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

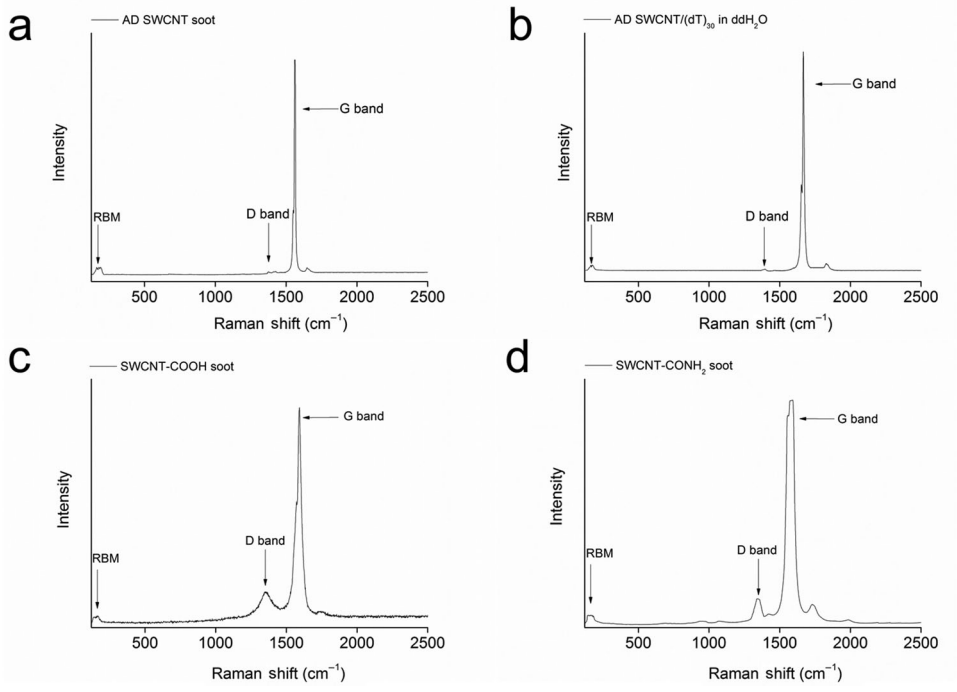

Figure 1. Raman spectra recorded using 514 nm laser for (a) AD SWCNT soot, (b) AD SWCNT/$(dT)_{30}$ dispersed in water, (c) SWCNT-COOH soot, and (d) SWCNT-$CONH_2$ soot.

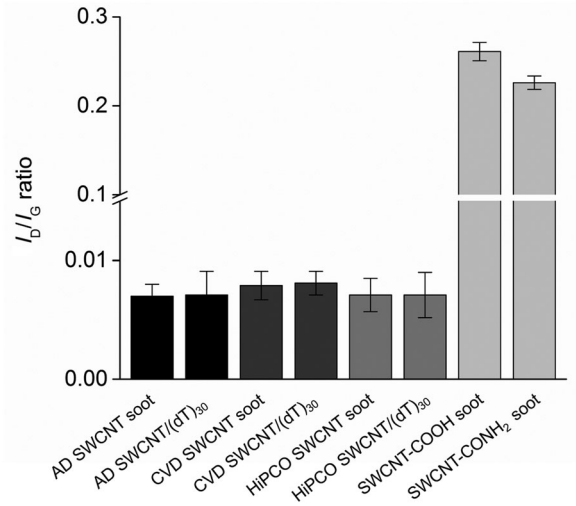

Figure 2. I_D/I_G ratio of different types of SWCNTs. Error bars represent SD from three independent repeats of the same experiments.

for SWCNT-COOH and Figure 1d for SWCNT-$CONH_2$. The D-band in SWCNT Raman spectra results from defect along SWCNT and covalently functionalized SWCNTs show a clear D-band signal around 1350 cm^{-1}.[30] At a given laser wavelength, this ratio is directly proportional to the density of defects on SWCNT surface.[29] As a result, the quality of an SWCNT sample is evaluated by comparing the Raman intensities between D and G bands, characterized by the I_D/I_G ratio.[30] For high-quality samples, the I_D/I_G ratio is often below 0.01. As shown in Figure 1, the D-bands for AD SWCNT soot and AD SWCNT dispersed in water with $(dT)_{30}$ (SWCNT/$(dT)_{30}$) are both very subtle. In contrast, both SWCNT-COOH and SWCNT-$CONH_2$ show apparent D-bands around 1350 cm^{-1}. The I_D/I_G ratio for AD SWCNT soot was 0.007 ± 0.001 (mean ± SD throughout), consistent with being a high-quality SWCNT sample without many defects. The I_D/I_G ratio for AD SWCNT/$(dT)_{30}$ was 0.007 ± 0.002, which is identical to that of AD SWCNT soot within error, indicating that 1 h sonication using a tabletop sonicator (~3 W) does not introduce any significant covalent modification of AD SWCNTs. In contrast, SWCNT-COOH and SWCNT-$CONH_2$ showed average I_D/I_G ratio of 0.261 ± 0.010 and 0.226 ± 0.008, respectively, indicating that both of these functionalized SWCNTs contain significant covalent modifications on their surface. Figure 2 shows the values of I_D/I_G ratio for the different SWCNT samples that we have prepared via sonication for 1 h at ~3 W in comparison with various SWCNT soots. For all these samples except the two made-to-functionalized SWCNTs, they all have an I_D/I_G ratio that is less than 0.01. There are no changes in I_D/I_G ratio within error before and after sonication for 1 h at ~3 W.

Effect of Sonication Time and Power on the Quality of SWCNT Dispersion

We have shown previously that a long sonication time or high sonication power during the dispersion of SWCNT leads to a significant reduction in SWCNT length.[20] This breakage of SWCNT as a result of ultrasonic processing may lead to covalent modification of SWCNTs, especially at the broken ends of individual SWCNTs. To investigate the effect of sonication time and power on the quality of dispersed SWCNTs, we measured Raman spectra for samples dispersed under various conditions, and calculated the I_D/I_G ratio for each sample for the comparison. As shown in Figure 3a for AD, CVD, and HiPCO SWCNTs dispersed in the presence of $(dT)_{30}$ at various powers, there is a small but measureable trend of increase in I_D/I_G ratio with increasing sonication power. This small trend of increase of I_D/I_G ratio to 0.008, 0.009, and 0.007 for AD, CVD, and HiPCO SWCNTs, respectively, as the power was increased from 3 to 80 W. Similarly, as shown in Figure 3b for AD, CVD, and HiPCO SWCNTs dispersed in the presence of $(dT)_{30}$ in a tabletop sonicator (~3 W) but with various lengths of sonication time, there is also a small but measureable trend of increase in I_D/I_G ratio with increasing sonication time. This small trend of I_D/I_G ratio to 0.008, 0.009, and 0.007 for AD, CVD, and HiPCO SWCNTs, respectively, as the time was increased from 30 min to 12 h. Regardless, all these I_D/I_G ratios remained below 0.01 throughout the different sonication procedures. These data suggest that although higher power or longer time of sonication will lead to more covalent modifications of SWCNTs (oxidation of sp^2 carbon to sp^3 for example), which likely occur around the broken ends of individual SWCNTs, the overall effect is very small and the dispersed SWCNTs remain in high quality.

Effects of Antioxidants on SWCNT Dispersion

Although extensive studies on SWCNT dispersion in the presence of ssDNA suggest that ssDNA [including $(dT)_{30}$] adsorbs

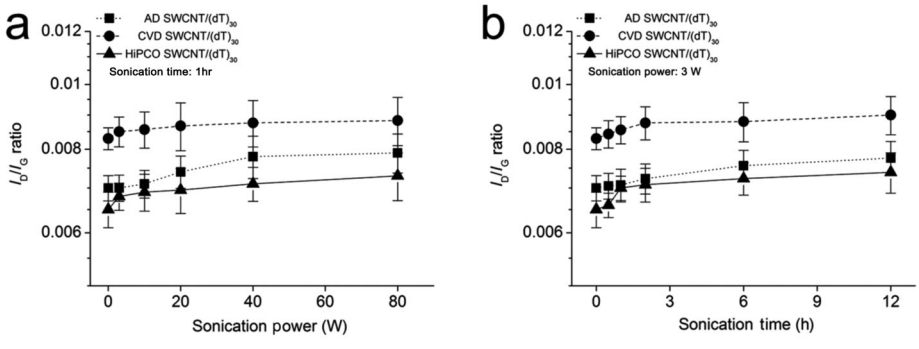

Figure 3. The impact of sonication power (a) and sonication time (b) on the quality of dispersed SWCNTs. The I_D/I_G ratios for SWCNT samples dispersed under various conditions are shown for AD, CVD, and HiPCO SWCNT/$(dT)_{30}$, as a function of sonication time in (a) or a function of sonication power in (b). Error bars represent SD from three independent repeats of the same experiments.

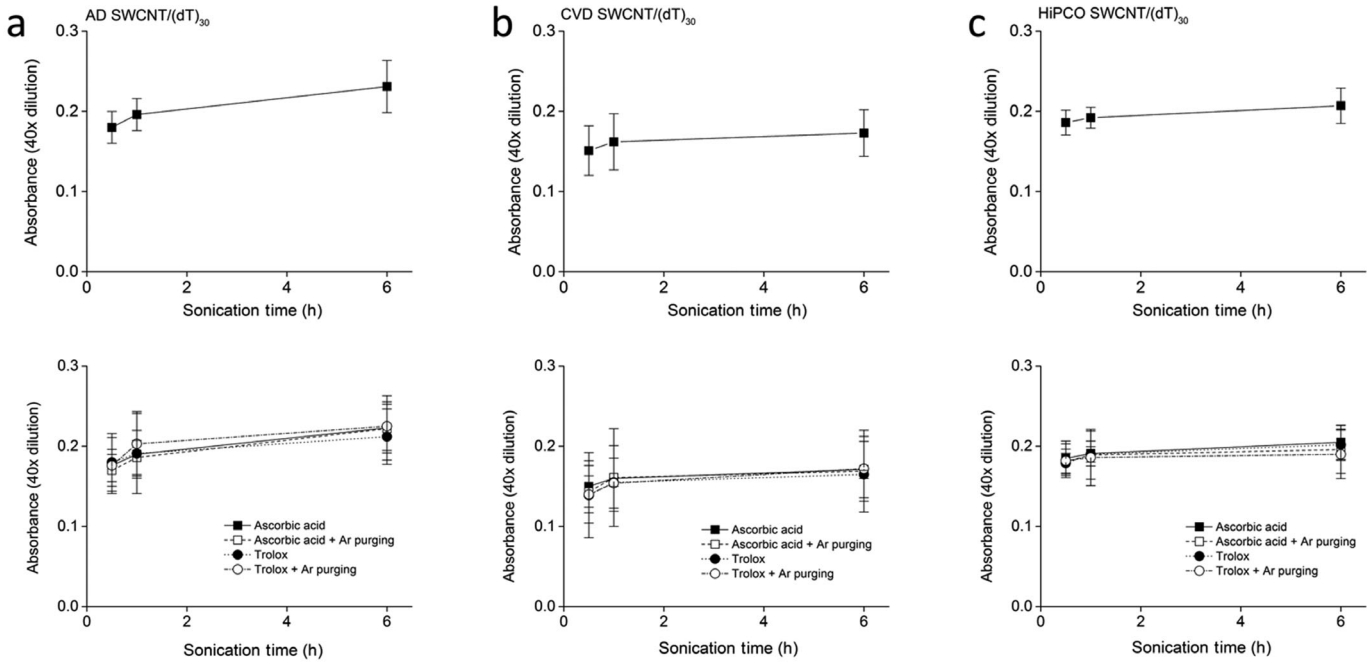

Figure 4. The dispersion of SWCNT samples in the absence (a) or presence of various antioxidants (b). The amount of dispersed SWNCTs was monitored by absorbance at 1023 nm. Using a sonication power of ~3 W, the amount of SWCNTs dispersed into an aqueous solution as a function of sonication time was shown in (a), (b), and (c) for AD, CVD, and HiPCO SWCNT/$(dT)_{30}$, respectively. The top panels are for dispersions in ddH_2O without antioxidants, and the bottom panels are for dispersions in ddH_2O in the presence of ascorbic acid or trolox, with or without argon purging. Error bars represent SD from three independent repeats of the same experiments.

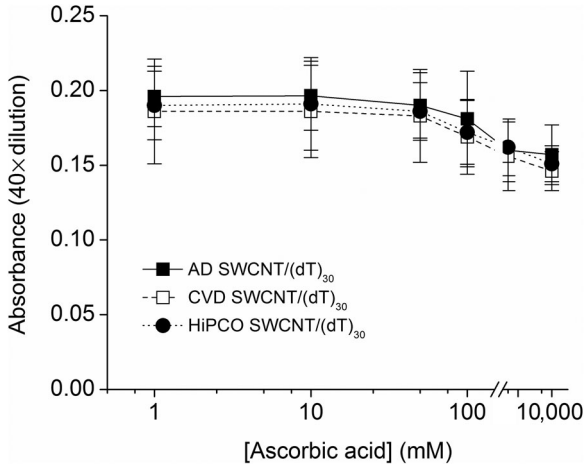

Figure 5. Ascorbic acid concentration-dependent dispersion (absorbance at 1023 nm) of AD, CVD, and HiPCO SWCNTs in the presence of $(dT)_{30}$. Error bars represent SD from three independent repeats of the same experiments. The sonication time is 1 h.

onto the surface of SWCNTs via π-stacking but not covalent attachment,[19,35] Hines[27] reported that dispersion of SWCNTs with $(dT)_{30}$ was achieved through covalent modification of SWCNT surface; free radicals generated during sonication leads to covalent attachment of ssDNA to SWCNT surface, which facilitates the dispersion of SWCNT in an aqueous solution. They showed that SWCNTs were not dispersed with $(dT)_{30}$ in the presence of antioxidants such as ascorbic acid or trolox (chemical structures shown in Figure S1, Supplementary material).[27] The explanation they provided was that antioxidant quenches free radicals during sonication, which prevent covalent functionalization of SWCNTs and covalent attachment of $(dT)_{30}$ to SWCNT surface. To test this possibility, we examined the dispersion of SWCNTs with $(dT)_{30}$ in the presence of either ascorbic acid or trolox. The amount of SWCNTs dispersed into the aqueous solution was measured by UV–Vis absorbance spectra as we showed previously.[20] As shown in Figure 4, SWCNTs can be dispersed into an aqueous solution in the presence of either antioxidant. The extent of dispersion was quantitatively similar to that without antioxidant (top panels). Moreover, a systematic study on the dependence of SWCNT dispersion on the concentration of ascorbic acid only shows less than 25% decrease in SWCNT dispersion even at very high concentrations of ascorbic acid (Fig. 5). We further tested the dispersion of SWCNTs in the presence of either antioxidant plus purging with argon to minimize the formation of free radicals during sonication. Throughout, all these different dispersion conditions produced similar extent of SWCNT dispersion, less than 3% difference was observed in the amount of SWCNTs dispersed under various conditions. This conclusion is true for all three types of SWCNTs that we have tested. Furthermore, we have quantitated the I_D/I_G ratios for all these samples prepared from sonication for 1 h at ~3 W. As shown in Figure 6, all these samples showed I_D/I_G ratios less than 0.01, and there were no changes in these values within error in the presence or absence of antioxidant. These results suggest that covalent attachment of $(dT)_{30}$ to the SWCNTs is unlikely to be the mechanism of SWCNT dispersion by $(dT)_{30}$ because no significant differences were observed for SWCNT dispersion in the presence or absence of antioxidants. These results also indicate that under current sonication conditions, the impact of free radical formation on SWCNT dispersion is low.

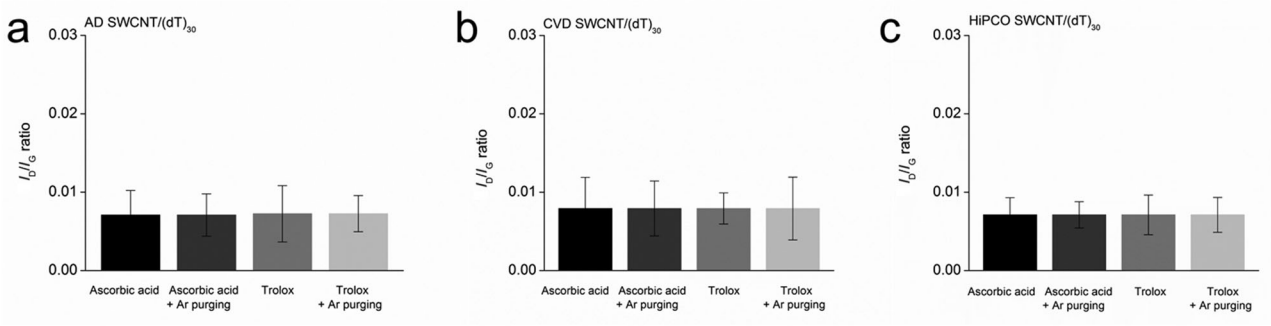

Figure 6. I_D/I_G ratios for (a) AD (b) CVD, and (c) HiPCO SWCNT/$(dT)_{30}$ after 1 h sonication using a tabletop sonicator (~3 W) in the presence of ascorbic acid or trolox, with or without argon purging. Error bars represent SD from three independent repeats of the same experiments.

CONCLUSIONS

Single-walled carbon nanotube dispersion into an aqueous solution using conventional sonication process (bath sonication power: 3–80 W, sonication time: 1–6 h) did not induce noticeable covalent modification of SWCNTs compared with SWCNT soot without sonication. Longer sonication time or higher sonication power does lead to more defects on SWCNTs; however, this increase in defect sites is less than 10% and thus not significant. SWCNT sonication in the presence of antioxidants and with argon purging suggests that blocking free radical formation has no apparent effect on SWCNT dispersion, and that dispersion of SWCNTs by ssDNA is through noncovalent π-stacking instead of convalent conjugation to SWCNT surface. Overall, our data provide evidence that sonication method during dispersion of SWCNT in an aqueous solution as we described does not induce noticeable surface damage to SWCNTs.

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