ROS EvaLuation for Series of CNTs Using the ESR Method and The Effects of CNT Morphology

S. Tsuruoka[1*], K. Takeuchi[2], K. Koyama[2], K. Fujisawa[2], H. Matsumoto[3], N. Saito[4], Y. Usui[2], D. W. Porter[5], V. Castranova[5], M. Endo[1]

[1] Shinshu University, Research Center for Exotic Nanocarbons, 4-17-1 Wakasato,Nagano, Japan, s_tsuruoka@shinshu-u.ac.jp
[2]Shinshu University, Faculty of Engineering, 4-17-1, Wakasato, Nagano, Japan,
[3]Department of Organic and Polymeric Materials Tokyo Institute of Technology, 2-12-1-S8-27 Ookayama, Meguro-ku, Tokyo, Japan
[4]Department of Applied Physical Therapy, Shinshu University, School of Health Sciences, 3-1-1 Asahi, Matsumoto, Nagano, Japan
[5]National Institute for Occupational Safety and Health, Morgantown, USA

ABSTRACT

Carbon nanotubes (CNTs) are becoming important materials in industry. It is a concern that CNTs may induce carcinogenic responses through pulmonary exposure. It has been recently reported that CNTs scavenge radical oxygen species (ROS) depending on their morphology. ROS production has been utilized for toxicological evaluations. The present work specifically investigates ROS scavenging capabilities using the series of CNTs and their derivatives with surface modifications. Those ROS scavenging properties were measured by ESR with 5,5-Dimethyl-1-pyrroline N-oxide (DMPO). Highly crystallized, mechanically chopped, and mechanically de-bulked CNTs were evaluated. Furthermore, their surface modification by nitric acid and ozone was compared with untreated CNTs.

Keywords: ROS, ESR, CNTs, surface morphology, functionalization

1 INTRODUCTION

Carbon nanotubes (CNTs) are becoming important materials in industry. It is a concern that CNTs may induce carcinogenic responses through pulmonary exposure. It has been recently reported [1-6] that CNTs scavenge radical oxygen species (ROS) depending on their morphology. ROS production has been utilized for toxicological evaluations. Although the electron charge transfer seems the noticeable phenomena of toxicological chemical reactions, a comprehensive evaluation of ROS scavenging capabilities using a variety of CNTs has not been demonstrated well. The present work specifically investigates ROS scavenging capabilities using the series of CNTs and their derivatives with surface modifications. Those ROS scavenging properties were measured by ESR with 5,5-Dimethyl-1-pyrroline N-oxide (DMPO) [7-13]. Highly crystallized, mechanically chopped, and mechanically de-bulked CNTs were evaluated. Furthermore, their surface modification by nitric acid and ozone was compared with untreated CNTs. Interestingly, the ROS scavenging rate was not significantly influenced by mechanical treatments, but depended on crystallization at high temperature. The results suggest that the electron transfer on the CNT surface is the fundamental mechanism of ROS scavenging. Dangling bonds are not a key factor for scavenging, though. ROS measurement is affected by surfactant concentration and the CNT/hydrogen peroxide ratio. The surface modification may lead to safety by design for CNTs.

2 MATERIALS AND METHODS

2.1 CNTs

More than 10 kinds of CNTs were prepared including surface modified and functionalized ones. As shown in Table 1, they consist of a cup-stack type of MWCNTs (cs-CNTs) and their derivatives, and usual MWCNTs that are functionalized with a functional group of -COOH. Particular CNTs identified as CS1 were the pivotal ones in the present study and were prepared and characterized intensively. CNTs identified as CS2 through CS5 were prepared to investigate effects of surface morphological changes. CS11 through CS14 were prepared to investigate effects of surface modifications by CS1 functionalized. Titanium dioxide (Evonik Degussa P25) was used as a reference. Figure 1 shows a transmission electron micrograph of CS1. As CS1 has a lot of dangling bonds on the surface in comparison with usual CNTs, it was assumed that physical treatments might give quantitative differences by significantly causing morphological changes as suggested by Fubuni et al. [14].

2.2 Measuring Method

First of all, dispersibility of the prepared CNTs was tested to avoid agglomeration during the Electron Spin Resonance (ESR) measurements. All CNTs were reasonably dispersed in a DMPO and hydrogen peroxide solution with surfactant, with no clumping by CNT agglomeration in the pipet of ESR equipment observed. The generation and scavenging of ROS by CNTs were measured at room temperature by ESR (JES-FA100, JEOL) using DMPO as the spin trapping agent. ESR settings were: frequency 9415.404 MHz, power 0.998 mW, field center 335 mT, sweep time 2 min., width +/- 5 mT, and modulation frequency 100 kHz. Fresh frozen DMPO (Dojindo Laboratories, Kumamoto Japan) was thawed at room temperature and diluted to 100 mM with ultrapure water. Hydrogen peroxide (hydrogen peroxide 30.0-35.5 mass%, Wako Pure Chemical Industries, Ltd. Japan) was diluted to 0.971 mM. Ferrous chloride (Iron (II) Chloride Tetrahydrate, Wako Pure Chemical Industries, Ltd. Japan) was dissolved into ultrapure water at 0.0314 mM. Surfactant was Sodium Dodecyl Benzensulfonate supplied by Kanto Chemical Co., Inc. Japan, diluted to 4.59 mM. CNTs listed in Tables 1 were dispersed into aqueous solutions listed in Table 2 depending on the measurement items. All of measuring samples were prepared just before ESR measurement to avoid any change over time. Each sample was measured five and more times repeatedly and averaged after elimination of the highest and lowest values.

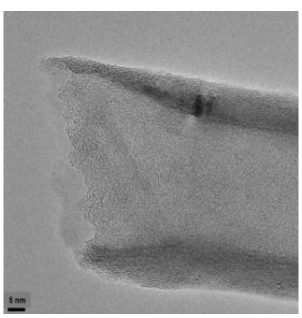

Figure 1. Transmission electron micrograph of a cup-stack type MWCNT (CS1).

3 RESULTS AND DISCUSSION

Figure 2 shows a typical ESR spectrum of ROS generation by the Fenton reaction and quenching by CNTs. CNTs tested and TiO_2 in the absence of ferrous chloride did not produce ROS. Degenaration rate of OH radicals corresponded to a CNT concentration. Degeneration rate of OH radicals with a change of CNTs concentration is plotted in Figure 3. The result shows that scavenging of OH radicals was proportional to a concentration of CNTs. OH radicals generated by the Fenton reaction between $FeCl_2$ and hydrogen peroxide were apparently quenched by CNTs in a concentration dependent manner. On the other hand, surfactant apprently affected degeneration rate of OH radicals in Figure 4 in which scavenging rate is proportional to a surfactant concentration.

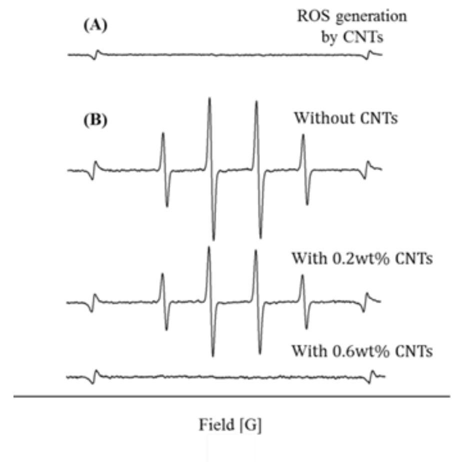

Figure 2. Typical ESR spectrum intensity of free radical adducts with DMPO using CS1; (A) OH radicals generated by CNTs without $FeCl_2$. Mixture A was used. (B) OH radicals generated by the Fenton reaction with $FeCl_2$. The more radicals were scavenged with the higher CNTs concentration. Mixture B was used.

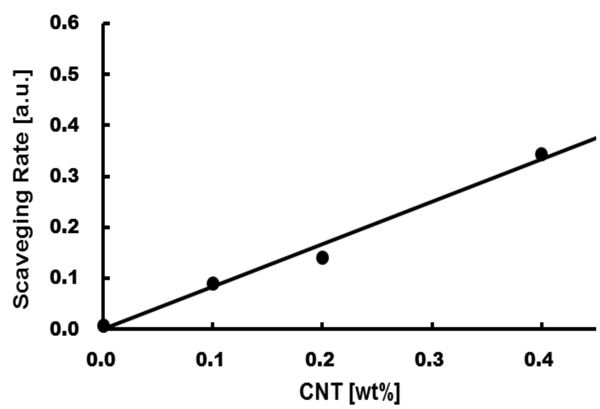

Figure 3. A change of scavenging rate of OH radicals with a CNTs (CS1) concentration change in an aqueous solution consisting of DMPO, hydrogen peroxide, $FeCl_2$ and surfactant (Mixture D). The CNTs are cup-stack type Multi-walled Carbon Nanotubes and the average diameter and length are at 100 nm and 5 μm, respectively. The rate was measured by ESR with OH radical – DMPO adduct.

Table 1. CNT properties used for ROS evaluations

CNTs	Description	SSA[a] (m^2/g)
CS1	Cup-Stack type MWCNT, diameters: outer = 80 ~100 nms, inner = 50 ~ 70 nms, length = 5 µms ave., purity > 95wt%	49.0
CS2	Graphitized CS1 annealed at 2800 °C	49.5
CS3	Mechanically chopped CS1, average length = 1.5 µm	46.9
CS4	Mechanically chopped CS1, average length = 1.0 µm	74.2
CS5	Mechanically de-bulked CS1, slightly shorter length than CS1	45.8
CS11	Functionalized CS1 (Oxidized by HNO_3, and then disentangled)	-
CS12	Functionalized CS1 (Disentangled and then oxidized by HNO_3)	-
CS13	Functionalized CS1 by ozone (processed mildly)	-
CS14	Functionalized CS1 by ozone (processed strongly)	-
CW1	MWCNTs	-
CW1F	Functionalized CW1 with *-COOH*	-

[a] SSA stands for specific surface area measured by BET isotherm adsorption method.

Table 2. Measuring Solutions: combinations of chemicals

Solutions	$FeCl_2$ 0.0314 mM	CNTs w/ surfactant 4.59 mM	DMPO 100 mM	Surfactant 4.59 mM	H_2O_2* 0.971 mM	Ultrapure water	Total volume
Mixture A	-	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.5 ml
Mixture B	0.1 ml	0. 1 ml	0.1ml	-	0.1 ml	0.1 ml	0.5 ml
Mixture C	0.4 ml	None	0.4 ml	0.4 -0.8 ml	0.4 ml	Balance	2.0 ml
Mixture D	0.4 ml	0 -0.4 ml	0.4 ml	Balance	0.4 ml	0.4 ml	2.0 ml

*Nominal concentration was determined as 33 wt%

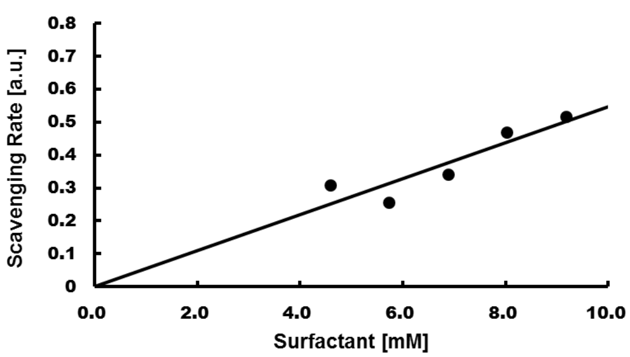

Figure 4. A change of scavenging rate of OH radicals with a surfactant concentration change in an aqueous solution consisting of DMPO, hydrogen peroxide, $FeCl_2$ and surfactant without CNTs. The rate was measured by ESR with OH radical – DMPO adduct. CS1 and Mixture C were used.

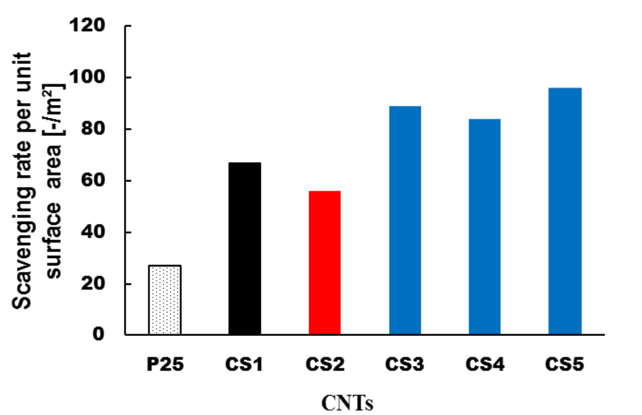

Figure 5. Scavenging rate of OH radicals with surface modified MWCNTs as shown in Table 1. Mixture B was used. The rate was measured by ESR with OH radical – DMPO adduct.

The relationship between CNT surface morphology and OH radical scavenging was evaluated using CS1 and its derivatives. Figure 5 shows results normalized by the individual specific surface area. It demonstrates that surface morphology has an effect on OH radical scavenging i.e., augmentation of scavenging. Figure 5 suggests that the shortest CNT (CS4) exhibited the lower scavenging rate, and that radical scavenging corresponds to length in addition to the surface properties. Meanwhile the graphitization that increases crystallinity and decreases the number of dangling bonds apparently affected scavenging.

The present study demonstrates that CNT scavenging rate depends on the surface morphology and length. The present ROS measuring method by ESR is simple and reproducible but chemical reactions of CNTs with DMPO have to be reviewed carefully because pyrroline types of organic chemicals are used to disperse CNT in aqueous solutions. Additionally, a surfactatnt concentration has to be carefully determined to conduct ROS measurement with CNTs. Thus, further investigation is necessary to determine effects by surface morphology and the role of electron charge transfer on the CNT surface with and without dangling bonds.

4 CONCLUSION

More than 10 kinds CNTs were evaluated to determine their ROS generation and scavenging rate. CNTs tested degenerated OH radicals. The scavenging rate depended on surface morphology. The result supports the assumption discussed by Fubini et al. [14] and Fenoglio et al. [2]. On the other hand, the OH radical degeneration reaction is far more complicated than predicted. Parameters to describe CNT scavenging characteristics have to include surface morphology, such as the number of dangling bonds, length, and functional groups attached CNT surface. The results suggest necessity of the further investigation of the role of impurities and CNT surface features, and surfactant effect in hydroxyl radical generation or scavenging.

ACKNOWLEDGEMENT

We thank GSI Creos Corporation to provide the specific CNT samples and evaluate their properties. Also we thank Prof. N. Wu at Dept of Mechanical & Aerospace Engineering, West Virginia University to provide carbonated MWCNTs. This work was supported by a Grant-in-Aid for Specially Promoted Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT) (No. 19002007) (ME), the Exotic Nanocarbon Project, Japan Regional Innovation Strategy Program by the Excellence, JST (Japan Science and Technology Agency) (ME, ST, KT).

DISCLAIMER

The findings and conclusions in this article are those of the authors and do not necessarily represent the view of the National Institute for Occupational Safety and Health.

REFERENCES

[1] Shvedova, A., et al., *J. Toxicol. Environ. Health.* **A 66**, 1909-1926, 2003.
[2] Fenoglio, I., et al., *Free. Rad. Biol. Med.* **40**, 1227-1233, 2006.
[3] Fenoglio, I., et al., *Chem. Res. Toxicol.* **21**, 1690-1697, 2008.
[4] Murray A.R., et al., *Toxicology* **257**, 161-171, 2009.
[5] Porter, D.W., et al., *Toxicology* **268**, 136-147, 2010.
[6] Tsuruoka, S., et al., *J. physics, Conference Series* (in print)
[7] Harbour, J.R., et al., *Can. J. Chem.* **52**, 3549-3553, 1974.
[8] Felix, C.C., et al., *Biochemical Biophysics Res. Comm.* **84**(2), 335-341, 1978.
[9] Fimkelstein, E., et al., *J. Am. Chem. Soc.* **102**, 4994-4999, 1980.
[10] Buettner, G.R., *Free Rad. Biol. Med*. **3**, 259-303, 1987.
[11] Krusic, P.J., et al. *J. Am. Chem. Soc.* **113**, 6274-6275, 1991.
[12] Makino, K., et al., *Can. J. Chem.* **70**, 2818-2827, 1992.
[13] Jones, C.M., et al., *J. Chem. Soc., Perkin Trans.* **2**, 2044-2051, 2002.
[14] Fubini, B., et al., *Nanomedicine* **6**, 899-920, 2011.