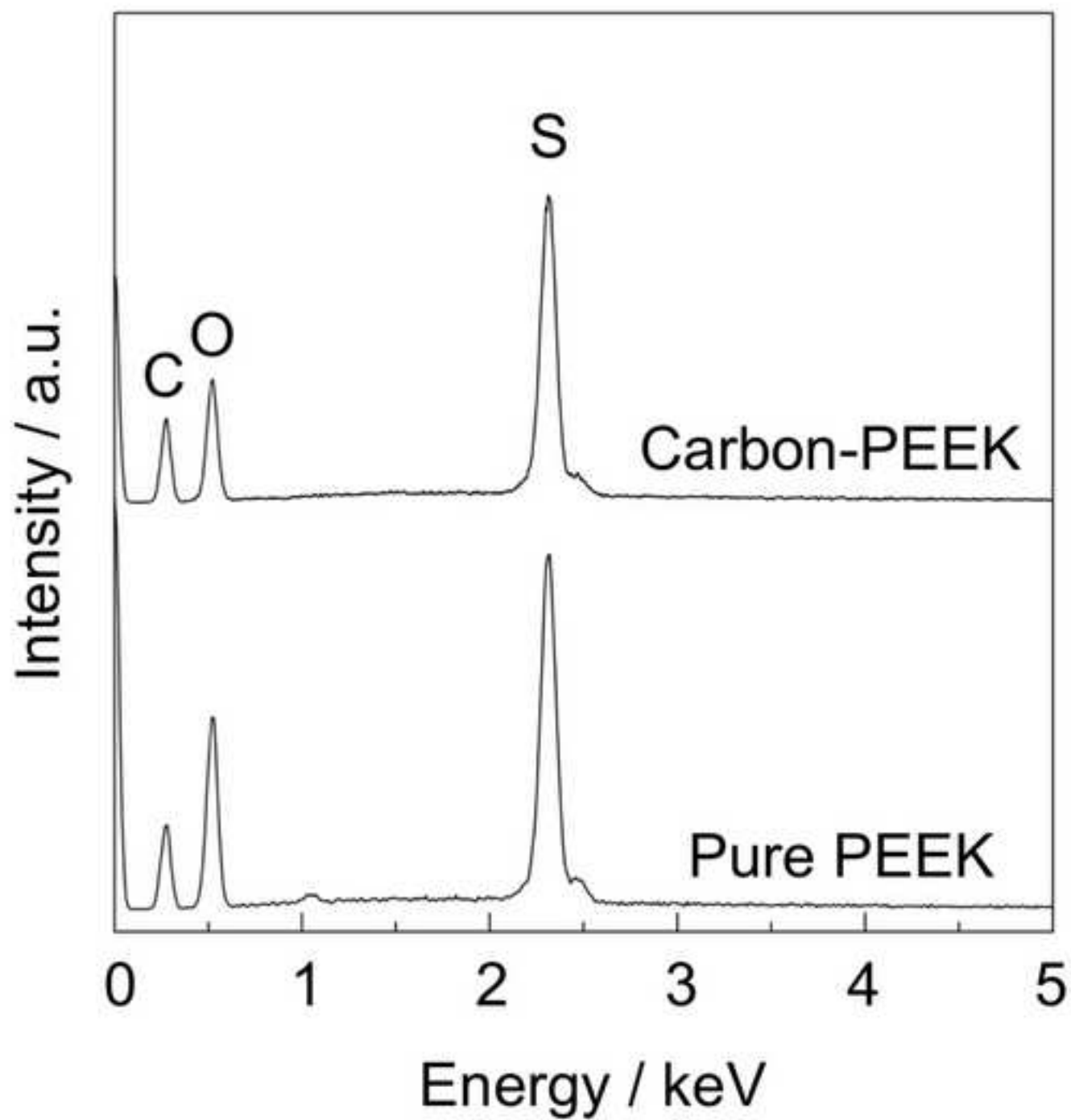


Fig. 2

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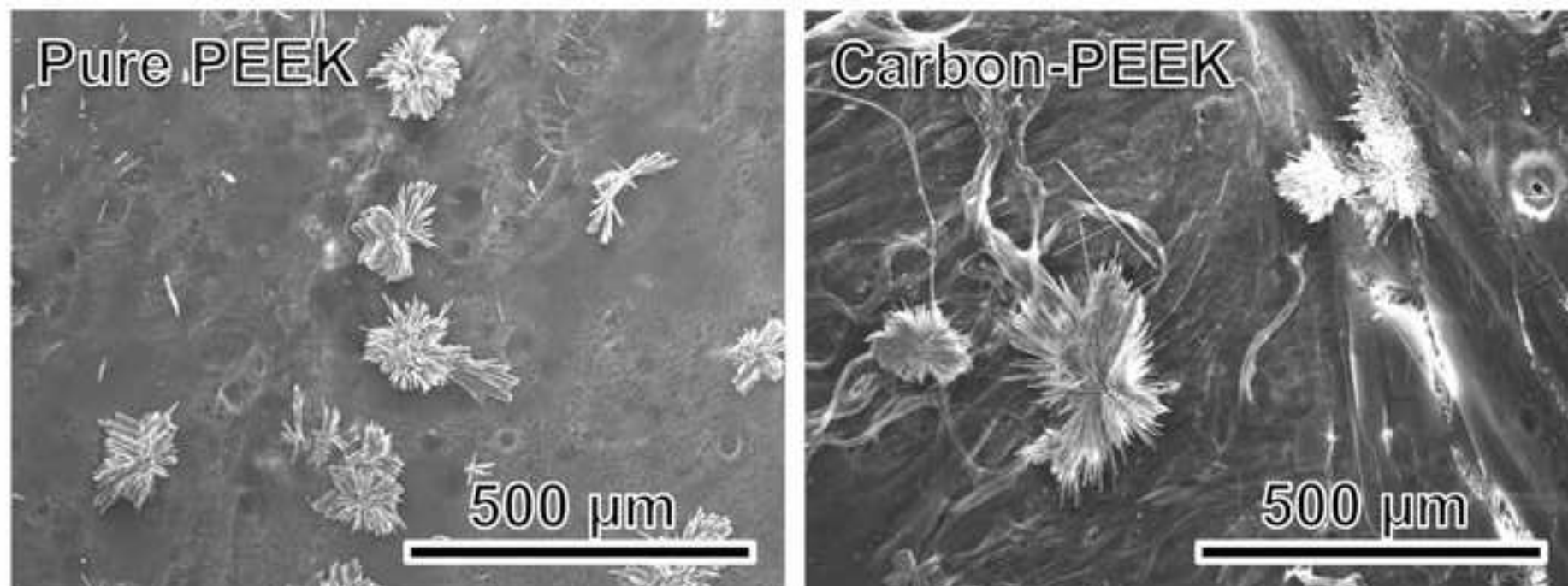


Fig. 3

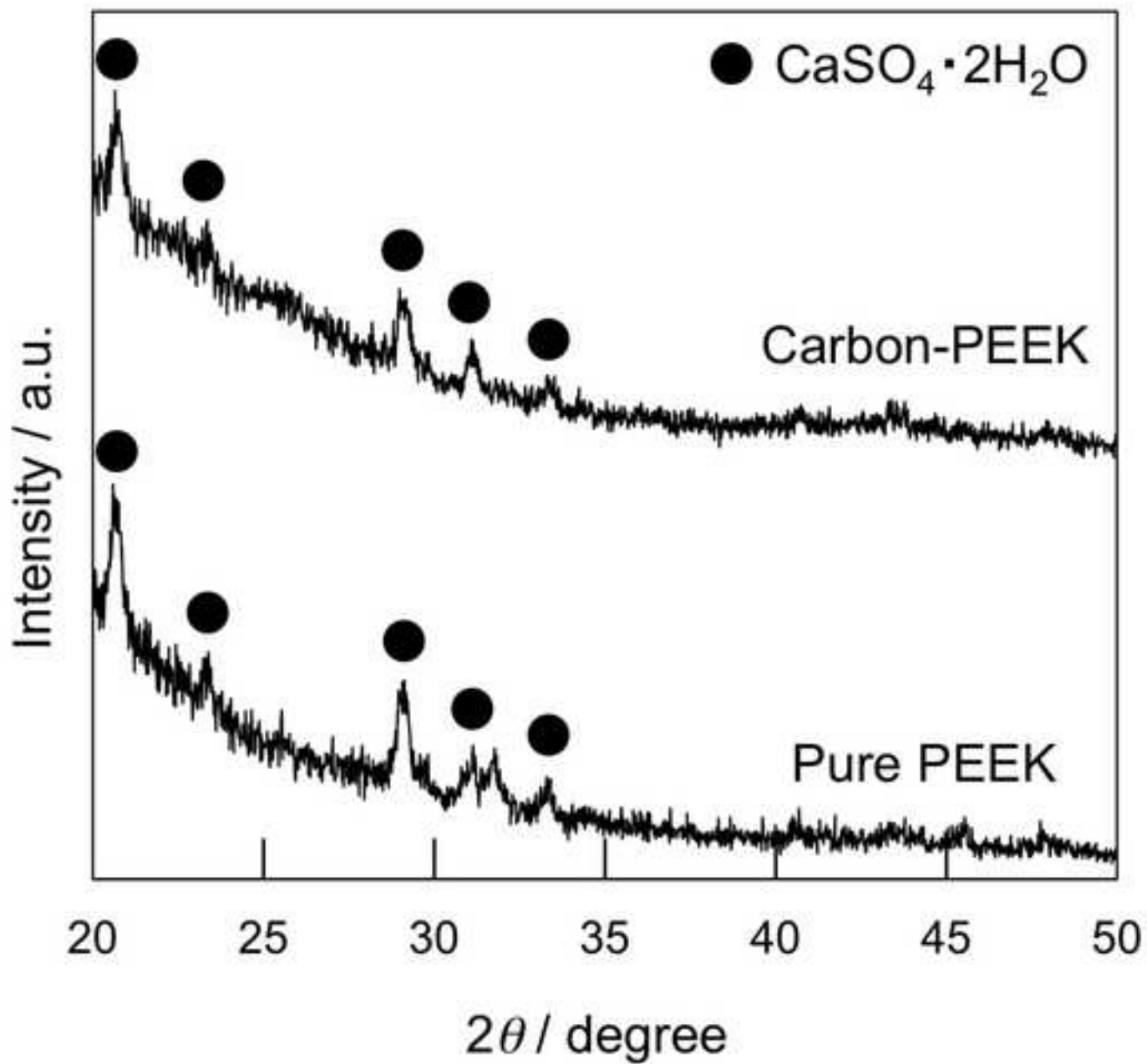


Fig. 4

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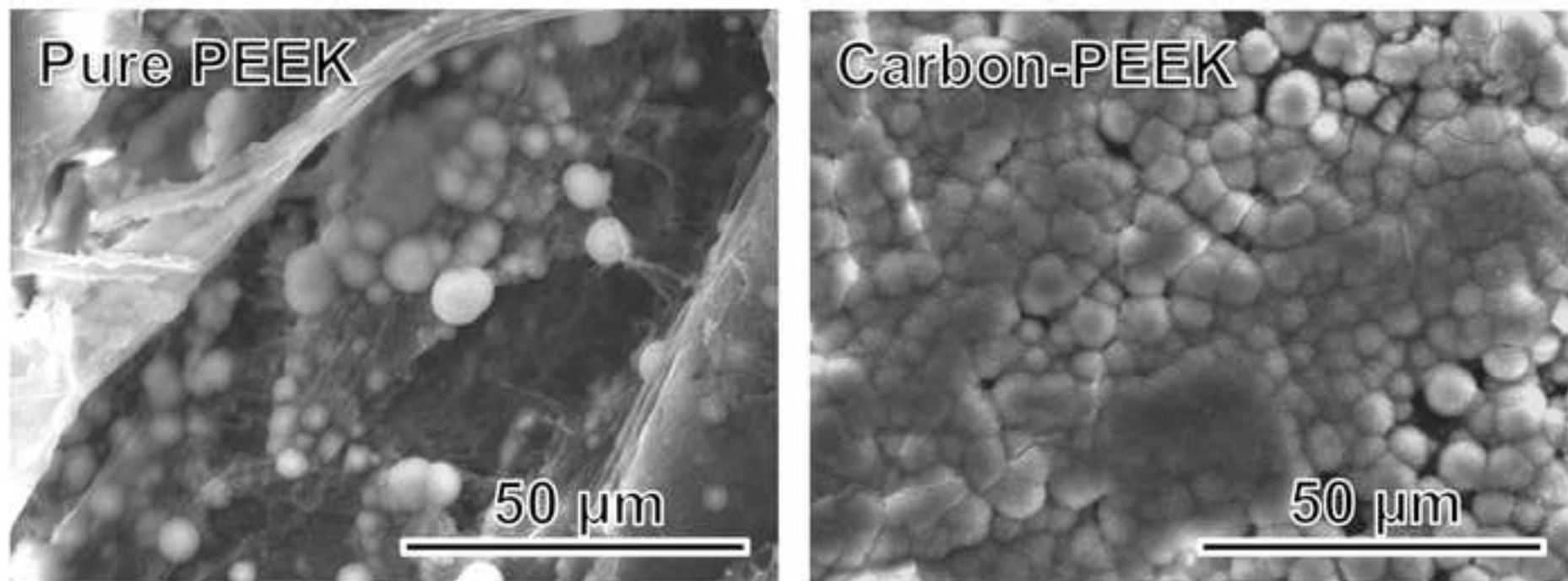


Fig. 5

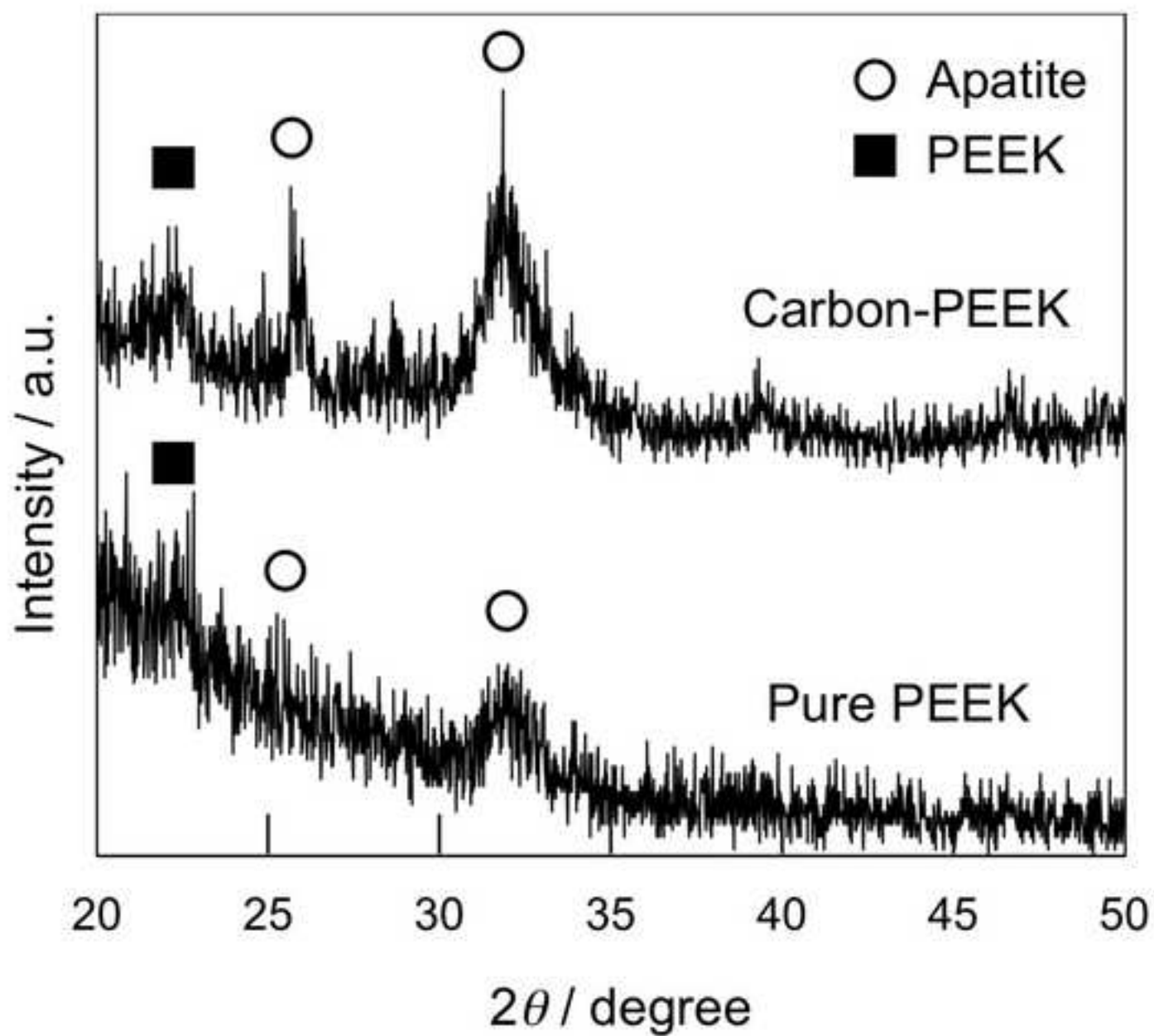


Fig. 6

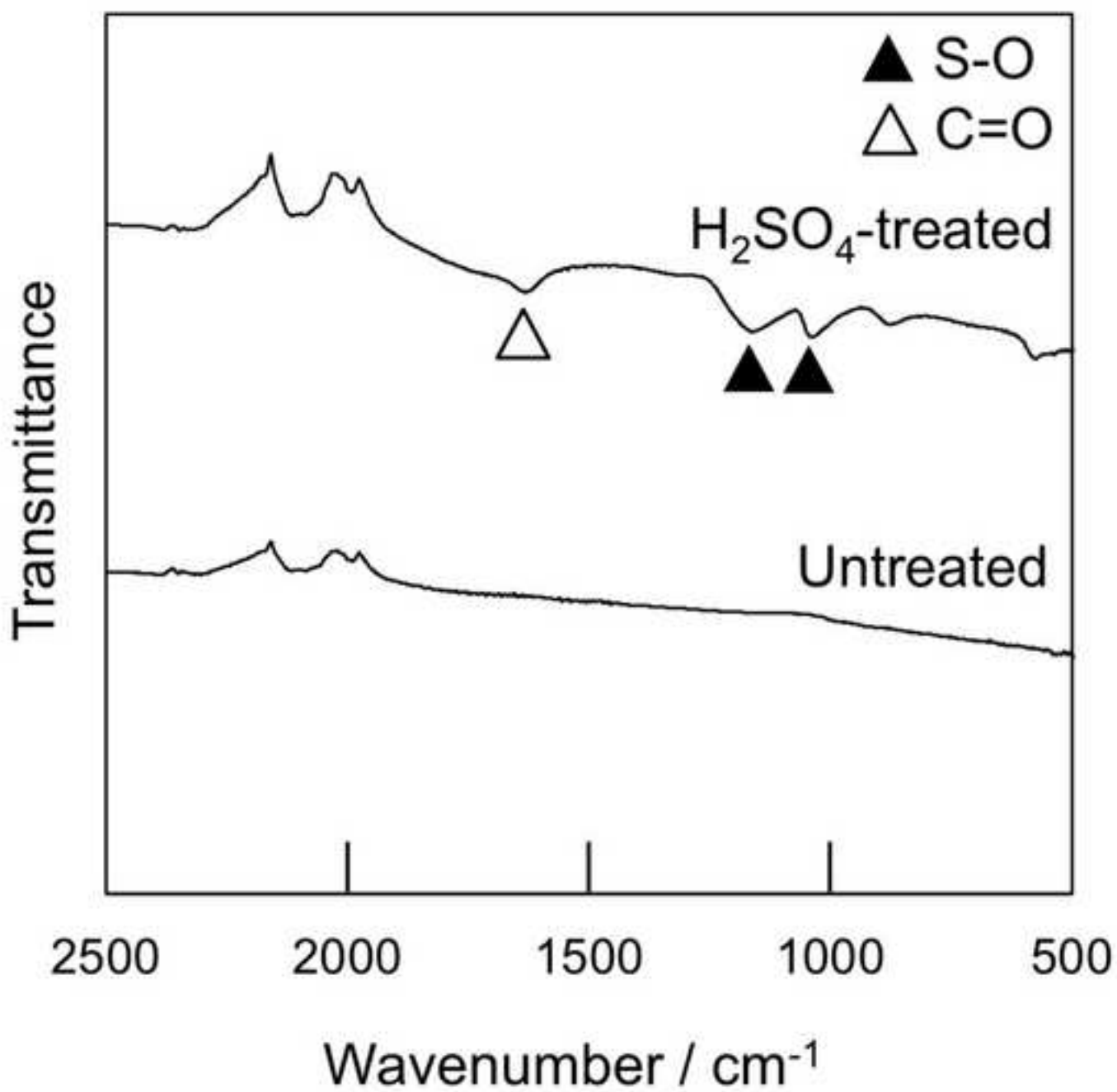


Fig. 7

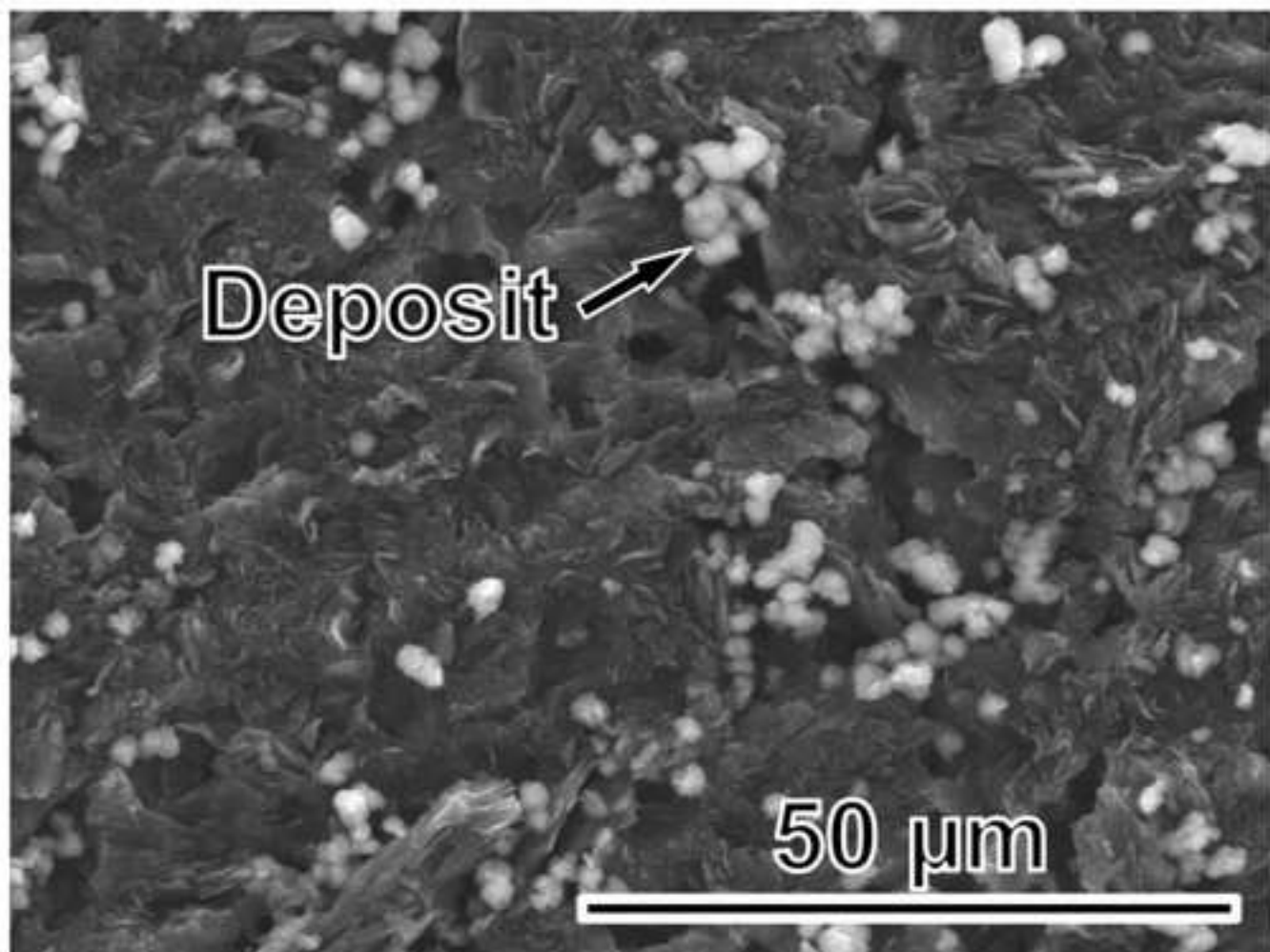


Fig. 8