5.5. Lipid peroxidation inhibitory activity

Rat liver microsomes were prepared from a phenobarbital-treated Wistar male rats, as previously described.[28] The incubation mixtures contained microsomes (0.56 mg protein) and the test compounds in a mixture of 690 μL of 0.1 M sodium phosphate buffer (pH 7.4, 0.1 mM EDTA) and 300 μL of ethanol were cooled in ice-water bath until lipid peroxidation initiation. *tert*-Butylhydroperoxide (1 mM) in distilled water (10 μL) was added into the incubation mixture to initiate lipid peroxidation and the mixture was incubated at 37°C for 20 min. After incubation, peroxidation reaction was terminated by 100 mL of BHT in ethanol (2%). Two milliliters of trichloroacetic acid (15%) and thiobarbituric acid (0.38%) in 0.25 M hydrochloric acid were added into the incubation mixture and then incubated at 85 °C for 20 min. The precipitate was removed by centrifugation (3000 rpm, 20 °C, 20 min) and the difference between the absorbance at 535 and 600 nm of supernatant was measured to gain a relative TBARS formation.[29] The IC_{50} values were determined by average of the results obtained by independent 3 trials for each test compound.

5.6. Cell culture

HL60 cells were cultured in the RPMI-1640 medium supplemented with 5% heat-inactivated fatal bovine serum, 100 IU/mL penicilline and 100 mg/mL streptomycin at 37 °C in a 5% CO_2 incubator with a humidified atmosphere.

5.7. Measurement of intracellular oxidative stress

HL60 cells were seeded on six-well multi-plates (1.0×10^6 cel/2 mL/well) and treated with DCFH-DA (10 μM) for 15 min under 5% CO_2 atmosphere at 37 °C. After pre-incubation, the cells were washed with phosphate buffered saline(−) and medium was exchanged. The cells were re-seeded on new six-well multi-plates (1.0×10^6 cells/well) and pre-incubated with test compounds in DMSO (10 μM, final concentration of DMSO was 1.0 v/v%) for 1 h under the same conditions. Then hydrogen peroxide (200 μM) was added to cells and incubated for 1 h under the same conditions. In control group, Millipore water (10 μL) was added instead of hydrogen peroxide. After incubation, cells were collected and centrifugated at 1000 rpm for 5 min. The pellet was re-suspended in 1 mL of BD FACS flow™ (BD, Japan) and cellular-fluorescence was quantified using a BD FACSCalibur™ flow cytometer (BD, Japan) with excitation and emission settings of 488 and 530 nm, respectively. FACS analysis was performed about 10,000 cells for each sample. The obtained data were analyzed by BD CellQuest™ Pro.

5.8. Cytotoxicity assay

HL60 cells were seeded on six-well multi-plates (1.0×10^6 -cells/well) and the test compounds (100 μM) in DMSO (final concentration of DMSO was 1 v/v%) were added followed by incubation for 24 h at 37 °C under 5% CO_2 atmosphere. After incubation, the cells were collected and centrifugated at 1000 rpm for 5 min. The medium was replaced by the same volume of phosphate buffered saline(−) and the cells were re-suspended. The suspension was mixed with trypan blue dye and viable cells were counted by dye exclusion method with a Vi-CELL™ (Beckman Coulter Inc.). The cell viability was expressed relative to the vehicle control (DMSO only) group ($n = 3$).

Acknowledgments

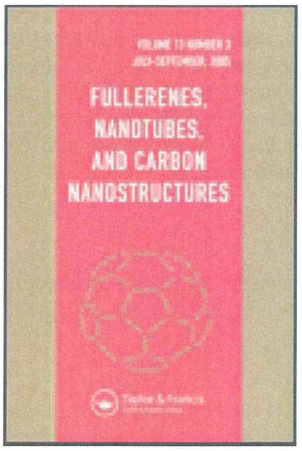
This work was supported by Platform for Drug Discovery, Informatics, and Structural Life Science from the Ministry of Education, Culture, Sports, Science and Technology, Japan (MEXT), 2012–2016, Strategic Research Foundation at Private Universities of the MEXT, 2011–2015 and Graduate School Doctoral Student Grant-in-Aid Program 2011 and 2012 from Keio University.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.10.021.

References and notes

1. For a recent review, see: (a) Uttara, B.; Singh, A. V.; Zamboni, P.; Mahajan, R. T. *Curr. Neuropharmacol.* **2009**, *7*, 65; (b) Reuter, S.; Gupta, S. C.; Chaturvedi, M. M.; Aggarwal, B. B. *Free Radical Biol. Med* **2010**, *49*, 1603; (c) Marchi, E. D.; Baldassari, F.; Bononi, A.; Wieckowski, M. R.; Pinton, P. *Oxid. Med. Cell. Longev.* **2013**, *2013*, 564961; (d) Lin, M. T.; Beal, M. F. *Nature* **2006**, *443*, 787; (e) Li, H.; Horke, S.; Förstermann, U. *Trends Pharm. Sci.* **2013**, *34*, 313.
2. Houkin, K.; Nakayama, N.; Kamada, K.; Noujou, T.; Abe, H.; Kashiwaba, T. *J. Stroke Cerebrovasc. Dis.* **1998**, *7*, 315.
3. Otomo, E. *Cerebrovasc. Dis.* **2003**, *15*, 222.
4. Yoshino, H.; Kumura, A. *Amyotroph. Lateral Scler.* **2006**, *7*, 241.
5. For a recent review, see: Steinhubl, S. R. *Am. J. Cardiol.* **2008**, *101*, 14D.
6. Hooper, D. C.; Spitsin, S.; Kean, R. B.; Champion, J. M.; Dickson, G. M.; Chaudhry, I.; Koprowski, H. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 675.
7. Ashtari, F.; Bahar, M.; Aghaei, M.; Zahed, A. *J. Clin. Neurosci.* **2013**, *20*, 676.
8. Liu, B.; Shen, Y.; Xiao, K.; Tang, Y.; Cen, L.; Wei, J. *Neurol. Res.* **2012**, *34*, 163.
9. Davis, J. W.; Grandinetti, A.; Waslien, C. I.; Ross, G. W.; White, L. R.; Morens, D. M. *Am. J. Epidemiol.* **1996**, *144*, 480.
10. Ascherio, A.; LeWitt, P. A.; Xu, K.; Eberly, S.; Watts, A.; Matson, W. R.; Marras, C.; Kieburtz, K.; Rudolpj, A.; Bogdnov, M. B.; Schwid, S. R.; Tennis, M.; Tanner, C. M.; Beal, M. F.; Lang, A. E.; Oakes, D.; Fahn, S.; Shoulson, I.; Schwarzschild, M. A. *Arch. Neurol.* **2009**, *66*, 1460.
11. Shen, C.; Guo, Y.; Lin, C.; Ding, M. *Can. J. Neurol. Sci.* **2013**, *40*, 73.
12. Yasuda, D.; Takahashi, K.; Kakinoki, T.; Tanaka, Y.; Ohe, T.; Nakamura, S.; Mashino, T. *MedChemComm* **2013**, *4*, 527.
13. King, L. J.; Parke, D. V.; Williams, R. T. *Biochem. J.* **1966**, *96*, 266.
14. Papy-Grcía, D.; Barbier, V.; Tournaire, M.; Cane, A.; Brugère, H.; Crumeyrolle-Arias, M.; Barritault, D. *Clin. Biochem.* **2003**, *36*, 215.
15. Crumeyrolle-Arias, M.; Tournaire, M. C.; Cane, A.; Launay, J. M.; Barritault, D.; Medvedev, A. *Biochem. Pharmacol.* **2004**, *67*, 977.
16. Cane, A.; Tournaire, M. C.; Barritault, D.; Crumeyrolle-Arias, M. *Biochem. Biophy. Res. Commun.* **2000**, *276*, 379.
17. Crumeyrolle-Arias, M.; Tournaire, M. C.; Rabot, S.; Malpaux, B.; Thiéry, J. C. *J. Neurosci. Res.* **2008**, *86*, 202.
18. Garden, S. J.; da Silva, R. B.; Pinto, A. C. *Tetrahedron* **2002**, *58*, 8399.
19. Popp, F. D. *J. Heterocycl. Chem.* **1982**, *19*, 589.
20. Hatanaka, M.; Takahashi, K.; Nakamura, S.; Mashino, T. *Bioorg. Med. Chem.* **2005**, *13*, 6763.
21. Finizio, A.; Guardo, A. D. *Chemosphere* **2001**, *45*, 1063.
22. Conde de la Rosa, L.; Schoemaker, M. H.; Vrenken, T. E.; Buist-Homan, M.; Havinga, R.; Jansen, P. L. M.; Moshage, H. *J. Hepatol.* **2006**, *44*, 918.
23. Kim, B. H.; Han, M. J.; Chung, A. S. *Free Radical Biol. Med.* **2001**, *30*, 686.
24. Tournier, C.; Thomas, G.; Pierre, J.; Jacquemin, C.; Pierre, M.; Saunier, B. *Eur. J. Biochem.* **1997**, *244*, 587.
25. Ha, J. S.; Sung, H. Y.; Lim, H. M.; Kwon, K. S.; Park, S. S. *Neurosci. Lett.* **2012**, *526*, 112.
26. For a previous review, see: Babich, H. *Environ. Res.* **1982**, *29*, 1.
27. Yamaji, K.; Sarker, K. P.; Maruyama, I.; Hizukuri, S. *Plant. Med.* **2002**, *60*, 16.
28. Ohe, T.; Mashino, T.; Hirobe, M. *Arch. Biochem. Biophys.* **1994**, *310*, 402.
29. Bernheim, F.; Bernheim, M. L.; Wilkur, K. M. *J. Biol. Chem.* **1948**, *174*, 257.



Fullerenes, Nanotubes and Carbon Nanostructures
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lfnn20

Radical-scavenging Ability of Hydrophilic Carbon Nanoparticles: From Fullerene to Its Soot

Ken Kokubo [a] , Shizuka Yamakura [a] , Yuji Nakamura [a] , Hiroshi Ueno [a] & Takumi Oshima [a]

[a] Division of Applied Chemistry , Graduate School of Engineering, Osaka University , Osaka , Japan

Accepted author version posted online: 02 Dec 2013.Published online: 16 Jan 2014.

To cite this article: Ken Kokubo , Shizuka Yamakura , Yuji Nakamura , Hiroshi Ueno & Takumi Oshima (2014) Radical-scavenging Ability of Hydrophilic Carbon Nanoparticles: From Fullerene to Its Soot, Fullerenes, Nanotubes and Carbon Nanostructures, 22:1-3, 250-261, DOI: 10.1080/1536383X.2013.812637

To link to this article: http://dx.doi.org/10.1080/1536383X.2013.812637

Fullerenes, Nanotubes, and Carbon Nanostructures, 22: 250–261, 2014

ISSN: 1536-383X print / 1536-4046 online
DOI: 10.1080/1536383X.2013.812637

Taylor & Francis
Taylor & Francis Group

Radical-scavenging Ability of Hydrophilic Carbon Nanoparticles: From Fullerene to Its Soot

KEN KOKUBO, SHIZUKA YAMAKURA, YUJI NAKAMURA, HIROSHI UENO AND TAKUMI OSHIMA

Division of Applied Chemistry, Graduate School of Engineering, Osaka University, Osaka, Japan

We have recently developed a facile synthetic method for highly water-soluble fullerene, so-called fullerenol, for the treatment of fullerene with hydrogen peroxide. This method was applied to fullerene soot to yield the corresponding new hydrophilic carbon materials, and the obtained products were subjected to infrared spectroscopy and elemental analysis. The DLS particle size analysis demonstrated the relatively high dispersion of hydrophilic fullerene soot with a diameter of ~70 nm in water, while the hydrophilic activated carbon obtained by the same treatment showed the larger aggregation with diameters of 200 and 970 nm. The surface analysis using FE-SEM showed the difference in morphology between fullerene soot and activated carbon as well as between before and after hydrophilic treatment of the soot with hydrogen peroxide. Moreover, this hydrophilic fullerene soot exhibited high antioxidant activity (%AOA) up to 87% compared with fullerenol $C_{60}(OH)_{36}$ (54%) and C_{60} (50%) evaluated by β-carotene bleaching method.

Keywords Fullerenol, fullerene soot, polyhydroxylation, carbon nanoparticle, radical scavenger

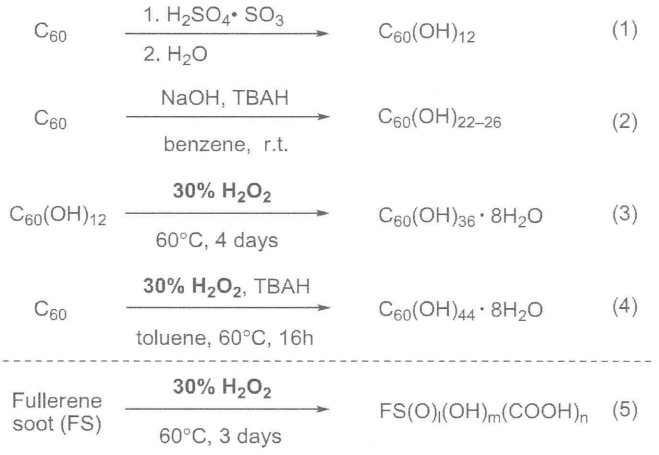
1. Introduction

Hydrophilic carbon particles have been used for black calligraphy ink since ancient times and are curently used in ink-jet printer ink. This material is readily available from soot at low cost and has the advantage of lightweight and relatively low toxicity. It can also be used as an adsorbent for undesired and toxic compounds, depending on the hybridization and surface area of the carbon structure, in the form of porous activated carbon (AC). Recently, the dispersion of fullerene, carbon nanotubes, graphene, nanodiamonds, and other nanocarbons in aqueous solution through the introduction of hydrophilic substituents or the use of surfactants has attracted growing attention (1–3). Hydrophilic carbon materials, especially nanoscale materials that disperse easily in aqueous solution, have great potential for applications in life science and materials chemistry because of their versatile properties and facile modifications. One promising candidate is polyhydroxylated fullerenes, so-called fullerenols (Scheme 1).

Among the reported synthetic methods for fullerenols with various number of hydroxyl groups developed so far (4,5), our method using hydrogen peroxide can produce the

This article is dedicated to Professor Takeshi Akasaka on the occasion of his 65th birthday.

Address correspondence to Ken Kokubo, Division of Applied Chemistry, Graduate School of Engineering, Osaka University, Suita, Osaka 565-0871, Japan. E-mail: kokubo@chem.eng.osaka-u.ac.jp



Scheme 1. Synthesis of various fullerenols.

highly hydroxylated and water-soluble fullerenols $C_{60}(OH)_{36}$ (6) and $C_{60}(OH)_{44}$ (7). These fullerenols are covered with many hydroxyl groups (an average 36 or 44 per 60 carbon atoms, forming a complex mixture of regioisomers) and thus are highly hydrophilic. However, due to the introduction of such a large number of hydroxyl groups, unavoidable high strain on fullerene cage would lead to the skeletal rearrangement along with C–C bond cleavage, forming holes at the fullerenyl cage. Therefore, such a fullerene multiadduct is not an *original* fullerene anymore but rather can be considered a "hydrophilic hollow carbon nanoparticle."

These hydrophilic modifications can also be successfully applied to endohedral fullerenes. Such external functionalization of endohedral metallofullerenes, especially to solubilize in polar solvents, can be a versatile and promising protocol for controlling the physicochemical properties as well as the static behavior of encapsulated metal ions. Whereas many metal-encapsulated endohedral fullerene derivatives have been well developed (8), only a few examples on fullerenol, $Gd@C_{60}(OH)_n$ and $Gd@C_{82}(OH)_n$, have been reported (9,10). More recently, we have succeeded in synthesizing Li-encapsulated fullerenols $Li^+@C_{60}O^-(OH)_n$ and found that it behaves as "cation-encapsulated anion nanoparticle" (11,12).

Although the structure can be defined only as an estimated average structure for these fullerenols, much interest and effort have been devoted to the studies of its antioxidant activity (13,14), the related bioactivities (15–17), the solubilizing agent for carbon nanotube (18), and other industrial applications (19,20). In order to know the scope and limitations of our hydrogen peroxide functionalization method, as well as to obtain other valuable carbon materials with similar properties, we focused our attention on fullerene soot (FS). One current method for producing fullerenes on a large industrial scale is a combustion method. Controlled combustion of toluene affords a large amount of soot from which fullerenes can be extracted with organic solvent. Residual soot after extraction, FS in the present study, is commercially available and appears to contain a mixture of fullerene precursors, such as the bowl-like sumanene or coronene structures, hemispheres of fullerene, or open-cage fullerenes entrapped in an amorphous carbon network and shows interesting properties (21,22). Therefore, FS seems to behave somewhat like pristine fullerene in chemical modification, although carbon nanotubes and graphite/graphene are known to

much more resist such modification due to the lack of reactive five-membered fused rings and strained structures.

In the present study, we extended our polyhydroxylation technique to other carbon materials, such as FS and AC, in order to solubilize them in water and characterized the obtained hydrophilic carbon materials by infrared spectroscopy (IR), elemental analysis, thermogravimetric analysis (TGA), and dynamic light scattering (DLS) measurements as well as FE-SEM surface observation (Scheme 1). We also investigated the antioxidant activity as assessed by β-carotene bleaching assay of these hydrophilic carbon materials.

2. Experimental

2.1. Synthesis of $C_{60}(OH)_{36}\bullet 8H_2O$

To the starting material $C_{60}(OH)_{10}$ (purchased from Frontier Carbon Corporation; 0.100 g), 30% hydrogen peroxide solution (10 mL) was added, and the mixture was vigorously stirred for 4 days at 60°C under air until the suspension turned into a clear yellow solution. After cooling the solution, a mixture of 2-propanol, diethyl ether, and hexane (50 mL each) was added gradually to yield a milky white precipitate. After careful centrifugation and decantation, the residual solid was washed twice with ~50 mL of diethyl ether using the general ultrasonication–centrifuge–decantation procedure. Drying of the residue under vacuum at room temperature overnight gave fullerenol (0.097 g, 67%) as a pale yellowish-brown powder.

2.2. Preparation of Hydrophilic Fullerene Soot (HFS)

A slurry of FS (Nanom Black™, purchased from Frontier Carbon Corporation, used as received; 1.0 g) in 30% hydrogen peroxide (20 mL) was stirred for 72 hours at 60°C under air. After centrifugation with additional deionized water (10 mL), the residual black solid was washed with methanol and diethyl ether. Drying under vacuum at room temperature overnight gave hydrophilic FS as a black solid (abbreviated as HFS1; 0.80 g). The aqueous solution obtained by centrifugation (~30 mL) was combined with 150 mL each of 2-propanol, diethyl ether, and hexane to precipitate a solid. Further washing with diethyl ether with centrifugation followed by drying under vacuum at room temperature overnight gave hydrophilic FS as a black solid (abbreviated as HFS2; 0.18 g).

2.3. Preparation of Hydrophilic Activated Carbon (HAC)

A slurry of AC (powder purchased from Wako, used as received; 1.0 g) in 30% hydrogen peroxide (20 mL) was stirred for 72 hours at 60°C under air. After centrifugation with additional deionized water (10 mL), the residual black solid was washed with methanol and diethyl ether. Drying under vacuum at room temperature overnight gave hydrophilic AC as a black solid (abbreviated as HAC; 0.74 g).

2.4. Measurements

Infrared spectra were recorded on a JASCO FT/IR-300E spectrometer. UV–visible spectra for β-carotene bleaching assay were recorded on a Shimadzu UV-1800 spectrometer. TGA charts were obtained with a Shimadzu TA-50 instrument. Particle size was measured by the dynamic light scattering (DLS) method using a Marvern Instruments Zetasizer Nano ZSl.

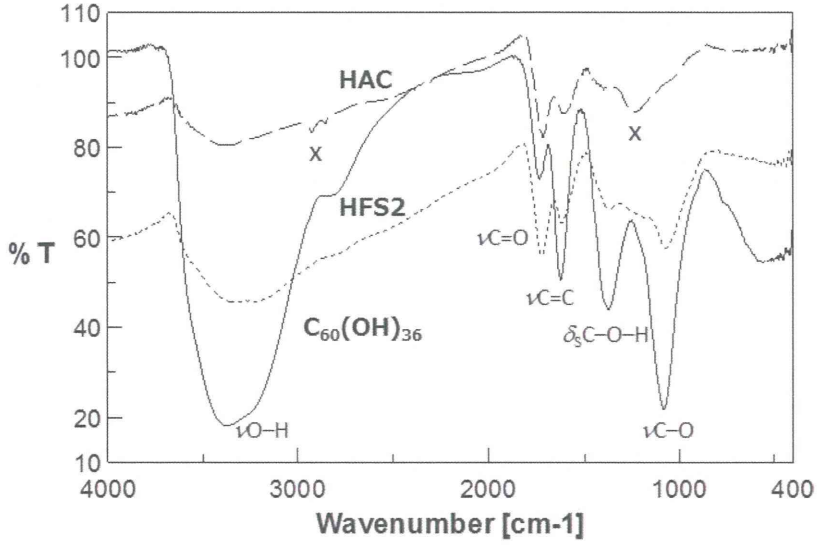


Figure 1. IR spectra of HAC, HFS2, and $C_{60}(OH)_{36}$.

FE-SEM observation was conducted using a JEOL JSM-6330FT with accelerating voltage of 20 kV. All the samples were used as a powder. AC and HFS samples were sputter coated by osmium oxide using Osmium Plasma Coater OPC60A before observation.

3. Results and Discussion

3.1. Characterization

The IR spectra of HAC, HFS2, and fullerenol $C_{60}(OH)_{36}$ were shown in Figure 1. No clear difference between the spectra of HFS1 (not shown) and HFS2 was observed. The characteristic four peaks for $C_{60}(OH)_{36}$, bands at 3400, 1625, 1375, and 1080 cm^{-1} assignable to νO–H, νC=C, δ_sC–O–H, and νC–O, respectively, were also observed in the spectrum of HFS2. In addition, the small band at 1730 cm^{-1} for $C_{60}(OH)_{36}$, assignable to νC=O, were significantly intensified in both spectra of HAC and HFS2. As reported for the oxidation of carbon nanotube using hydrogen peroxide, carboxylic group is mainly introduced instead of hydroxyl group (23,24). Graphene oxide is known to be formed by the oxidation of graphite using $KMnO_4/H_2O_2$ (25,26). In the spectrum of HAC, the new peaks at 2920 and 1240 cm^{-1} (marked as x) were observed, indicating the coexistence of different functional groups; e.g., epoxide. This is probably because of the different reactivity of AC toward oxidation due to the different sp^2/sp^3 carbon hybridization ratio and the different functional groups on the amorphous carbon surface compared with FS and C_{60}. The peak at 1240 cm^{-1} in HAC, assignable to epoxide ν_sC–O–C (26), was also observed in the spectrum of HFS as a weak broad band. This similarity as well as the apparent appearance of C=O bonds suggest that the carbon linkage of FS is similar to that of sp^2-hybridized fullerene as well as that of sp^3-like AC.

As shown in Figure 2, TGA chart of HFS1 is similar to that of $C_{60}(OH)_{36}$ under the same condition (6). This means that HFS1 has the secondary bound water, the amount of

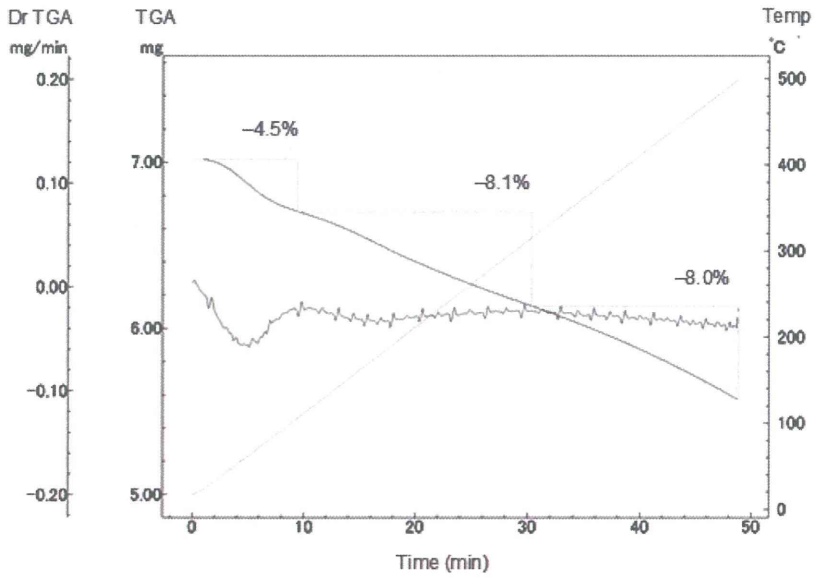


Figure 2. TGA chart for HFS1 heated at the rate of 10 °C/min under N_2.

which was estimated as 4.5wt% from the first weight loss up to 100°C. The second reduction (8.1wt%) might be attributed to the dehydration of the introduced hydroxyl groups; however, the amount was considerably less than that of $C_{60}(OH)_{36}$ (25wt%), consistent with the results of IR.

The results of elemental analysis and water content measurement for the synthesized carbon materials are summarized in Table 1. As compared with a pure carbon allotrope of fullerene C_{60}, both FS and AC contained some impure elements probably due to the oxygen-containing groups, lowering the carbon content. Treatment of FS and AC with hydrogen peroxide at 60°C for 3 days yielded the corresponding hydrophilic carbon materials, such as HFS and HAC, respectively. Their dispersion ability in water was greatly improved with decreasing carbon content and increasing hydrogen content. For example, HFS2, a higher polarity component of hydrophilic FS precipitated from aqueous solution after centrifugation, had lower carbon content (58%) than HFS1 (72%), which was the

Table 1
Elemental analysis and water content of carbon materials after treatment with H_2O_2

Materials	%C	%H	%N	Water content (wt%)[a]
C_{60}	>99	–	–	<0.1
fullerene soot (FS)	93.21	1.31	0.05	<1
activated carbon (AC)	84.20	2.36	0.62	–
hydrophilic FS (HFS1)	72.03	2.23	0.06	4.5
hydrophilic FS (HFS2)	58.37	3.01	0.12	–
hydrophilic AC (HAC)	57.92	3.26	0.32	–
$C_{60}(OH)_{36}\bullet 8H_2O$	48.06	3.61	–	8.9

[a]Measured by Karl-Fisher method for C_{60} and by thermogravimetric analysis (TGA) for others.

residual solid after centrifugation. The water-soluble fullerenol $C_{60}(OH)_{36}$ had the lowest carbon content (48%) as well as 8.9wt% entrapped water by hydrogen bond that could not be removed by usual drying under vacuum at room temperature. The similar water content was detected in hydrophilic FS, up to 4.5wt%, indicating the introduction of hydrophilic –OH/–COOH groups. The higher carbon content (93%) and the lower hydrogen content (1.3%) of FS as compared with the corresponding values for AC (84% and 2.4%, respectively) implied that the carbon structure of FS (e.g., sp^2-hybridized bowl-like sumanene or coronene structures coexisted with sp^3-hybridized amorphous carbon) was somewhat similar to that of sp^2-hybrlidized C_{60}, well consistent with the spectrum similarity of IR spectroscopy.

The particle size distribution of the prepared hydrophilic carbon materials was measured in water (0.1wt%) by DLS (Figure 3). The particle size distribution of $C_{60}(OH)_{36}$ was

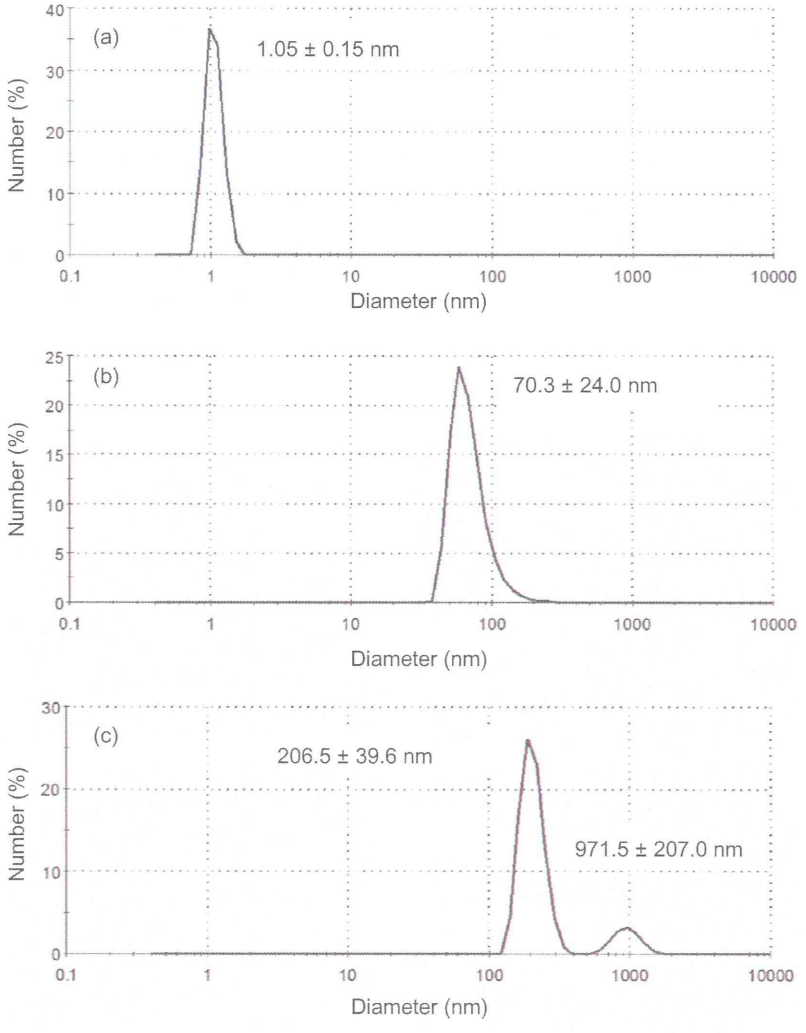


Figure 3. Particle size distribution of (a) $C_{60}(OH)_{36}$, (b) hydrophilic fullerene soot (HFS2), and (c) hydrophilic activated carbon (HAC) in water (0.1wt%) measured by DLS, expressed by size distribution in number.

rather narrow at 1.05 ± 0.15 nm, clearly indicating that $C_{60}(OH)_{36}$ was highly dispersed in water at the molecular level (ca. 1 nm). In contrast, HFS1 and HFS2 prepared from soot had relatively larger particle size distributions at 197.9 ± 84.7 and 70.3 ± 24.0 nm, respectively. However, HAC showed a much larger particle size distribution at 971.5 ± 207.0 nm along with smaller particles at 206.5 ± 39.6 nm. It is interesting that HFS1 showed higher dispersing property than HAC despite its higher carbon content (72%) than that of HAC (58%).

The surface morphology on chemical modification of FS was examined by using FE-SEM. While the raw FS showed the small particle-aggregated fine structure (Figure 4), the raw AC showed rather smooth surface (Figure 5b at lower magnification) with microporous structure (Figures 5c, d at higher magnification). After the treatment with hydrogen peroxide for FS, HFS1 showed the similar particle-aggregated structure (Figure 6). However, the particle-structure size was somewhat enlarged through the chemical modification process. It is also noted that the similar surface analysis of C_{60} showed far more different smooth morphology from those of FS and AC due to the higher crystallinity of C_{60} (Figure 7).

3.2. *Radical-scavenging Ability as Assessed by β-Carotene Bleaching Assay*

The β-carotene bleaching assay is a common method in the field of food chemistry for evaluating antioxidant activity (27). The method is based on the discoloration of yellow β-carotene solution due to disruption of the π-conjugation by addition of a lipid peroxyl radical (LOO•) or its precursor radical (L•) generated from the autoxidation of linoleic acid by heating in air. In view of the highly π-conjugated molecules, both fullerene and β-carotene seem to enable accurate evaluation of antioxidant activity; i.e., radical scavenging

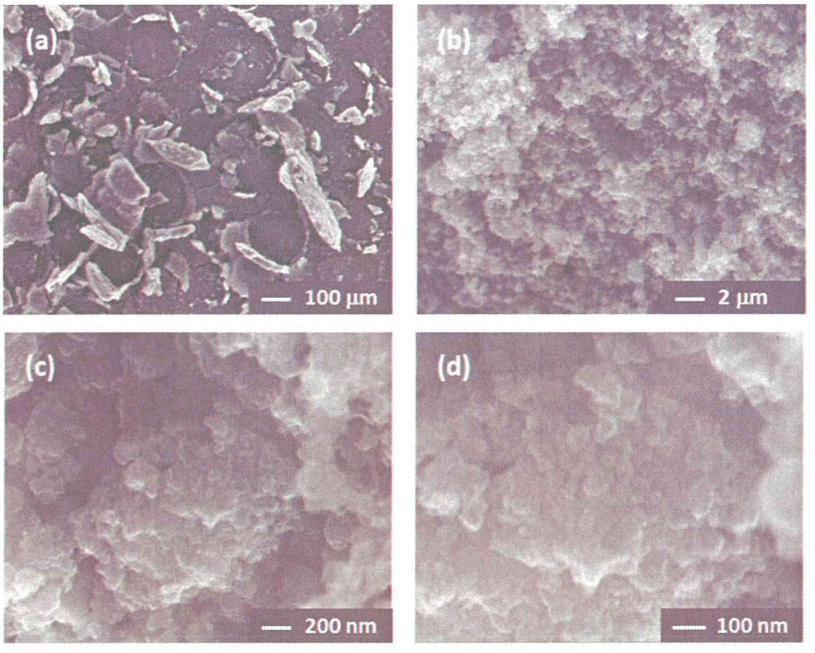


Figure 4. FE-SEM images of FS (a) × 90, (b) × 5,000, (c) × 50,000, and (d) × 100,000.