Facile Synthesis of Highly Water-Soluble Fullerenes More Than Half-Covered by Hydroxyl Groups

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ABSTRACT Using a novel hydrogen peroxide heating method, we synthesized milky white, water-soluble polyhydroxylated fullerenes (fullerenols) with 36–40 hydroxyl groups (estimated average) along with 8–9 secondary bound water molecules. The fullerenols exhibited high water solubility up to 58.9 mg/mL in a neutral (pH = 7) condition. Dynamic light scattering analysis showed a high dispersion property, to give a narrow particle size distribution within 0.7–2.0 nm.

KEYWORDS: fullerene · fullerenol · hydroxyl group · nanoparticle · water solubility

Water-soluble fullerenes are promising materials for use in the field of life science and as medicines or cosmetic ingredients in light of the expected high scavenging ability of their radical species.[1−6] For that reason, the development of facile synthetic methods to produce biocompatible water-soluble fullerenes on an industrial scale has been eagerly pursued. Because a polyhydroxylated fullerene, fullerenol, has been anticipated as a candidate for highly water-soluble fullerenes as a result of its medically applicable simple structure,[7,8] negligible toxicity,[2,9] expected neutral pH, and accessibility to further modifications,[10,23] in addition to its radical scavenging ability,[11−15] several synthetic methods for the fullerenols having various numbers of hydroxyl groups have been reported.[16−26] Generally, a fullerenol with fewer than 12 hydroxyl groups on a fullerene cage shows very poor water solubility,[16] whereas the fullerenols with more numerous hydroxyl groups (16 or 20–24) exhibit good water solubility.[21,26] However, although most water-soluble fullerenes studied were prepared using the latter method with NaOH as a hydroxylation reagent (or by a method using strong acid and then neutralized by NaOH), these water-soluble fullerenols might be restricted for practical use by unfavorable contamination of Na^+ ion, which is inevitably introduced during treatment with NaOH and which is difficult to remove by the usual manner, except for repeated gel column chromatography in these synthetic methods.[22,27] In this report, we describe the facile synthetic method of a novel milky white, non-Na-contaminated fullerenol, which has 36–40 hydroxyl groups, using hydrogen peroxide as a hydroxylation reagent. These compounds showed notable high water solubility up to 58.9 mg/mL.

RESULTS AND DISCUSSION

Fullerene has been called a "radical sponge" because of its extremely high reactivity to radical species.[28] Such a high radical reactivity makes it very attractive for use in a synthetic approach to polyfunctionalized fullerene derivatives because the radical addition can be a sophisticated strategy to introduce numerous functional groups in one step.[29,30] The addition of the hydroxyl radical to the double bonds of $C_{60}$is known to take place with a diffusion-controlled rate constant of *ca.* 10^{10} M^{-1} s^{-1}.[31] Therefore, we first investigated the reaction of fullerene C_{60} with hydroxyl radicals prepared from hydrogen peroxide under the Fenton conditions.[32] However, the reaction did not proceed, even for several weeks, probably because of the insolubility of C_{60} in an aqueous solution. We then conducted a similar reaction using 12-hydroxylated fullerenol (**1**) instead of C_{60}, heating at 60 °C. Results showed that a homogeneous yellow aqueous solution was obtained after 2–4 days, even in the absence of Fe^{2+} Fenton catalyst. Although the Fe^{2+} catalyst might be effective to accelerate the reaction, complete removal of the catalyst from the product fullerenol is somewhat difficult.[33] For that reason, we optimized the

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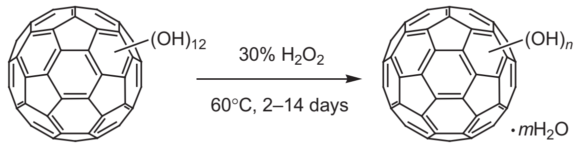


1

2: n = 36, m = 8
3: n = 40, m = 9
(Estimated average structure)

Scheme 1.

above reaction conditions satisfactorily without the catalyst.

The Na^+-free fullerenol $C_{60}(OH)_{12}$ (**1**) was prepared using Chiang's oleum method by polycyclosulfation of C_{60}, followed by hydrolysis in the presence of water at 85 °C;[16] the structure was confirmed using IR spectroscopy, and the number of hydroxyl groups was determined using fast atom bombardment mass spectroscopy (FAB MS), along with elemental analysis. The suspension of fullerenol **1** (0.100 g) in 30% aqueous H_2O_2 (10 mL) was then stirred vigorously at 60 °C under air for several days (Scheme 1). The reddish brown suspension became a transparent yellow solution within 2–4 days, although a slight difference in the color and the number of hydroxyl groups (*e.g.*, n = 32–36 for **2**) was observed, depending on the differences in the lot number of the starting 12-hydroxylated fullerenol, the reaction scale, heating conditions, and/or the efficiency of stirring because of an inevitable solid–liquid heterogeneous reaction. To this solution was added a mixture of 2-propanol, diethyl ether, and hexane (each 50 mL) as poor solvents, to afford a yellowish brown to milky white precipitation of desirable fullerenol. After careful centrifugation and decantation, the residual solid was washed twice with 50 mL of diethyl ether using the general ultrasonic centrifuge decantation procedure. Drying of the residue under vacuum at room temperature for 18 h gave the novel water-soluble fullerenol **2** (0.097 g, 67%[34]) as a yellowish brown to milky white powder. Fullerenol **3** was obtained similarly as a milky white powder at prolonged reaction time of up to 2 weeks (0.103 g, 68%[34]).

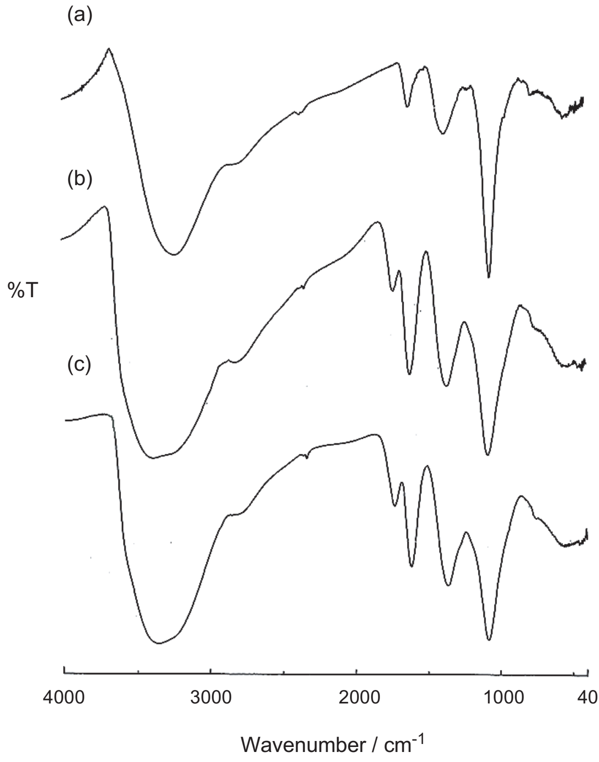


Figure 1. IR spectra of (a) fullerenol $C_{60}(OH)_{12}$ (1), (b) $C_{60}(OH)_{36} \cdot 8H_2O$ (2), and (c) $C_{60}(OH)_{40} \cdot 9H_2O$ (3).

The IR spectra of fullerenols **2** and **3** are shown in Figure 1 along with the spectrum of starting fullerenol **1**.[16] These spectral patterns closely resemble each other, although the relative peak intensities differ somewhat, suggesting a difference in the numbers of introduced hydroxyl groups. With a broad O−H band around 3400 cm^{-1}, the spectra showed three characteristic bands at 1080, 1370, and 1620 cm^{-1} assigned for νC−O, δ_SC−O−H, and νC═C absorption. These four broad bands are invariably reported as diagnostic absorptions of various fullerenols.[16–26] A small peak around 1720 cm^{-1} implies the existence of carboxylic group, which might be formed by further oxidation (in our oxidative conditions) of a hydroxyl group associated with C−C bond cleavage of the fullerene nucleus or of a carbonyl group formed by the known pinacol rearrangement of vicinal hydroxyl groups of fullerenol.[35,36] This presumption was supported by the fact that the treatment of fullerenol **2** with NaOH caused the disappearance of the carbonyl peak, possibly because of the low wavenumber shift to *ca.* 1600 cm^{-1} by the formation of the carboxylate anion; *i.e.*, the carbonyl peak might be hidden by the large peak at 1620 cm^{-1}. Alternatively, Chiang *et al.* reported the plausible incorporation of a hemiketal moiety, which can be transformed into a cyclic ketone moiety in the presence of an acid, although its mechanism of formation remains unclear.[35] They also reported a reversible IR spectral change between the hemiketal and the corresponding ketone by changing the pH. However, not only the carbonyl peak around 1700 cm^{-1} but also three other peaks at 1000–1600 cm^{-1} were changed completely because of the transformation of the hemiketal structure, although our spectrum of **2** showed no such drastic change. It is difficult to determine which structure it is. A peak at about 170 ppm in the solid-state ^{13}C NMR for a similar fullerenol revealed a little about the extent on the cage of carboxylic units rather than a ketone moiety.[23] Such partial decomposition must not be crucial because the generally strong *C*═O absorption is smaller than the generally weak or medium other *C*═C or C−O absorption, as shown also by the results of elemental analysis (see below).

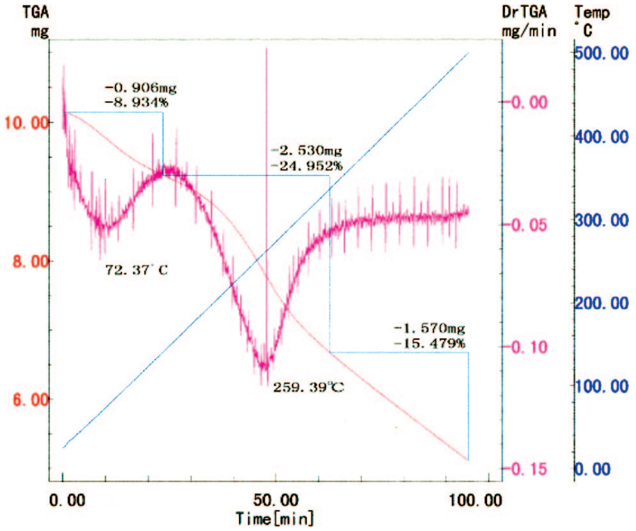


Figure 2. Thermogravimetric analysis of fullerenol 2. Heating rate was 5 °C/min under N_2flow.

To determine the number of hydroxyl groups introduced for these fullerenols **2** and **3**, we tried to measure the molecular weight using FAB and MALDI-TOF MS. However, the mass spectra of these fullerenols were not clearly obtained, in contrast to those of **1**.[16,37] We estimated their average structure on the basis of results of elemental analysis along with a water content measurement. The average structures of some fullerenols that have been reported previously were estimated using only elemental analysis;[21,26] most fullerenols were not calculated as hydrated formulas.[16–27] However, especially for fullerenols with larger numbers of substituted hydroxyl groups, one can readily imagine the existence of a substantial amount of secondary water bound to the plural hydroxyl groups on a fullerene surface. Such tightly entrapped water cannot be dissociated by the usual heating up to about 150 °C.[35] Therefore, we conducted thermogravimetric analysis of fullerenol **2** using a TGA spectrometer (Figure 2).

The weight loss of fullerenol **2** was observed in three temperature ranges; *i.e.*, room temperature to 130 °C, 130–350 °C, and >350 °C. The first loss (8.9 wt %) is assigned to the secondary bound water, as reported by Chiang *et al.*[35] The second reduction (25 wt %) might be attributed to dehydration of the introduced hydroxyl groups, *e.g.*, by possible thermal pinacol rearrangement. The reduction at the highest temperature (>350 °C) might be attributed to decomposition of the fullerene nucleus. The weight reduction profile observed for fullerenol **3** was similar to that of the starting fullerenol **1**. The first weight losses are 9.6 (rt −130 °C) and 12.0 wt % (rt−250 °C), respectively, although the pristine C_{60} is quite stable (no weight loss) up to 400 °C.[38]

As shown in Table 1, the average structure of fullerenol **2** was deduced as $C_{60}(OH)_{36} \cdot 8H_2O$ using elemental analysis along with the water content measurement. It would be calculated as $C_{60}(OH)_{44}$ (C, 49.06; H, 3.02) if the average structure were estimated using elemental analysis alone. Consequently, measuring the secondary bound water is very important to evaluate the number of hydroxyl groups introduced. Similarly, the average structure of fullerenol **3** was determined as $C_{60}(OH)_{40} \cdot 9H_2O$. It is reasonable to infer that the prolonged reaction time resulted in the further introduction of hydroxyl groups. Furthermore, it is noteworthy that the water solubility of fullerenol **3** was much higher than that of **2** because of the greater number of hydroxyl groups of the former, although the numbers of bound water molecules are fundamentally equal for the two. Wang *et al.* reported the high water solubility of fullerenol $C_{60}(OH)_{16}$ as greater than 200 mg/mL, despite the moderate number of hydroxyl groups.[21] However, such a type of water-soluble fullerenol might include a few sodium ions because of (1) the synthetic process using NaOH as hydroxylation or neutralization reagent and (2) the difficulty in complete removal of sodium ion from the weakly acidic or chelation-natured fullerenol.[22,27] Because the simple acidification of fullerenol must induce the acid-catalyzed pinacol rearrangement, it is difficult to re-

TABLE 1. **Elemental Analysis, Water Content, Water Solubility, and Average Structure of Fullerenols 2 and 3**

average structure	elemental analysis (%)[a]	water content (wt %)[a,b]	solubility (mg/mL)[c]
$C_{60}(OH)_{36} \cdot 8H_20$ (**2**)	C, 48.06; H, 3.61 (C, 48.79; H, 3.54)	8.9 (9.7)	17.5
$C_{60}(OH)_{40} \cdot 9H_20$ (**3**)	C, 46.26; H, 3.68 (C, 46.11; H, 3.74)	9.6 (10.4)	58.9

[a] Values in parentheses are calculated data. [b] Water content was determined by thermogravimetric analysis. [c] Water solubility at 25 °C in neutral water (pH 7).

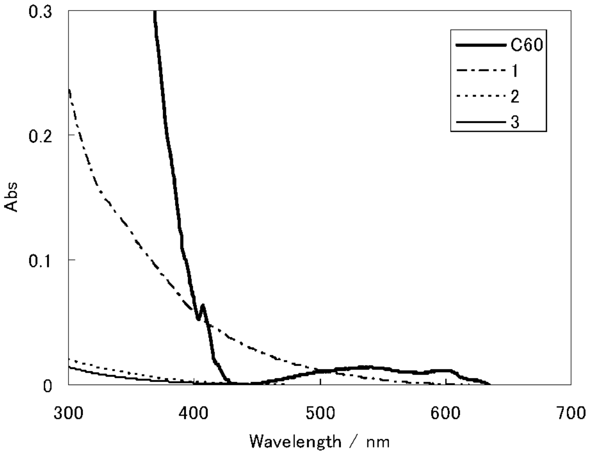
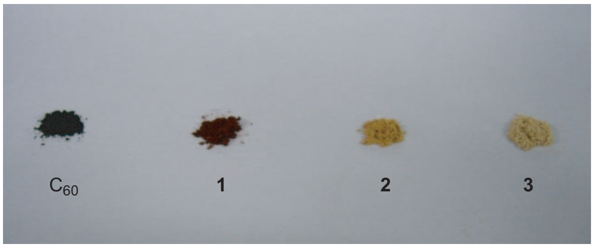


Figure 3. UV–vis spectra of C_{60} and fullerenols 1–3: C_{60} in toluene, $C_{60}(OH)_{12}$ (1) in 1,4-dioxane, and aqueous solutions of $C_{60}(OH)_{36} \cdot 8H_2O$ (2) and $C_{60}(OH)_{40} \cdot 9H_2O$ (3) (each 3.33 mg/L).

(a) Powder



(a) Solution

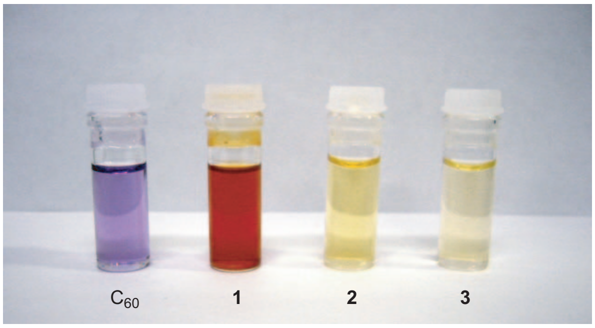


Figure 4. Colors of water-soluble fullerenols 1–3 (a) and their solution (b): C_{60} in toluene, $C_{60}(OH)_{12}$ (1) in 1,4-dioxane, and aqueous solutions of $C_{60}(OH)_{36} \cdot 8H_2O$ (2) and $C_{60}(OH)_{40} \cdot 9H_2O$ (3).

move the sodium ion completely without using a column chromatography process.

The UV–visible absorption spectra of fullerenols **2** and **3** in water (3.33 mg/L; 2.3 and 2.1 μM, respectively) are shown in Figure 3. Compared to those of pristine C_{60} in toluene and fullerenol **1** in dioxane (each 3.33 mg/L; 4.6 and 3.3 μM, respectively), fullerenols **2** and **3** are almost transparent in the visible region, implying their considerably decreased π-conjugation (average number of *C*═C double bonds in C_{60}, **1**, **2**, and **3** is 30, 24, 12, and 10, respectively). Indeed, the colors of these water-soluble fullerenols **2** and **3** are blue-shifted and far different from those of pristine C_{60}, as shown for a powder or in solution (Figure 4). The highly polyfluorinated fullerene derivatives, for example $C_{60}F_{36}$ and $C_{60}F_{48}$, are also known to become pale yellow because of the significant loss of the fullerene double bonds, as observed in the present study.[39,40]

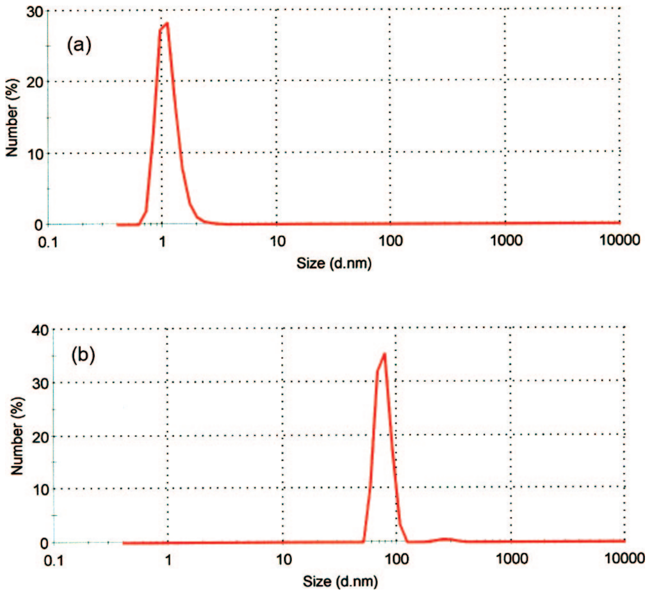


Figure 5. DLS analysis of fullerenol 2 in water (0.1 wt %) (a) and as produced (b) in the presence of NaOH (1 mM, pH 12), expressed by size distribution in number.

The particle size of **2** in the 0.1 wt % aqueous solution[41] was measured using dynamic light scattering (DLS) analysis. A chart showing the size distribution by number is presented in Figure 5. Using MOPAC PM3 calculation, the diameter of a possible isomer of $C_{60}(OH)_{36}$,[42] as estimated from the O−O atomic distance between the farthest hydroxyl groups on both poles, was almost 1.0 nm. The narrow distribution of the particle size, 0.7–2.0 nm, clearly indicates that fullerenol **2** is highly dispersed in a molecular level and that the possible aggregation of fullerenols, as is known for many fullerene derivatives[43,44] and even for some fullerenols,[7,8,45] is not so prevalent. The latter is inferred because the present fullerenol **2** might be highly surrounded by the solvent–water molecules, which are attributable to the strong hydrogen-bonding with the introduced hydroxyl groups; consequently, the intermolecular hydrophobic interaction (or π−π interaction) between C_{60} cores is overwhelmed. It is interesting to note that the aggregation size was controlled by changing the pH of the solution. The addition of NaOH to the yellowish solution of **2** up to pH 12 changed the color of solution to light brown. The DLS measurement of the basic solution revealed a high extent of aggregation (50−100 nm) of the fullerenol (Figure 5b), although the addition of HCl (pH 2.6) essentially did not affect the particle size. The observed phenomenon was rationalized on the basis of the strong interaction between the metal cation (Na^+) and the fullerenol, leading to the aggregation or finally the precipitation.[33]

Interestingly, in contrast to the high dispersion property of **2** in diluted aqueous solution, the aggregation phenomenon was observed during deposition. Addition of a mixture of 2-propanol, diethyl ether, and hexane (5:5:5 to fullerenol aqueous solution in v/v) as a poor solvent into the reasonably concentrated aqueous solution of the fullerenol **2** or **3** gradually brought about deposition of the fullerenol. Scanning probe microscopy (SPM) surface analysis of the powder of fullerenol **2** revealed its nanoscale spherical structure to be about 50 nm, implying the formation of fullerenol aggregation during deposition (Figure 6). Therefore, the addition of the poor solvent probably reduced the solvation of the fullerenol by water molecules and increased the hydrophobic interaction between intermolecular fullerene cores to afford such a spherical aggregation, which finally developed into deposition. Therefore, results suggest that the aggregation size of the fullerenol might be controlled by the polarity and the nature of the solvent.

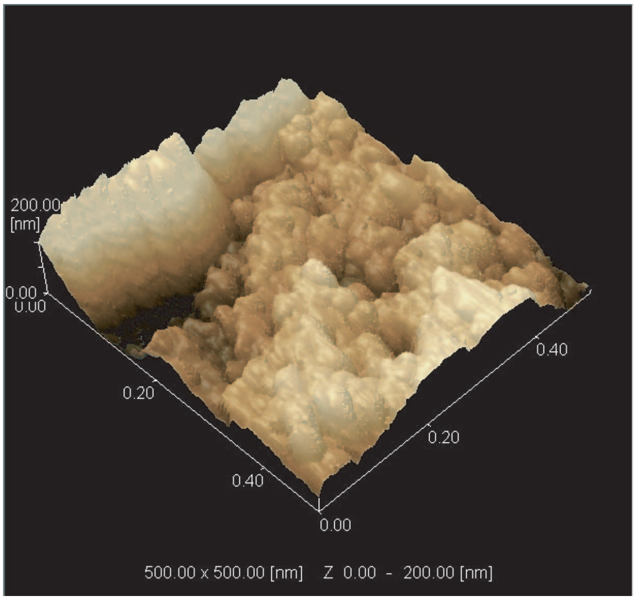


Figure 6. SPM surface analysis for a powder of fullerenol 2.

Although we estimated the average structure of fullerenol **2** as much as we could in the present work, the exact structure of each fullerenol constituting an isomeric mixture with various numbers and positions of hydroxyl groups remains under investigation. The actual structure might contain some epoxy or hemiketal oxygens as well as carbonyl oxygen on the fullerene surface, as previously proposed for some fullerenols.[24,25,27,35] Alternatively, our synthetic method using hydrogen peroxide might partly introduce a $-$OOH group[46] along with a $-$OH group. We cannot rule out these possibilities because these functional groups are generally difficult to distinguish using IR spectroscopy. However, results of both elemental analysis and thermogravimetric analysis suggest that these introduced impurity groups[25] are quite few (*e.g.*, calculated elemental analysis for $C_{60}(OH)_{36}O_4 \cdot 8H_2O$: C, 46.76; H, 3.40). In addition, the resultant good water solubility is apparently supported by the higher number of hydroxyl groups.

With respect to the exact structure of fullerenols, Cai[47] and Wilson[27] independently reported the radical nature of fullerenol $C_{60}H_2(OH)_{24}$ and $Na^+_n[C_{60}O_x(OH)_y]^{n-}$ (where $n = 2$–3, $x = 7$–9, and $y = 12$–15), respectively, as confirmed by electron spin resonance (ESR) spectroscopy. It is supposed that only a trace amount of alkali metal contamination can produce the radical anion of C_{60}, and the above two fullerenols seem to be contaminated by the employed alkali metal ions. In contrast, our fullerenol is synthesized under metal-ion-free conditions and thus is expected not to contain such metal ions. We tested our compound by ESR measurement. Surprisingly, however, the present fullerenol **2** also showed a clear ESR signal ($g = 2.0033$) at room temperature in a solid-state measurement, in good agreement with the results of Cai and Wilson (Figure 7). The g value was calibrated by using a Mn^{2+} marker and was slightly different from the value observed for $C_{60}H_2(OH)_{24}$ at 300 K ($g = 2.0026$).[47] The observed paramagnetic property may be due to the some radical species in the fullerenol mixture, and the observed g value suggests the existence of a stable radical such as cyclopentadienyl radical on the fullerene.[27,28]

CONCLUSIONS

In conclusion, we have developed a facile method for the synthesis of water-soluble polyhydroxylated [60]fullerenes bearing more than 30 hydroxyl groups—over half the number (60) of carbons—using hydrogen peroxide instead of sodium hydroxide. The estimated average structure of these fullerenols was determined as $C_{60}(OH)_{36} \cdot 8H_2O$ or $C_{60}(OH)_{40} \cdot 9H_2O$, depending on the reaction time. The obtained fullerenol showed high water solubility up to 58.9 mg/mL and high dispersion on a molecular level. In our preliminary experiment, the fullerenol also exhibited a potent antioxidant activity as a cosmetic ingredient and a high ability to polish the surface of a copper wafer when applied as a chemical mechanical polishing slurry. A study of these applications and continuous improvement of the present synthetic method are now in progress.

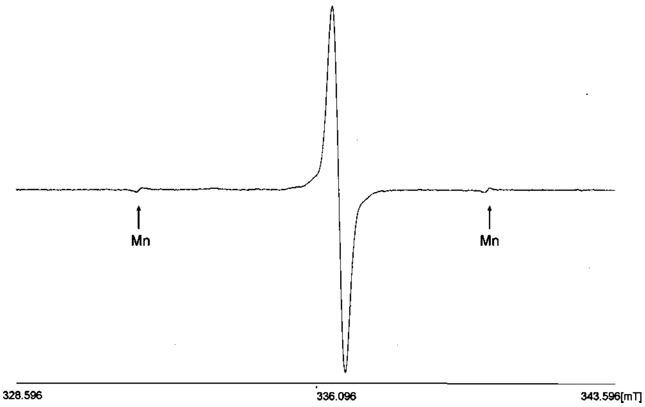


Figure 7. ESR spectrum for solid-state fullerenol 2 at 298 K.

METHODS

Materials and Analysis. Fullerene C_{60} was purchased from Frontier Carbon Corp. or Tokyo Chemical Industry Co., Ltd. The fullerenol $C_{60}(OH)_{12}$ (**1**) was synthesized by the previously reported method.[16] The product was confirmed by IR and FAB mass spectra as well as an elemental analysis. Anal. Calcd for $C_{60}(OH)_{12} \cdot 5H_2O$: C, 71.01; H, 2.19. Found: C, 71.84; H, 2.34. Other reagents and organic solvents were all commercially available and used as received. IR spectra were measured with a JASCO FT/IR-300E instrument, and TGA spectra were obtained with a Shimadzu TA-50 instrument. UV–visible spectra were recorded on a JASCO V-550 spectrometer. DLS analysis was performed on a Marvern Instruments Zetasizer Nano ZSI instrument. SPM surface analysis was performed on a Shimadzu SPM-9600 instrument. ESR measurement was performed on a JEOL X-band ESR spectrometer (JES-ME-LX). The *g* value was calibrated by using a Mn^{2+} marker.

Synthesis of $C_{60}(OH)_{36} \cdot 8H_2O$ (2). To a 30% hydrogen peroxide solution (10 mL) was added starting material $C_{60}(OH)_{12}$ (**1**, 0.100 g), 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 of the solution, the addition of a mixed solvent of 2-propanol, diethyl ether, and hexane (each 50 mL) gradually yielded a milky white precipitate. After careful centrifugation and decantation, the residual solid was washed twice with *ca.* 50 mL of diethyl ether by the general ultrasonic centrifuge/decantation procedure. Drying of the residue under vacuum at room temperature for 18 h gave fullerenol **2** (0.097 g, 67%) as a pale yellow-brown powder.

Synthesis of $C_{60}(OH)_{40} \cdot 9H_2O$ (3). Similar treatment of $C_{60}(OH)_{12}$ (**1**, 0.100 g) for a prolonged reaction time (2 weeks) at 60 °C and the same workup as above provided fullerenol **3** (0.103 g, 68%) as a milky white powder.

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Supporting Information Available: FAB mass spectrum of **1** and TGA chart for pristine C_{60}, **1**, and **3**. This material is available free of charge *via* the Internet at http://pubs.acs.org.

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