The Impact of Sonication on the Surface Quality of Single-Walled Carbon Nanotubes

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ABSTRACT: Sonication process is regularly adopted for dispersing single-walled carbon nanotubes (SWCNTs) in an aqueous medium. This can be achieved by either covalent functionalization of SWCNTs with strong acid or by noncovalent functionalization using dispersants that adsorb onto the surface of SWCNTs during dispersion. Because the dispersion process is usually performed using sonication, unintentional free radical formation during sonication process may induce covalent modification of SWCNT surface. Herein, we have systematically investigated the status of SWCNT surface modification under various sonication conditions using Raman spectroscopy. Comparing I_D/I_G (Raman intensities between D and G bands) ratio of SWCNTs under various sonication conditions suggests that typical sonication conditions (1–6 h bath sonication with sonication power between 3 and 80 W) in aqueous media do not induce covalent modification of SWCNT surface. In addition, we confirm that SWCNT dispersion with single-stranded DNA (ssDNA) involves noncovalent adsorption of ssDNA onto the surface of SWCNTs, but not covalent linkage between ssDNA and SWCNT surface. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:2594–2599, 2015

Keywords: single-walled carbon nanotubes; sonication; surface modification

INTRODUCTION

There is an ever-increasing interest in the use of single-walled carbon nanotubes (SWCNTs) for pharmaceutical applications, either as potential drug or gene delivery tools[1–4] or as tissue scaffolds.[5,6] Many of these applications are owing to their unique mechanical, electrical, and thermal properties. SWCNTs typically exist as water-insoluble bundles.[7,8] For various pharmaceutical applications, it is almost inevitable that one needs to disperse SWCNT bundles into an aqueous medium in order to be biologically compatible. Aggregated instead of dispersed SWCNTs have been shown to induce toxicity *in vivo*.[9] Thus, there is a need for methods to disperse and stabilize SWCNTs in aqueous media.

Two types of methods have been developed in the literature for the dispersion of SWCNTs in aqueous media. The first type involves the treatment of SWCNTs with strongly oxidative acids,[10–12] which oxidize the surface of SWCNTs and give rise to hydrophilic groups that afford the dispersion of SWCNTs in aqueous media.[13] Although efficient, this strong acid treatment and the resulting highly modified surface of SWCNTs are not usually compatible with downstream applications.[14,15] Modification of SWCNT surface through noncovalent interactions is thus desired to achieve dispersion in aqueous media.[16,17] This method involves the use of a dispersant molecule, which is typically amphiphilic in nature.[18–21] Sonication (3–15 W, 1 h) of SWCNTs with dispersant in a pH ~6 deionized water (ddH_2O) system induces adsorption of dispersant onto the surface of SWCNTs via π-stacking or Van der Waals interactions.[18–20] The hydrophilic groups in the dispersant molecules thus afford the dispersion of SWCNTs.

Although this noncovalent functionalization of SWCNT surface does not involve any covalent modification of SWCNT surface,[22–24] the impact of sonication procedure itself on SWCNT surface is less certain. Sonication in an aqueous solution is known to generate free radicals such as hydroxyl radical ($OH\cdot$) and the super-oxide ion (O_2^-).[25,26] These highly reactive species may chemically modify the sp^2 carbons on the surface of SWCNTs, and further mediate the covalent attachment of dispersant molecules on SWCNT surface. Indeed, Hines[27] reported that covalent linkage between SWCNT and short single-stranded DNA (ssDNA) may occur after sonication process in water. Although sonication procedure is widely adopted in SWCNT community for their dispersion in aqueous media, the extent of covalent modification of SWCNT surface through sonication has not been quantified or reported. For pharmaceutical applications where the molecules of interest are associated with SWCNT surface through noncovalent adsorption for delivery into biological milieu, the covalent attachment of these molecules will unavoidably compromise the release of these molecules. Therefore, in this paper, we have carried out a systematic study on the effect of sonication on SWCNT surface modification, the results of which should be useful for the application of SWCNTs as drug or gene delivery tools.

EXPERIMENTAL

Preparation of Individually Dispersed SWCNTs

One milligram of as-prepared SWCNTs soot produced from arc discharge method (AD SWCNTs; Helix Materials Solution, Richardson, Texas), or chemical vapor deposition (CVD; SES Research, Houston, Texas), or high-pressure carbon monoxide process (HiPCO, Super purified; Unidym, Sunnyvale, California) was added to 1 mL distilled water and ddH_2O (Synergy UV, Millipore, Massachusetts) together with 1 mg of a ssDNA oligo, $(dT)_{30}$ (IDT, Coralville, Iowa) in a 1.5-mL centrifuge

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tube. The mixture was then subjected to bath sonication under various conditions as indicated throughout the paper. The sonication was performed either using a tabletop sonicator (Table Ultra Sonic Cleaner, FS-20H; Thermo Fisher Scientific, Waltham, Massachusetts) with an output power ~3 W for various amount of time as indicated, or using a variable-power sonication system (Ultrasonic Processor, S-4000; Misonix, Farmingdale, New York) for 1 h at various output power settings (10, 20, 40, and 80 W). For sonication in the latter system, 15 s of sonication was followed by 5 s idleness for each step, and the step was repeated until the total on-time reached 1 h. For both sonication apparatus, ice was constantly added to the water bath surrounding the centrifuge tube to prevent overheating throughout the entire sonication process. Dispersed SWCNTs were centrifuged at 17,000*g* for 1 h at room temperature (Sorvall Legend Micro 17; Thermo Fisher Scientific). Supernatants were collected, and the fraction of individually dispersed SWCNTs was estimated by recording absorbance at 1023 nm using a UV/Vis spectrophotometer (UV-1800; Shimadzu, Kyoto, Japan), as we described previously.[20]

Raman Spectroscopy of SWCNTs

Ten microliter of dispersed SWCNT samples in ddH_2O was placed onto aluminum foil on a glass slide ($75 \times 25 \times 1$ mm^3; Thermo Fisher Scientific) and analyzed using two different wavelengths of laser (514 and 633 nm, inVia Raman microscope; Renishaw Inc., Hoffman Estates, Illinois). The microscope was operated at 1% laser power with a 100× objective lens (BX41; Olympus, Tokyo, Japan) and 30 s exposure time. For each sample, the Raman peak intensities for D-band (I_D, ~1350 cm^{-1}) and G-band (I_G, ~1590 cm^{-1}) were taken directly from the spectra, and their ratios were calculated as reported.[28–32] Carboxylated SWCNTs (SWCNT-COOH, or P3-SWNT from Carbon Solutions Inc., Riverside, California) and amide-functionalized SWCNTs (SWCNT-$CONH_2$, or P9-SWNT from Carbon Solutions Inc.) were used and measured as the original soot without dispersion.

Dispersion of SWCNTs in the Presence of Ascorbic Acid or Trolox

One milligram of AD, CVD, or HiPCO SWCNTs was dispersed in 1 mL of ascorbic acid or trolox (antioxidant)[33,34] aqueous solution in the presence of 1 mg of $(dT)_{30}$ through sonication using the tabletop sonicator at ~3 W for various amount of time as indicated. The concentration of ascorbic acid or trolox was 0.5 mg/mL unless otherwise noted. To reduce the concentration of O_2 in the solution that may facilitate the generation of radicals, we also conducted sonication after argon purging. This was performed by placing the 1.5-mL centrifuge tube with sample mixtures in a vacuum desiccator. With the tube cap open, we pulled with house vacuum for 1 h. Argon stream was then applied for 1 min. The cap of the tube was then closed and sealed with Parafilm (Bemis NA, Neenah, Wisconsin) before sonication. All reagents were from Sigma–Aldrich (Saint Louis, Missouri) unless specified.

RESULTS AND DISCUSSION

Raman Spectroscopy of SWCNTs

To test whether sonication process induces covalent modification of SWCNTs, Raman spectra[28–32] of AD, CVD, and HiPCO SWCNTs after dispersion with $(dT)_{30}$ (1 h at 3 W) were analyzed and compared with SWCNTs before dispersion (SWCNT soot). Typical Raman spectra of AD SWCNT before and after dispersion were shown in Figures 1 a and 1b, respectively. For comparison, we also collected the Raman spectra for SWCNT soot that were functionalized by the manufacturer, as shown in Figure 1c

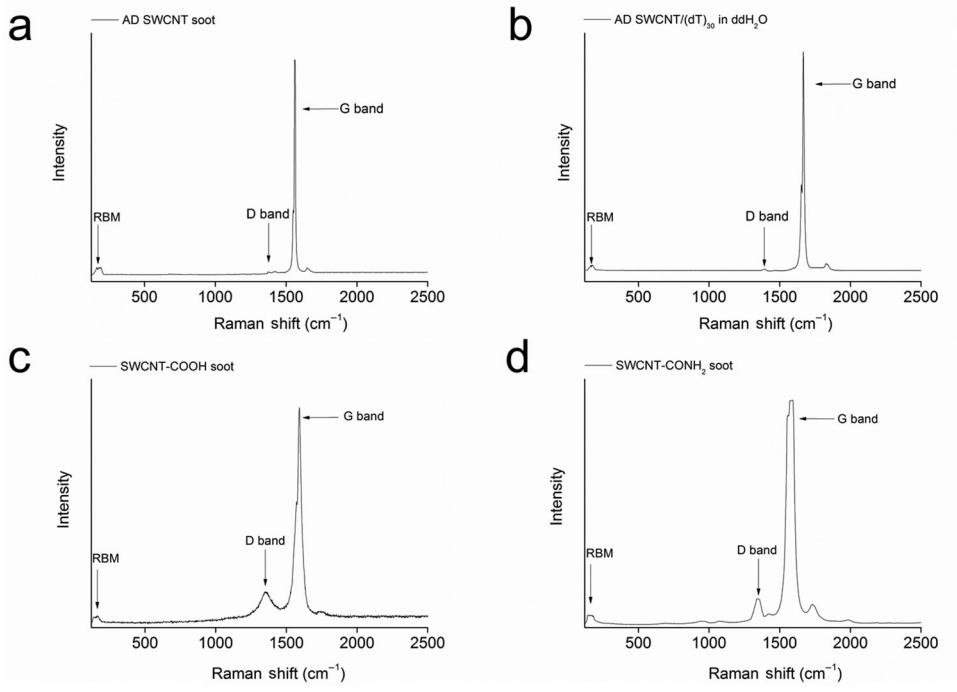

Figure 1. Raman spectra recorded using 514 nm laser for (a) AD SWCNT soot, (b) AD SWCNT/$(dT)_{30}$ dispersed in water, (c) SWCNT-COOH soot, and (d) SWCNT-$CONH_2$ soot.