A Facile High-speed Vibration Milling Method to Water-disperse Single-walled Carbon Nanohorns

Chunying Shu,[†] Jianfei Zhang,[†] Jiechao Ge,[†] Jae Hyun Sim,[†] Brian G. Burke,[⊥]
Keith A. Williams,[⊥] Nichole M. Rylander,[‡] Tom Campbell,[§] Alex Puretzky,[#]
Chris Rouleau,[#] David B. Geohegan,[#] Karren More,[#] Alan R. Esker,[†] Harry W. Gibson,[†]
and Harry C. Dorn*,[†]

[†]*Chemistry Department, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061,*
[‡]*Department of Mechanical Engineering and School of Biomedical Engineering and Sciences, Virginia Polytechnic
Institute and State University, Blacksburg, Virginia 24061,* [§]*Institute for Critical Technology and Applied Science
(ICTAS), Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24060,* [⊥]*Department of
Physics, University of Virginia, Virginia 22903, and* [#]*Center for Nanophase Materials Sciences and SHaRE
Facility, Oak Ridge National Laboratory, Oak Ridge Tennessee, 37831*

Received September 2, 2009. Revised Manuscript Received November 9, 2009

A high-speed vibration milling (HSVM) method was applied to synthesize water dispersible single-walled carbon nanohorns (SWNHs). Highly reactive free radicals ($HOOCCH_2CH_2\bullet$) produced from an acyl peroxide under HSVM conditions react with hydrophobic SWNHs to produce a highly water dispersible derivative (f-SWNHs), which has been characterized in detail by spectroscopic and microscopic techniques together with thermogravimetric analysis (TGA) and dynamic light scattering (DLS). The carboxylic acid functionalized, water-dispersible SWNHs material are versatile precursors that have potential applications in the biomedical area.

Introduction

Carbon nanomaterials have been attracting great attention as novel biomaterials for potential use in the field of nanomedicine.[1] Single-walled carbon nanohorns (SWNHs) reported by Iijima et al. in 1999 are similiar in structure to single-walled carbon nanotubes (SWNTs).[2] In contrast to SWNTs, high-purity SWNHs are produced by laser electric arc vaporization of pure graphite without the use of metal catalysts, thereby making them very cost-effective. SWNHs are typically composed of tubes of about $2-3$ nm in diameter and 50 nm long with closed conical tips.[3] During the synthesis process, SWNHs aggregate within spherical structures with diameters between 40 and 200 nm. The aggregate diameter depends on the individual SWNHs length which can be adjusted from roughly $5-50$ nm by limiting the growth time with an adjustable pulse-width laser.[4] The specific surface area can easily be increased from ~500 m²/g to over 2000 m²/ g by oxidative activation treatments, which open slit pores to access the inner surface of the SWNHs. The large specific surface areas and variable porosity of SWNHs make them

promising candidates for hydrogen and methane storage,[5−8] and catalyst electrodes in fuel cells.[9] Because SWNHs are free of metal contamination, they also can be used as laser therapeutic agents,[10,11] antiviral materials,[12] carriers in drug delivery systems (DDS),[13−18] and magnetic resonance imaging (MRI) contrast agents.[19]

*Corresponding author. E-mail: hdorn@vt.edu.
(1) Bekyarova, E.; Ni, Y.; Malarkey, E. B.; Montana, V.; McWilliams, J. L.; Haddon, R. C.; Parpura, V. *J. Biomed. Nanotechnol.* **2005,** *1,* 3.
(2) Iijima, S.; Yudasaka, M.; Yamada, R.; Bandow, S.; Suenaga, K.; Kokai, F.; Takahashi, K. *Chem. Phys. Lett.* **1999,** *309,* 165.
(3) Miyawaki, J.; Yudasaka, M.; Azami, T.; Kubo, Y.; Iijima, S. *ACS Nano* **2008,** *2,* 213.
(4) Puretzky, A. A.; Styers-Barnett, D. J.; Rouleau, C. M.; Hu, H.; Zhao, B.; Ivanov, I. N.; Geohegan, D. B. *Appl. Phys. A: Mater. Sci. Process.* **2008,** *93,* 849.
(5) Murata, K.; Kaneko, K.; Kanoh, H.; Kasuya, D.; Takahashi, K.; Kokai, F.; Yudasaka, M.; Iijima, S. *J. Phys. Chem. B* **2002,** *106,* 11132.

(6) Tanaka, H.; Kanoh, H.; El-Merraoui, M.; Steele, W. A.; Yudasaka, M.; Iijima, S.; Kaneko, K. *J. Phys. Chem. B* **2004,** *108,* 17457.
(7) Bekyarova, E.; Murata, K.; Yudasaka, M.; Kasuya, D.; Iijima, S.; Tanaka, H.; Kahoh, H.; Kaneko, K. *J. Phys. Chem. B* **2003,** *107,* 4681.
(8) Murata, K.; Hashimoto, A.; Yudasaka, M.; Kasuya, D.; Kaneko, K.; Iijima, S. *Adv. Mater.* **2004,** *16,* 1520.
(9) Yoshitake, T.; Shimakawa, Y.; Kuroshima, S.; Kimura, H.; Ichihashi, T.; Kubo, Y.; Kasuya, D.; Takahashi, K.; Kokai, F.; Yudasaka, M.; Iijima, S. *Phys. B: Condens. Matter* **2002,** *323,* 124; Proceed. of the Tsukuba Symposium on Carbon Nanotube in Commemoration of the 10th Anniv. of its Discovery, Tsukuba, Japan, Oct 3−5, 2001.
(10) Miyako, E.; Nagata, H.; Hirano, K.; Makita, Y.; Nakayama, K. I.; Hirotsu, T. *Nanotechnology* **2007,** *18,* 475103 (1−7).
(11) Zhang, M.; Murakami, T.; Ajima, K.; Tsuchida, K.; Sandanayaka, A. S. D.; Ito, O.; Iijima, S.; Yudasaka, M. *Proc. Natl. Acad. Sci. U.S.A.* **2008,** *105,* 14773.
(12) Miyako, E.; Nagata, H.; Hirano, K.; Sakamoto, K.; Makita, Y.; Nakayama, K.; Hirotsu, T. *Nanotechnology* **2008,** *19,* 075106(1−6).
(13) Muralkami, T.; Ajima, K.; Miyawaki, J.; Yudasaka, M.; Iijima, S.; Shiba, K. *Mol. Pharmaceutics* **2004,** *1,* 399.
(14) Xu, J. X.; Yudasaka, M.; Kouraba, S.; Sekido, M.; Yamamoto, Y.; Iijima, S. *Chem. Phys. Lett.* **2008,** *461,* 189.
(15) Ajima, K.; Yudasaka, M.; Murakami, T.; Maigne, A.; Shiba, K.; Ijima, S. *Mol. Pharmaceutics* **2005,** *2,* 475.
(16) Venkatesan, N.; Yoshimitsu, J.; Ito, Y.; Shibata, N.; Takada, K. *Biomaterials* **2005,** *26,* 7154.
(17) Matsumura, S.; Ajima, K.; Yudasaka, M.; Iijima, S.; Shiba, K. *Mol. Pharmaceutics* **2007,** *4,* 723.
(18) Murakami, T.; Sawada, H.; Tamura, G.; Yudasaka, M.; Iijima, S.; Tsucbida, K. *Nanomedicine* **2008,** *3,* 453.
(19) Miyawaki, J.; Yudasaka, M.; Imai, H.; Yorimitsu, H.; Isobe, H.; Nakamura, E.; Iijima, S. *Adv. Mater.* **2006,** *18,* 1010.

Dispersion of this hydrophobic carbon nanomaterial under aqueous media is a foremost challenge and prerequisite to facilitate applications in the biomedical area. To date, a wide variety of approaches to make carbon nanomaterials dispersible in water have been reported, i.e., both covalent (chemical) and noncovalent modifications.[20−22] The noncovalent modifications to carbon nanomaterials include using surfactants,[23−27] polymers,[17,25,28−31] and biopolymers.[32−36] Covalent modifications have the advantage of being more robust during manipulation and processing in comparison to the noncovalent dispersants. Currently, there are two main strategies used to attach functional groups to carbon nanomaterials. The first one consists of oxidative treatment using strong acid solutions, leading to carboxyl groups at the tips and defect sites of the SWNHs.[37,10] The second type of covalent functionalization is based on addition reactions to SWNT[38−40] or SWNHs.[41−47] Covalent modification is often time-consuming and complicated because of the low solubility and reactivity of SWNHs in organic solvents.[38] Recently, Rubio et al. explored a microwave irradiation method to functionalize SWNHs,[48] which dramatically reduces chemical waste and reaction times. Herein, we report a facile strategy to functionalize SWNHs by a high speed vibration milling (HSVM) method,[49] which is solvent-free, simple, and highly efficient to render the highly hydrophobic nanomaterial water dispersible by covalent modification with carboxylic acid groups. The resultant product is a versatile precursor to SWNHs based nanomaterials for applications in materials and health sciences.

Experimental Details

Materials. Long, metal-free SWNHs (estimated carbon purity ≥95%, metal free) were synthesized by Nd:YAG (λ = 1.064 μm, 20 ms pulse width, 90 J/pulse, 5Hz repetition rate) laser vaporization of carbon targets into argon (1atm) at 1100 °C.[50] Succinic acid acyl peroxide was synthesized as previously reported;[51] Pluronic F127 was purchased from Invitrogen-Molecular Probes.

General Characterization Methods. The proportion of functional groups on f-SWNHs was estimated by thermogravimetric analysis (TGA Q5000) in an inert nitrogen atmosphere from room temperature to 600 °C. In a typical experiment, 6−7 mg of material were in a sample pan and the temperature was equilibrated to room temperature. Subsequently, the temperature was increased to 600 °C at a rate of 5 °C/min and the weight changes were recorded. Fourier transform infrared spectroscopic (FTIR) measurements using an attenuated total reflectance (ATR) sample stage were performed on a Perkin-Elmer spectrometer. Raman spectra were measured on a a JY UV−Vis single monochromator (633 nm line) spectrometer with a CCD detector. UV−Vis spectra were measured on a Cary 50 (Bio, Varian, Inc.). The transmission electron microscopy (TEM) images were obtained on a Philips EM 420 at an accelerating voltage of 100 kV. X-ray photoelectron spectroscopy (XPS) measurements were taken with a PHI Quantera SXM scanning photoelectron spectrometer microprobe using Al Kα radiation. Dynamic light scattering (DLS) measurements were carried out by an ALV/CGS-3 compact geniometer system using a He−Ne laser of λ_0 = 632.8 nm as a light source and ALV/LSE-5003 multi-τ digital correlator with 288 channels. Autocorrelation functions were collected at six different scattering angles (30, 45, 60, 75, and 90°) at 25 °C. CONTIN analysis[52] was used to obtain the hydro-

(20) Klumpp, C.; Kostarelos, K.; Prato, M.; Bianco, A. *Biochim. Biophys. Acta* **2005**, *1758*, 404; presented at the Conference on Mechanisms of Carrier-Mediated Intra-Cellular Delivery of Therapeutics, Montpellier, France, Aug 25−26, 2005.
(21) Niyogi, S.; Hamon, M. A.; Hu, H.; Zhao, B.; Bhowmik, P.; Sen, R.; Itkis, M. E.; Haddon, R. C. *Acc. Chem. Res.* **2002**, *35*, 1105.
(22) Sun, Y. P.; Fu, K. F.; Lin, Y.; Huang, W. J. *Acc. Chem. Res.* **2002**, *35*, 1096.
(23) O'Connell, M. J.; Bachilo, S. M.; Huffman, C. B.; Moore, V. C.; Strano, M. S.; Haroz, E. H.; Rialon, K. L.; Boul, P. J.; Noon, W. H.; Kittrell, C.; Ma, J. P.; Hauge, R. H.; Weisman, R. B.; Smalley, R. E. *Science* **2002**, *297*, 593.
(24) Islam, M. F.; Rojas, E.; Bergey, D. M.; Johnson, A. T.; Yodh, A. G. *Nano Lett.* **2003**, *3*, 269.
(25) Moore, V. C.; Strano, M. S.; Haroz, E. H.; Hauge, R. H.; Smalley, R. E.; Schmidt, J.; Talmon, Y. *Nano Lett.* **2003**, *3*, 1379.
(26) Richard, C.; Balavoine, F.; Schultz, P.; Ebbesen, T. W.; Mioskowski, C. *Science* **2003**, *300*, 775.
(27) Chen, R. J.; Zhang, Y. G.; Wang, D. W.; Dai, H. J. *J. Am. Chem. Soc.* **2001**, *123*, 3838.
(28) Shvartzman-Cohen, R.; Nativ-Roth, E.; Baskaran, E.; Levi-Kalisman, Y.; Szleifer, I.; Yerushalmi-Rozen, R. *J. Am. Chem. Soc.* **2004**, *126*, 14850.
(29) Sinani, V. A.; Gheith, M. K.; Yaroslavov, A. A.; Rakhnyanskaya, A. A.; Sun, K.; Mamedov, A. A.; Wicksted, J. P.; Kotov, N. A. *J. Am. Chem. Soc.* **2005**, *127*, 3463.
(30) Didenko, V. V.; Moore, V. C.; Baskin, D. S.; Smalley, R. E. *Nano Lett.* **2005**, *5*, 1563.
(31) Star, A.; Stoddart, J. F.; Steuerman, D.; Diehl, M.; Boukai, A.; Wong, E. W.; Yang, X.; Chung, S. W.; Choi, H.; Heath, J. R. *Angew. Chem., Int. Ed.* **2001**, *40*, 1721.
(32) Zheng, M.; Jagota, A.; Semke, E. D.; Diner, B. A.; McLean, R. S.; Lustig, S. R.; Richardson, R. E.; Tassi, N. G. *Nat. Mater.* **2003**, *2*, 338.
(33) Muralkami, T.; Fan, J.; Yudasaka, M.; Iijima, S.; Shiba, K. *Mol. Pharmaceutics* **2006**, *3*, 407.
(34) Dieckmann, G. R.; Dalton, A. B.; Johnson, P. A.; Razal, J.; Chen, J.; Giordano, G. M.; Munoz, E.; Musselman, I. H.; Baughman, R. H.; Draper, R. K. *J. Am. Chem. Soc.* **2003**, *125*, 1770.
(35) Ortiz-Acevedo, A.; Xie, H.; Zorbas, V.; Sampson, W. M.; Dalton, A. B.; Baughman, R. H.; Draper, R. K.; Musselman, I. H.; Dieckmann, G. R. *J. Am. Chem. Soc.* **2005**, *127*, 9512.
(36) Zorbas, V.; Smith, A. L.; Xie, H.; Ortiz-Acevedo, A.; Dalton, A. B.; Dieckmann, G. R.; Draper, R. K.; Baughman, R. H.; Musselman, I. H. *J. Am. Chem. Soc.* **2005**, *127*, 12323.
(37) Ziegler, K. J.; Gu, Z. N.; Peng, H. Q.; Flor, E. L.; Hauge, R. H.; Smalley, R. E. *J. Am. Chem. Soc.* **2005**, *127*, 1541.
(38) Peng, H.; Alemany, L. B.; Margrave, J. L.; Khabashesku, V. N. *J. Am. Chem. Soc.* **2003**, *125*, 5174.
(39) Georgakilas, V.; Tagmatarchis, N.; Pantarotto, D.; Bianco, A.; Briand, J. P.; Prato, M. *Chem. Commun.* **2002**, 3050.
(40) Georgakilas, V.; Voulgaris, D.; Vazquez, E.; Prato, M.; Guldi, D. M.; Kukovecz, A.; Kuzmany, H. *J. Am. Chem. Soc.* **2002**, *124*, 14318.
(41) Tagmatarchis, N.; Maigne, A.; Yudasaka, M.; Iijima, S. *Small* **2006**, *2*, 490.
(42) Cioffi, C.; Campidelli, S.; Brunetti, F. G.; Meneghetti, M.; Prato, M. *Chem. Commun.* **2006**, 2129.
(43) Isobe, H.; Tanaka, T.; Maeda, R.; Noiri, E.; Solin, N.; Yudasaka, M.; Iijima, S.; Nakamura, E. *Angew. Chem., Int. Ed.* **2006**, *45*, 6676.
(44) Pagona, G.; Karousis, N.; Tagmatarchis, N. *Carbon* **2008**, *46*, 604.
(45) Pagona, G.; Tagmatarchis, N.; Fan, J.; Yudasaka, M.; Iijima, S. *Chem. Mater.* **2006**, *18*, 3918.
(46) Pagona, G.; Sandanayaka, A. S. D.; Araki, Y.; Fan, J.; Tagmatarchis, N.; Charalambidis, G.; Coutsolelos, A. G.; Boitrel, B.; Yudasaka, M.; Iijima, S.; Ito, O. *Adv. Funct. Mater.* **2007**, *17*, 1705.
(47) Cioffi, C.; Campidelli, S.; Sooambar, C.; Marcaccio, M.; Marcolongo, G.; Meneghetti, M.; Paolucci, D.; Paolucci, F.; Ehli, C.; Rahman, G. M. A.; Sgobba, V.; Guldi, D. M.; Prato, M. *J. Am. Chem. Soc.* **2007**, *129*, 3938.
(48) Rubio, N.; Herrero, M. A.; Meneghetti, M.; Díaz-Ortiz, A.; Schiavon, M.; Prato, M.; Vazquez, E. *J. Mater. Chem.* **2009**, *19*, 4407.
(49) Braun, T.; Rausch, H.; Biro, L. P.; Zsoldos, E.; Ohmacht, R.; Mark, L. *Chem. Phys. Lett.* **2003**, *375*, 522.
(50) Puretzky, A. A.; Styers-Barnett, D. J.; Rouleau, C. M.; Hu, H.; Zhao, B.; Ivanov, I. N.; Geohegan, D. B. *Appl. Phys. A* **2008**, *93*, 849.
(51) Clover, A. M.; Houghton, A. C. *Am. Chem. J.* **1904**, *32*, 43.
(52) Ju, R. T. C.; Frank, C. W.; Gast, A. P. *Langmuir* **1992**, *8*, 2165.

Scheme 1. Depiction of Functionalization of SWNHs (above) and Photographs of Dispersions of f-SWNHs in PBS, Water, and Acetone (0.5 mg/mL), As Well As Pristine SWNHs in Water (bottom)[a]
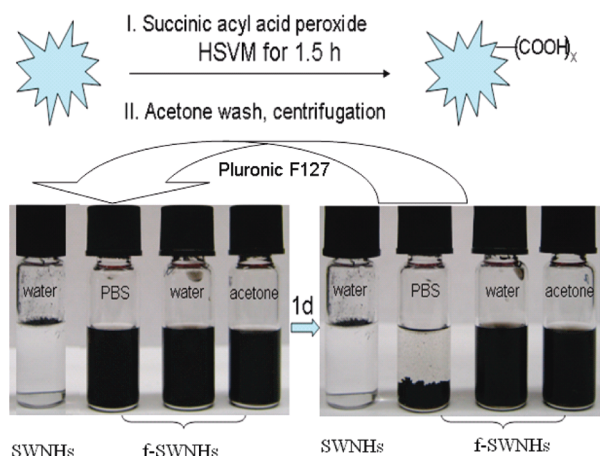

[a] The f-SWNHs in PBS precipitated quickly, but can be resuspended with the addition of Pluronic F127.

dynamic radius based on the diffusion coefficient and size distribution.

Results and Discussion

Functionalized SWNHs (f-SWNHs) were synthesized by the HSVM method. Briefly, 5 mg of SWNHs were mixed with 100 equiv. (mass ratio) of succinic acid acyl peroxide. The resulting mixture was put in a stainless steel capsule and shaken vigorously for 1.5 h (SPEX 8000 Mixer/Mill, 1725 rpm), leading to an ultrafine powder. This ultrafine powder was washed with a large amount of acetone and centrifuged to remove any organic residues not associated with the SWNHs. The supernatant was carefully decanted. This process was repeated three times and the solid was washed with pure water once. The final product was sonicated in pure water or acetone for 20 min to obtain a dark suspension. The f-SWNHs are stable in pure water and acetone for at least several months. Scheme 1 shows the process for the production of the f-SWNHs and the resultant stable dispersion in water (0.5 mg/mL) and in acetone (0.5 mg/mL).

In phosphate buffered saline (PBS) solution, a transparent dark solution (0.5 mg/mL) was obtained after sonication. Unfortunately, most of the f-SWNHs precipitated from the PBS solution after 30 min because of the presence of the salt. It is reported that Pluronic F127 is a good stabilizer in similar formulations and exhibits biological nontoxicity at concentrations less than 1%.[53] Herein, we found that the precipitate in PBS or 0.9% $NaCl_{aq}$ can be dispersed to give a stable homogeneous suspension with the addition of 0.25% Pluronic F127. The solubility of f-SWNHs in water, PBS/0.25% Pluronic F127, and 0.9% $NaCl_{aq}$/0.25% Pluronic F127 is up to 0.1 mg/mL.
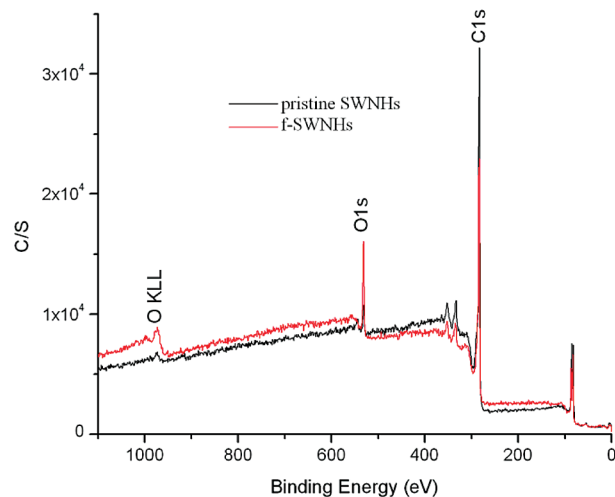
Figure 1. XPS survey of f-SWNHs (red line) and pristine SWNHs (black line).

As shown in Figure 1, XPS survey results reveal that the O/C atomic ratio of as-prepared f-SWNHs (13.5:86.5) dramatically increases relative to that of the pristine SWNHs (3.3:96.7) and no other impurities were detected except for gold from the substrate (gold foil). Considering the composition of the functional groups, $-CH_2CH_2-$ COOH, and the impurities in the pristine SWNHs (3.3%) from the XPS survey, the estimated payload of functional groups is ca. 0.4 mg/mg SWNHs.

TGA provides a quantitative estimate of the degree of the carboxylic acid functionalization of the SWNHs. Pristine SWNHs are thermally stable under nitrogen atmosphere up to 600 °C, though there is a 0.81% weight loss (Figure 2, black line). This minor weight loss is from defects or oxygen derived impurities, which has been confirmed by XPS analysis. In sharp contrast, there is a 24.5% weight loss of the f-SWNHs under the same conditions, indicating the loss of organic addends. Considering the impurity-derived weight loss and the weight loss of the f-SWNHs up to 600 °C, the percentage of the organic moiety attached to the SWNHs can be calculated[54] to be 0.31 mg/mg SWNHs.

Attenuated total reflectance infrared (ATR-IR) spectroscopy indicates the presence of the covalently attached moieties on the SWNHs. As Figure 3 shows, as-prepared f-SWNHs (black line) exhibit strong characteristic absorptions for $-CH_2-CH_2-$ (ν_{C-H} = 2917, 2843 cm^{-1}) and COOH ($\nu_{C=O}$ = 1727 cm^{-1}), indicating the existence of $-CH_2CH_2COOH$ groups. The low-intensity of free acid around 3300 cm^{-1} may be due to the dehydration of the free acid when the sample was heated at 80 °C in vacuum oven overnight. It is noteworthy that carboxylic acid groups ($-COOH$, $\nu_{C=O}$ = 1727 cm^{-1}) rather than carboxylate groups ($-COO^-$, $\nu_{C=O}$ = 1620 cm^{-1})[55] will facilitate further versatile modification.[38,56]

(53) Lynch, R. M.; Voy, B. H.; Glass, D. F.; Mahurin, S. M.; Zhao, B.; Hu, H.; Saxton, A. M.; Donnell, R. L.; Cheng, M. D. *Nanotoxicology* **2007**, *1*, 157.
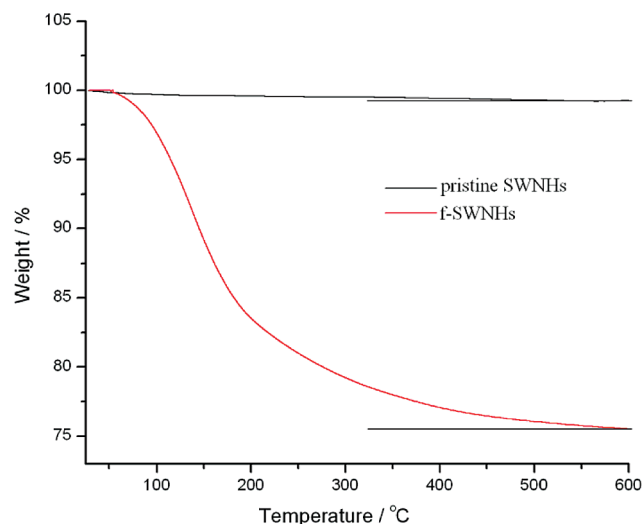
(54) Hudson, J. L.; Jian, H. H.; Leonard, A. D.; Stephenson, J. J.; Tour, J. M. *Chem. Mater.* **2006**, *18*, 2766.
(55) Chen, J.; Hamon, M. A.; Hu, H.; Chen, Y. S.; Rao, A. M.; Eklund, P. C.; Haddon, R. C. *Science* **1998**, *282*, 95.
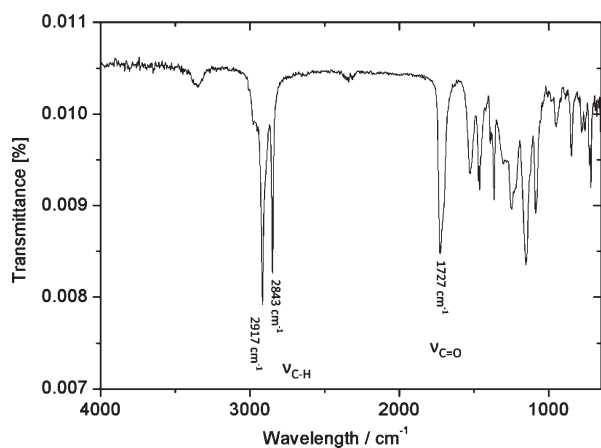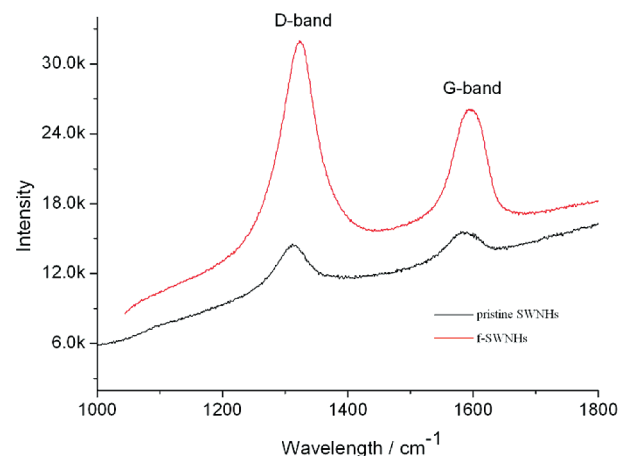(56) Fu, K. F.; Huang, W. J.; Lin, Y.; Zhang, D. H.; Hanks, T. W.; Rao, A. M.; Sun, Y. P. *J. Nanosci. Nanotechnol.* **2002**, *2*, 457.

Figure 2. Thermogravimetric analysis (N$_2$, 5 °C/min.) of f-SWNHs (red line) compared with SWNHs (black line).

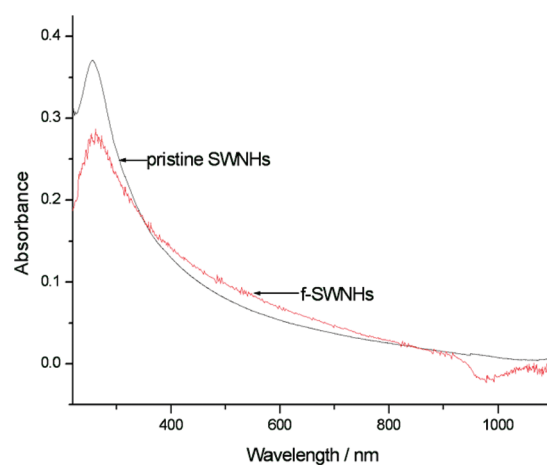**Figure 3.** ATR-IR spectrum of f-SWNHs.

Figure 4. Raman spectra of f-SWNHs (red line) and pristine SWNHs (black line).

Figure 5. UV−Vis−NIR spectral comparison between SWNHs−COOH in water (red line) and pristine SWNHs in 1,2-dichloroethane (black line).

Figure 6. Representative TEM images of (a) SWNHs and (b) f-SWNHs. Accelerating voltage: 100 kV.

The Raman spectrum of f-SWNHs exhibits one broad peak at 1593 cm^{-1} ("G-band"), assigned to tangential vibrations in the sp^2-bonded carbon network and another broad peak at 1323 cm^{-1} ("D-band"), attributed to the disruption of the basal plane lattice due to the conical terminated tips of the nanohorns as well as to sp^3 single-bonding carbon atoms existing within SWNHs aggregates.[57] These results are closely consistent with that of aryl diazonium functionalized SWNHs, which exhibit G-band and D-band absorptions at 1593, 1341 cm^{-1}, respectively.[44] The increased intensity around the D-band of f-SWNHs compared with pristine SWNHs indicates the generation of sp^3-hybridized carbon atoms in the SWNHs framework.[57]
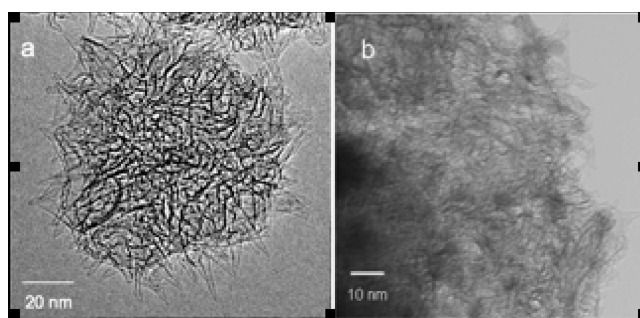
The UV−Vis−NIR spectrum (Figure 5) of the carboxylic acid functionalized SWNHs exhibits a featureless broad maximum at 256 nm, then monotonically decreases toward the NIR region,[44] similar to that of pristine SWNHs except for the NIR region. This weak absorption

of the f-SWNHs in the NIR region could be an important signature that will facilitate biomedical applications.[10]

TEM and DLS measurements were used to explore the morphological characteristics and particle-size distribution of the functionalized SWNHs. Typical TEM images of f-SWNHs as well as the pristine SWNHs are shown in Figure 6, indicating that the unique structure of SWNHs as well as their spherical aggregation were retained. Notably, the fraction of f-SWNHs seems to be

(57) Utsumi, S.; Honda, H.; Hattori, Y.; Kanoh, H.; Takahashi, K.; Sakai, H.; Abe, M.; Yudasaka, M.; Iijima, S.; Kaneko, K. *J. Phys. Chem. C* **2007**, *111*, 5572.
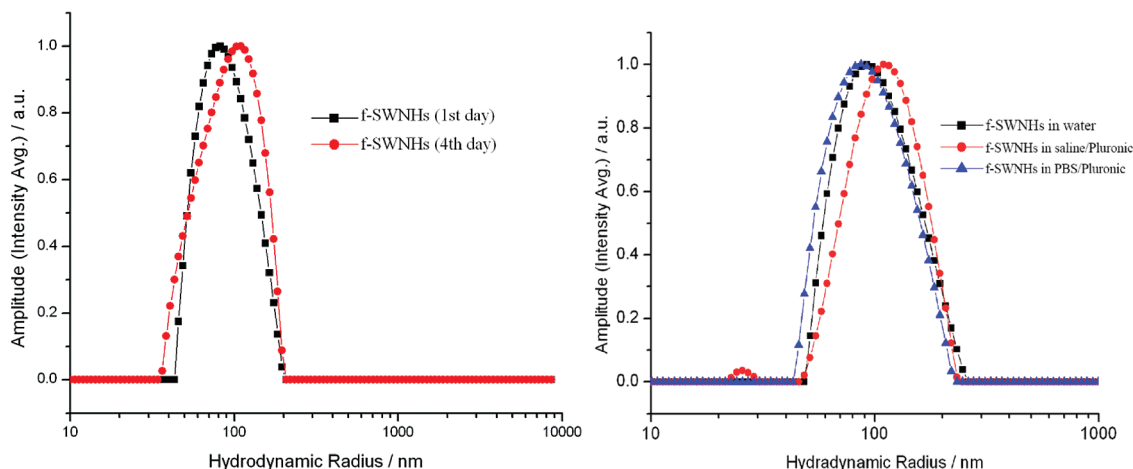
Figure 7. Intensity-weighted (intensity percent) size distribution profiles (90° scattering angle, 25 °C) of as-prepared f-SWNHs (black line) and SWNH−COOH stored at room temperature for four days (red line) in pure water (pH 7.0) (left) and f-SWNHs in different media (right).

less than that of pristine SWNHs, because of the damage from the high-speed vibration milling process.

The intensity-weighted size distribution of the f-SWNHs in pure water is clearly unimodal and the average hydrodynamic radii of the functionalized SWNH aggregates is ca. 80 nm for the fresh solution with an increase to 105 nm after 4 days storage at room temperature (Figure 7). Notably, the distribution of the stored solution spans from 35 to 200 nm, whereas that of fresh solution spans the range from 43 to 200 nm. The smaller aggregates from the stored solution may be ascribed to an aggregation/ deaggregation process. The hydrodynamic radii found in the current study are somewhat smaller than those reported for functionalized SWNHs (T7 Tag Ab-SWNHs) for which a hydrodynamic diameter of 210 nm was reported.[58] Significantly, our f-SWNHs are relatively stable, as confirmed by the DLS results. That is, after 4 days storage at room temperature, there is increase in the average hydrodynamic radius and broadening in the distribution. This result is consistent with the stability of f-SWNHs in pure water as Scheme 1 shows. In different media, the f-SWNHs have similar size distributions, as Figure 7 (right) shows, further indicating the stability of the f-SWNHs. The slight increase in average hydrodynamic radii in saline/0.25% Pluronic F127 relative to those in water and PBS/0.25% Pluronic

F127 may be due to electrostatic screening effects by the salt.

Conclusions

In summary, we have functionalized SWNHs with carboxylic acid groups by a facile solvent-free high-speed vibration milling method, which constitutes an efficient way for the chemical modification and solubilization of these materials. The method is complementary to other existing functionalization routes. Because the nanohorns are not deaggregated during the reaction and the round-shaped aggregates remain intact in solution, the water-dispersible f-SWNHs may be used in biotechnological applications, such as drug and gene delivery systems, as well as loading of the SWNHs side-walls with desirable functional groups for specific tissue-targeting.

(58) Miyako, E.; Nagata, H.; Hirano, K.; Sakamoto, K.; Makita, Y.; Nakayama, K.-i.; Hirotsu, T. *Nanotechnology* **2008**, *19*, 075106(1−6).