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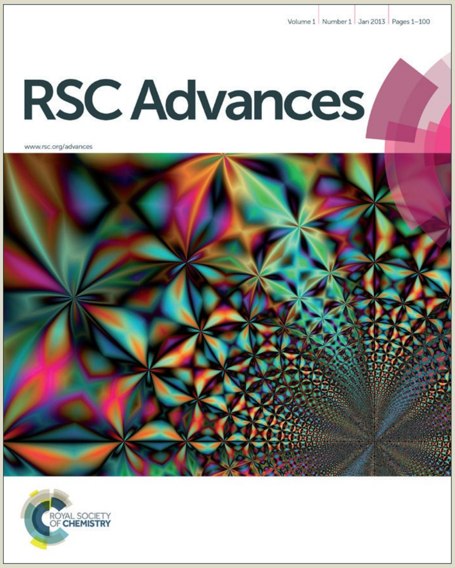

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ARTICLE

# A Simple Quantitative Estimate of the Number of Functional Groups on the Surfaces of Single-Walled Carbon Nanotubes

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Quynh Bui Vu Ngoc,[†] Myung-Soo Choi,[†] Woo-Jae Kim*

We develop a quantitative analytical method to estimate the number of functional groups on single-walled carbon nanotube (SWNT) surfaces by simply measuring the Raman features of functionalized SWNTs. We use diazonium chemistry to attach functional groups to the SWNT surfaces and calculate the number of functional groups on the SWNTs by measuring their densities and using analytical method developed in this study. We successfully establish a linear relationship between the independently estimated number of functional groups per carbon atom and the area ratio of the disorder mode (D peak, 1289 cm$^{-1}$) to the tangential mode (G peak, 1582 cm$^{-1}$) from the Raman measurements. From this study, we find out that the injected reagents do not completely react with SWNT after 0.0263 functional groups per carbon atom are already attached to SWNT surfaces, e.g., 2.354 functional groups per nm (6,5) SWNT, therefore above this range, there is no linear relationship between the number of functional groups on SWNTs estimated from injected reagent concentration and the D to G area ratio ($A_{D/G}$) of Raman feature. However, the number of functional groups per carbon atom ($n_f$) estimated using the analytical method developed in this study linearly depends on the area ratio of D to G peaks of Raman spectrum even above this range ($n_f = 0.02{\cdot}A_{D/G}$), therefore this simple and quick estimation scheme can provide an analytical basis for a variety of SWNT applications. We take into account the decrease of number of surfactants by the coverage of functional groups on SWNTs in this scheme, and also verified that this relation is valid irrespective of SWNT diameter distributions.

## Introduction

Single-walled carbon nanotubes (SWNTs) are nanometer-diameter cylinders comprising a single graphene sheet wrapped into a tubular structure.[1] SWNTs possess a unique electrical conductivity, charge carrier mobility, high surface area, and chemical reactivity, as well as sensitivity,[2–6] and have therefore been proposed and used as novel platforms for electrodes in fuel cells, supercapacitors, and lithium ion batteries, chemical and biological sensors, drug delivery systems, etc. Some of these applications require that functional groups be added to the surfaces of SWNTs to make use of their novel properties. Specific molecules attached to their surfaces maximize their performance in various applications.[7] For example, carboxyl groups attached to SWNTs by acid treatment enable solution processing of SWNTs and furthermore, these groups on SWNTs can react with other chemical species, such as SOCl and carbodiimide, to produce functional materials that have the potential to react with other compounds.[8,9] Hydrophilic polymers attached to oxidized SWNTs using poly(ethylene glycol) can yield stable biocompatible CNT-polymer conjugates for use in biological applications.[10–12] Diazonium groups selectively attached to metallic over semiconducting SWNTs modify electronic structures of SWNTs and furthermore, enable SWNT separation by electronic types for various applications.[13]

Therefore, the functionalization of SWNTs has been extensively investigated to explore the chemistry of SWNTs for potential applications. While both covalent and non-covalent functionalizations have been attempted, the covalent approach provides better stability and reproducibility in devices fabricated from these materials.[7,14–17] For this purpose, various covalent functionalization reactions of SWNTs, including diazonium-based functionalization,[13] 1,3-dipolar cycloaddition,[18,19] and the carboxylic acid derivatization protocol[20,21] have been proposed and used in many applications, including solar cells,[22] bio and chemical sensors,[20] drugs and gene deliveries,[15] [20]nanostructured electronic devices,[23] nanofiltration,[24] catalyst supports.[19]

In addition to the development of novel functionalization schemes, it is also critical to estimate the number of functional groups attached to the SWNT surfaces in these applications because the number of functional groups on the SWNTs determines the quantitative performance of an SWNT-based device,[25,26] e.g., the amount of a drug that can be stored in the

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*Department of Chemical and Biological Engineering, Gachon University, Seongnam, Gyeonggi-do 461-701, Korea*
*****Corresponding author. wjkim@gachon.ac.kr (W-J.K.), Phone: 82-31-750-8514. Fax: 82-31-750-8516.

[†] These authors contributed equally.

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SWNTs,[27–29] the maximum sensitivity of a biosensor,[30,31] and most importantly, the threshold beyond which SWNT properties are completely destroyed upon functionalization. However, it is very difficult to achieve precise control over the reactions of such additions due to the complexity of the solubility, hydrophobicity and reactivity issues of SWNTs and reagents; therefore, it is very difficult to characterize the surfaces of functionalized SWNTs.[19]

The area ratio of the disorder mode (D peak, 1289 $cm^{-1}$) to the tangential mode (G peak, 1582 $cm^{-1}$) from the Raman measurements of functionalized SWNTs has been widely used to study the extent of surface functionalization of SWNTs. This method is a simple and powerful tool to investigate the surfaces of functionalized SWNTs, however, it usually gives a qualitative information of SWNT surfaces, rather than quantitative information by itself. Therefore, various complex characterization techniques, including UV/vis/NIR, Raman, TGA, XPS, IR, AFM, and TEM,[32,33] have been employed to investigate and estimate the extent of the covalent functionalization of SWNTs; however, simple quantitative analytical tools that can estimate the number of functional groups on SWNTs have not been proposed yet. As a consequence, theoretical and experimental support for characterizing SWNT functionalization is urgently needed.[34]

In this study, we develop a quantitative analytical method for estimating the number of functional groups on SWNT surfaces by simply measuring the Raman features of functionalized SWNTs. We used radical additions employing diazonium chemistry as a way to covalently attach functional groups to the SWNTs. Covalent functionalization using radical addition is particularly useful because the functional groups are predominantly attached to the SWNTs as a monolayer; therefore, functional groups can be directly attached to the SWNT surfaces. We also previously showed that covalently attached functional groups can alter the densities of individual single-walled carbon nanotubes (SWNTs) in a highly controllable manner.[35] Therefore, we prepared various functionalized SWNTs with different numbers of functional groups per carbon atom using diazonium reactions and measured their densities using the density-induced ultracentrifugation method.[36] The disorder mode (D band) representing surface defects increased in comparison with the tangential mode (G band) in the Raman spectra as the extent of the surface functionalization of SWNTs increased; therefore, the number of functional groups per carbon atom is correlated with the area ratio of the D band to the G band of various functionalized SWNTs.

In this paper, we present the first independent measure of the number of functional groups on SWNTs with different chemical conversions by examining their Raman spectra and densities and establish a linear relationship between the number of functional groups per carbon atom and the area ratio of the disorder mode (D peak, 1289 $cm^{-1}$) to the tangential mode (G peak, 1582 $cm^{-1}$) measured in the Raman spectra for a simple and reliable quantitative estimation of the number of functional groups attached to each carbon nanotube by making Raman spectroscopy-based measurements. We anticipate that this correlation will allow for a more rigorous analysis of the covalent functionalization of SWNTs in various applications.

## Experimental Section

**Control sample preparation.** We used CoMoCAT SWNT samples (SG65, SWeNT) as a control due to their narrow chirality distributions. More than 90% of these SWNTs have diameters ranging from 0.7 to 0.9 nm; therefore, we can minimize the density differences among the SWNTs, which leads to a more accurate estimate of the density of the functional groups on the SWNTs. The CoMoCAT SWNTs were dissolved in deionized water using 1% (w/v) sodium dodecyl sulfate. The SWNTs were dispersed in aqueous solution using a homogenizer (T18 basic, ultra-turrax) and ultra-sonicator (VCX-750, Sonics & Materials) for 1 hour. After that, the SWNTs were centrifuged (SW 32.1 Ti, Beckman Coulter) for 4 hours at 30,000 rpm and the supernatant solution, containing singly suspended SWNTs, was collected and used for further experiments.[37]

**SWNT surface functionalization.** 4-nitrobenzene diazonium tetrafluoroborate salt (Sigma-Aldrich) was used to functionalize the SWNTs. 4-nitrobenzene diazonium salt was added to 20 ml of the SWNT solution to attach 4-nitrophenyl functional groups to the SWNTs.[35,38] The reaction was performed at 50 °C for 20 hours and four different concentrations of diazonium, 0.11, 0.17, 0.23, 0.29 mM, were used to attach different numbers of functional groups to the SWNTs. Then, four of the reacted SWNT samples were dialyzed with an aqueous solution containing 2% (w/v) sodium cholate surfactant for 24 hours. The extent of the SWNT functionalization of each sample was investigated using UV-vis-NIR absorption (Lambda 750, Perkin-Elmer) and Raman spectroscopy (DM 500i, Dongwoo optron).

**Estimating the density of the functionalized SWNTs.** We performed density-induced separations of various functionalized SWNTs using an approach similar to the one reported in our previous study.[35] A density gradient was created in the centrifuge tube to determine the density of the functionalized SWNTs. From the bottom to the top of the centrifuge tube, we created the following layers: the first layer was 3 ml of the non-ionic medium iodixanol (OptiPrep, 60% (w/v) iodixanol, Sigma-Aldrich), the second layer was 1 ml of functionalized SWNTs with an iodixanol concentration of 50% (w/v), the third layer was iodixanol with a concentration varying from 29.5% to 48.5% (w/v) to create a density gradient, and the top layer was deionized water. All of the solutions in the centrifuge tube had a 2% (w/v) sodium cholate surfactant content. All of the samples were centrifuged at 22°C and 32000 rpm for 22 hours using a swinging bucket rotor (SW 32.1 Ti, Beckman Coulter). A fraction recovery system was used to fractionate the centrifuged samples with 200 µl each time.

## Results and Discussion

**Attachment of different numbers of functional groups to the SWNTs.** Four functionalized SWNT samples with different numbers of functional groups attached to their surfaces were prepared. Figure 1 shows the UV-vis-NIR absorption spectra of the four functionalized SWNT samples prepared by the injection of 0.11, 0.17, 0.23, or 0.29 mM nitrobenzene diazonium reagent solutions and of a non-functionalized SWNT sample as a reference. Compared with the peak intensities of the control sample, which represent the first van Hove transitions of metallic SWNTs ($E_{11}^{M}$ at 400-500 nm) and the second and first van Hove transitions of semiconducting SWNTs ($E_{22}^{SC}$ at 500-850 nm and $E_{11}^{SC}$ at 850-1350 nm, respectively), all of the peaks of the four functionalized SWNT samples were completely decayed. These results indicate that semiconducting SWNTs, which comprise more than 90% of the SWNTs used in this study, and a trace amount of metallic SWNTs completely react with the nitrobenzene diazonium reagent, leading to the covalent attachment of nitrophenyl functional groups to the SWNT surfaces.[13,39,40] Although we anticipate that SWNTs reacted with diazonium in higher concentrations have more functional groups on their surfaces than those reacted with lower reagent concentrations, it is not possible to observe these differences between functionalized SWNTs using UV-vis-NIR absorption spectra, as shown in Figure 1, because reactions at SWNT surfaces at concentrations above the threshold (0.11 mM, as shown for the sample in Figure 1 and Figure S1) result in the localization of valence electrons, ensuring that they are no longer free to participate in photoabsorption, which leads disappearance of the spectrum features. Above this threshold, the spectrum does not change even though the number of functional groups on the SWNTs increases, as demonstrated in the results for the 0.17, 0.23 and 0.29 mM reacted samples shown in Figure 1.

To investigate the optical differences originating from the different numbers of functional groups attached to the surfaces of the four functionalized SWNT samples, we performed Raman measurements of the four functionalized SWNT samples and a non-functionalized SWNT sample as a control. The results are shown in Figure 2. We monitored the disorder mode (D peak, 1289 cm$^{-1}$) and the tangential mode (G peak, 1582 cm$^{-1}$) for all of the samples using 632.8 nm excitation to investigate the extent of the SWNT functionalization. In the case of the control sample, the D peak, which represents surface defects originating from the covalent attachment of functional groups, was not observed, which indicates that there was no functional group on the control sample used in this study. In contrast, for all four of the functionalized SWNT samples, the disorder mode (D peak, 1289 cm$^{-1}$) in the Raman spectra increased when compared with the tangential mode (G peak, 1582 cm$^{-1}$), and it is obvious that the D/G area ratio increased as the concentration of the diazonium reagent increased from 0.11 mM to 0.29 mM in the reacted samples. These results indicate that the number of nitrophenyl groups covalently attached to the SWNT surfaces increases with the concentration of the nitrobenzene diazonium reagent. We successfully prepared functionalized SWNTs that have D/G area ratios ranging from 1.49 to 2.08.

**Estimating the number of functional groups per carbon atom.** We previously showed that the densities of both non-functionalized and functionalized SWNT-surfactant assemblies can be

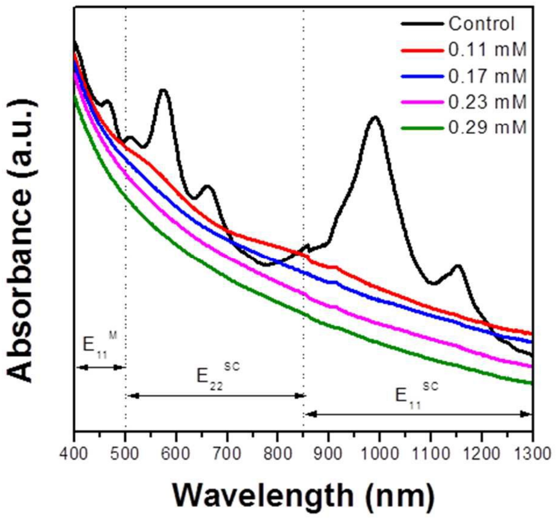

**Figure 1**. UV-vis-NIR absorption spectra of functionalized SWNT samples with different concentrations of 4-nitrobenzene diazonium tetrafluoroborate salt and a control sample as a reference.

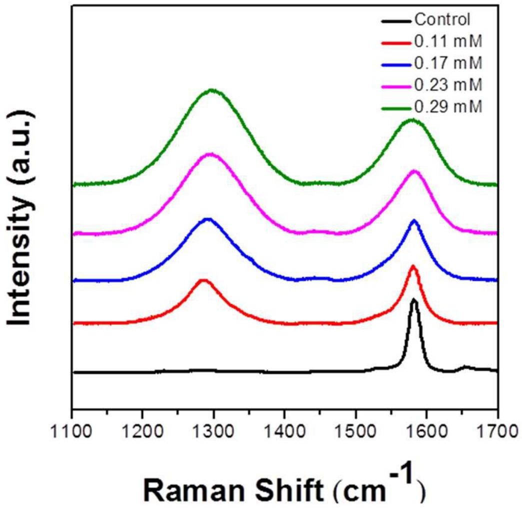

**Figure 2**. Raman excitation measurements (632.8 nm) for functionalized samples with different concentrations of diazonium reagents and a control sample as a reference.

successfully calculated using a model developed previously,[35] and that these predicted values are in good agreement with the densities of functionalized SWNTs measured experimentally using the density-induced centrifugation method.

In this study, we estimate the number of functional groups attached to an SWNT surface per one carbon atom of a carbon nanotube for all four of the functionalized SWNT samples based on their densities as measured by the density-induced centrifugation method.

The effective density of a surfactant-carbon nanotube assembly, whether non-functionalized or functionalized, can be calculated using estimates of its total mass and volume as follows. The total mass of a functionalized SWNT-surfactant assembly is the sum of the masses of the surfactant, the carbon atoms in the SWNTs and the functional groups attached to the SWNT surfaces. The total volume of a functionalized SWNT-surfactant assembly is the sum of the volumes of the hydrated surfactant, the cylindrical carbon nanotube and the functional groups attached to SWNT surfaces.

Because the average diameter of the CoMoCAT SWNTs used in this study is approximately 0.757 nm, we estimated the density of the non-functionalized and functionalized SWNTs based on (6,5) SWNT, which has a diameter of 0.757nm. The mass of a non-functionalized SWNT ($M_{(n,m)}$) can be calculated using the number of carbon atoms per nanometer ($n_c$)[41,42] and the number of surfactant molecules per nanometer ($n_s$),[13,43] as shown in the following equation for the total mass per unit length ($M_{(n,m)}$).[35]

$$M_{(n,m)} = n_s M_s + n_c M_c \tag{1}$$

$M_s$ is the molecular weight of a surfactant molecule, $M_c$ is the atomic weight of carbon and $M_f$ is the molecular weight of a nitrophenyl group. The general formula for the volume of the assembly per unit length is

$$V_{(n,m)} = n_s V_s + \frac{1}{4}\pi {d_{eff}}^2 \tag{2}$$

$$d_{eff} = d_{(n,m)} + 2r_c \tag{3}$$

where the effective diameter of a (6,5) SWNT-surfactant assembly ($d_{eff}$) is calculated using $d_{(n,m)}$, the diameter of a (6,5) SWNT molecule (0.757 nm), and $r_c$, the van der Waals radius of a carbon atom in an aromatic molecule (~1.72 Å).[35,44] The anhydrous molecular volume of one of the sodium cholate surfactant molecules used in this study ($V_s$) can be found in the literatures.[45–47] However, anhydrous surfactant molecules impart little buoyancy to carbon nanotubes. Therefore, we accounted for one hydration shell when calculating the apparent molecular volume of sodium cholate (1018 Å$^3$/molecule).[35] Accordingly, the buoyant density of a generic nanotube is given by

$$\rho_{(m,n)} = \frac{M_{(\mathrm{n,m})}}{V_{(\mathrm{n,m})}} = \frac{n_{\mathrm{S}} M_{\mathrm{S}} + n_{\mathrm{C}} M_{\mathrm{C}}}{n_{\mathrm{S}} V_{\mathrm{S}} + \frac{1}{4}\pi {d_{eff}}^2} \tag{4}$$

All the parameters, necessary to calculate the density of non-functionalized as well as functionalized SWNTs, can be found in the literatures, except the number of surfactant molecules per nanometer ($n_s$) of SWNTs. Recent studies report the relationship between the number of sodium cholate surfactant molecules and the diameter of SWNT per unit length, based on the buoyancy-induced density measurements,[36,49] however, these numbers vary among the literatures because the buoyancy of SWNT-surfactant assembly is affected by various parameters, e.g., diameter distributions of SWNTs, surfactant concentrations, pretreatment conditions specified to each experiment, etc. Therefore, we re-estimated the number of surfactant molecules per unit length of carbon nanotube ($n_s$) based on the measured density of (6,5) SWNTs, which is 1157.4 kg/m$^3$ (as shown in Figure 3 and Table 1) under the experimental conditions of this study using eq (5), re-arranged from eq (4), and the result is 1.4707.

$$n_{\mathrm{s}} = \frac{n_{\mathrm{C}} M_{\mathrm{C}} - \frac{1}{4}\pi {d_{eff}}^2 \rho_{(m,n)}}{\rho_{(m,n)} V_{\mathrm{S}} - M_{\mathrm{S}}} \tag{5}$$

The densities of SWNTs functionalized with 4-nitrophenyl groups were then calculated using the estimated value of $n_s$ for un-functionalized (6,5) SWNTs. The molecular mass and apparent molecular volume (115.6 Å$^3$/molecule)[35,48] of the 4-nitrophenyl group was included in the SWNT-surfactant system for calculating the densities of the functionalized SWNTs. From eqs (1), (2) and (4) we determined the values of $M_{(n,m)}$, $V_{(n,m)}$, and $\rho_{(n,m)}$, respectively. The formula relating to the number diazonium to the density of the functionalized SWNTs is

$$\rho_{(m,n),functionalized} = \frac{M_{(n,m)}}{V_{(n,m)}} = \frac{M_{(n,m)} + n_f M_f}{V_{(n,m)} + n_f V_f} \tag{6}$$

Where $n_f$ is the number of 4-nitrophenyl groups per carbon atom (which needs to be calculated) and $M_f$ is the molecular weight of the 4-nitrophenyl group. Therefore, the number of 4-nitrophenyl groups per carbon atom can be evaluated using the following expression

$$n_f = \frac{M_{(m,n)} - \rho_{(m,n),functionalized} \cdot V_{(m,n)}}{\rho_{(m,n),functionalized} \cdot V_f - M_f}$$

$$= \frac{(n_S M_S + n_c M_c) - \rho_{(m,n),functionalized} \cdot (n_S V_S + \frac{1}{4}\pi {d_{eff}}^2)}{\rho_{(m,n),functionalized} \cdot V_f - M_f} \tag{7}$$

**Measuring the SWNT Density.** The densities of the non-functionalized (control) and four functionalized SWNT samples were measured using density-induced centrifugation. The functionalized SWNT samples prepared as described in the experimental section and the control sample were centrifuged for 22 hours in the density gradient solution.

Figure 3 shows photographs of the centrifuge tubes of these five samples after centrifugation. The SWNTs moved from the top to the bottom of the centrifuge tubes as the extent of their functionalization increased, indicating that the density of SWNTs increases as the number of functional groups per carbon atom increases. We measured the density of each SWNT sample by

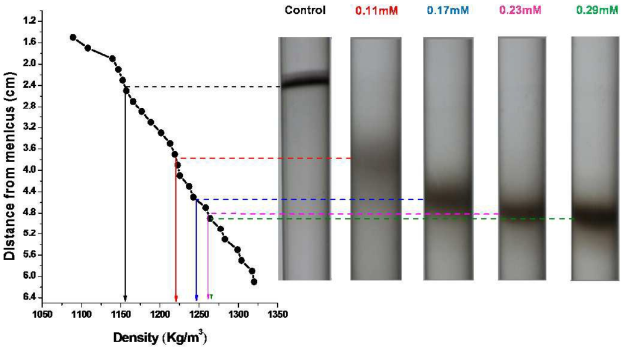

**Figure 3.** Density measurements of functionalized SWNTs and a control sample: the density profile after 22 hours of centrifugation is shown. The inset shows a photograph of the centrifuge tubes for the functionalized samples and the control sample after centrifugation.

matching the distance of each fraction from the meniscus with the final density of the solution (Figure S2). The densities of the control, 0.11 mM, 0.17 mM, 0.23 mM and 0.29 mM reacted samples were 1157, 1220, 1247, 1261 and 1266 kg/m$^3$, respectively. Using these density values and eq (7), we estimated the amount of diazonium per carbon atom for each functionalized SWNT sample.

In this calculation, one important aspect must be considered. That is, SWNT surface functionalization would affect or disturb the buoyancy-inducing organization of surfactants around each SWNT. Therefore, the number of surfactant molecule per unit length of SWNTs would be affected by the functional groups attached to SWNT surfaces, which lead to the changes in the number of surfactant per unit length of SWNTs for functionalized SWNTs.

**Table 1.** The number of functional groups per carbon atom for SWNTs, estimated using their measured densities and those calculated by the injected amount of reagents.

| Sample | Batch | Measured Density (Kg/m$^3$) | The estimated number of functional groups per carbon atom | The injected number of functional groups per carbon atom | D to G ratio |
|---|---|---|---|---|---|
| **Control** | 1st | 1157 | 0 | 0 | 0 |
| | | 1153 | 0 | | |
| | | 1155 | 0 | | |
| | | Aerage | 0 | | |
| | 2nd | 1151 | 0 | 0 | 0 |
| **0.11 mM** | 1st | 1220 | 0.0249 | 0.0280 | 1.386 |
| | | 1228 | 0.0279 | | |
| | | 1224 | 0.0262 | | |
| | | Average | 0.0263 | | |
| | 2nd | 1219 | 0.0241 | 0.0280 | 1.334 |
| **0.17 mM** | 1st | 1247 | 0.0355 | 0.0467 | 1.825 |
| | | 1255 | 0.0401 | | |
| | | 1251 | 0.0383 | | |
| | | Average | 0.0380 | | |
| | 2nd | 1237 | 0.0320 | 0.0467 | 1.569 |
| **0.23 mM** | 1st | 1261 | 0.0415 | 0.0654 | 2.029 |
| | | 1267 | 0.0459 | | |
| | | 1264 | 0.0444 | | |
| | | Average | 0.0439 | | |
| | 2nd | 1248 | 0.0368 | 0.0654 | 1.853 |
| **0.29 mM** | 1st | 1266 | 0.0445 | 0.0841 | 2.078 |
| | | 1271 | 0.0479 | | |
| | | 1269 | 0.0469 | | |
| | | Average | 0.0464 | | |
| | 2nd | 1258 | 0.0413 | 0.0841 | 2.006 |

We previously reported the linear relation between the SWNT diameter and the number of surfactants per unit length of SWNTs, which can be converted to the linear relationship between the number of carbon and surfactant per unit length of SWNTs (e.g., for (6,5) SWNT, 89.52 carbon atoms exist in 1 nm length).[49] When SWNTs are functionalized, the surfaces would be covered by functional groups, and the number of carbon atom, which are available for surfactant adsorption would decrease, leading to the decrease in the number of surfactant per unit length of carbon, as expressed in eq (8)

$$\boldsymbol{n_s = 0.016 \cdot n_c + 0.075} \tag{8}$$

Where, $n_c$ is the number of carbon per unit length of SWNT and $n_s$ is the number of surfactant per unit length of SWNT. We first estimate the $n_f$ using eq (7) by first inserting the initial $n_c$ of (6,5) SWNT, $n_s$ of non-functionalized SWNT, i.e., 1.4707, then get new $n_c$ value by subtracting initial $n_c$ by estimated $n_f$. The re-estimated $n_s$ using eq (8) is now smaller than initial $n_s$ due to the decreased $n_c$ value compared to initial $n_c$. We repeat this process and find out that $n_s$ is being converged to specified numbers, i.e., 1.4707 (0 mM), 1.4305 (0.11 mM), 1.4124 (0.17 mM), 1.4039 (0.23 mM), and 1.4011 (0.29 mM). From this results, we confirmed that the actual number of surfactant per nm of SWNT decreases upon SWNT functionalization, because the carbon atom, which was originally available for surfactant adsorption is covered by the functional group attached to carbon atom, leading to the decrease in the number of surfactant. We used this numbers to calculate the number of functional groups on SWNT per unit length (or per unit number of carbon).

The average estimated amount of diazonium per carbon atom in the functionalized SWNT samples is 0.0263 (0.11 mM), 0.0320 (0.17 mM), 0.0439 (0.23 mM), and 0.0464 (0.29 mM), as listed in Table 1. There are differences in the numbers of functional groups per carbon atom between the values estimated from the measured densities (filled black square in Figure 4(a)) and those estimated from the amount of diazonium injected during the experiments (hollow black square in Figure 4(a)); the former are always less than the latter, and these differences increase as the diazonium concentration increases, especially when more than approximately 0.0263 functional groups per carbon atom (i.e., 2.354 functional groups per nm SWNT for (6,5) SWNT) are attached to SWNT surfaces, as shown in Figure 4(a). These differences may originate because a small portion of the diazonium reagents can react by themselves as well as with the carbon nanotubes. Therefore, when the diazonium concentration is high, the probability, that the diazonium reacts with each other to form by-products rather than with the carbon nanotubes to form functionalized SWNTs, increases, leading to the large difference between the actual number of functional groups attached to the SWNT surfaces and the number injected during the reaction. This result strongly suggests that the number of functional groups on the SWNT surfaces must be estimated using a direct counting method, as performed in this study, rather than simply calculated from the diazonium concentration injected during the experiment.

**A linear relationship between the number of functional groups on the SWNTs and the D to G area ratios of Raman spectroscopy.** We measured the ratio of the areas of the D and G peaks of the functionalized SWNTs using Raman spectroscopy and found values of 1.386 (0.11 mM), 1.825 (0.17 mM), 2.029 (0.23 mM) and 2.078 (0.29 mM) for the four functionalized SWNT samples (the details are in Table 1 and supplementary information). We then correlated the D/G area ratios with the number of functional groups per carbon atom in the four functionalized SWNT samples, as measured by the estimates of their densities described in the previous section, which were 0.0263 (0.11 mM), 0.0380 (0.17 mM), 0.0439 (0.23 mM), and 0.0464 (0.29 mM), respectively. We were able to successfully establish a correlation between the D to G area ratios and the number of functional groups per carbon atom. The relationship between the D to G ratio and the amount of diazonium is shown in Figure 4(a). It is a linear relationship with a high reliability ($R^2$ = 0.996) and can be expressed as shown in eq (9).

$$n_f = 0.02 \cdot A_{D/G} \qquad (9)$$

We performed the same experiment using another batch of CoMoCAT SWNTs ($2^{nd}$ Batch of Figure 4(b)) and show the results in Figure 4(b) and Table 1. Although the same concentrations of diazonium reagents used in the $1^{st}$ Batch (Figure 4(a)) were injected, i.e., 0.11 mM, 0.17 mM, 0.23 mM and 0.29 mM, the extents of reactions were different from those of the $1^{st}$ Batch of CoMoCAT SWNTs (Figure 4(a)) as indicated by the different D to G area ratio of Raman spectrum for four samples, compared to those of $1^{st}$ Batch (the detail information is also represented in Figure S3 of the supplementary information). This result indicates that the control of extent of SWNT functionalization by adjusting reagent concentrations is difficult as mentioned in the earlier section of this manuscript. However, the number of attached functional groups per carbon atom for reacted SWNTs estimated using the iterations of eq (7) and (8) are in good agreement with eq (9) as shown in Figure 4(b), which indicates estimated number of attached functional groups on SWNT surface using analytical method developed in this study based on the measured density of functionalized SWNTs linearly depends on their D to G area ratio of Raman spectrum, while the number of functional groups on SWNT surfaces calculated by the injected concentrations of reagent does not. In addition, the above results suggest that our quantitative estimation model developed in this study is valid irrespective of batch types of SWNTs.

To confirm that the linear relationship between the D to G ratio and the number of attached functional groups per carbon atom of functionalized SWNTs developed in this study is also valid for other types of carbon nanotubes, we performed the same series of experiment using arc discharged SWNTs, which have larger diameters (1.3~1.5 nm) than CoMoCAT SWNTs (0.757 nm) used in this study, and show the result in Figure S5 and S6 in the supplementary information. The calculated number of functional groups per carbon atom for reacted arc discharged SWNTs using eq (9) is 0.0313, and the value estimated by experiment is 0.0306 are in good agreement, as also shown in Figure S6. This result confirms that our simple quantitative estimation model can be applied to various types of functionalized SWNT, irrespective of diameter distributions of SWNTs.

This method provides the independent measurement of *(n,m)* SWNTs with functional groups that have different chemical conversions. We can use the D to G area ratio ($A_{D/G}$) to evaluate the number of functional groups accurately using simple and fast Raman measurements.

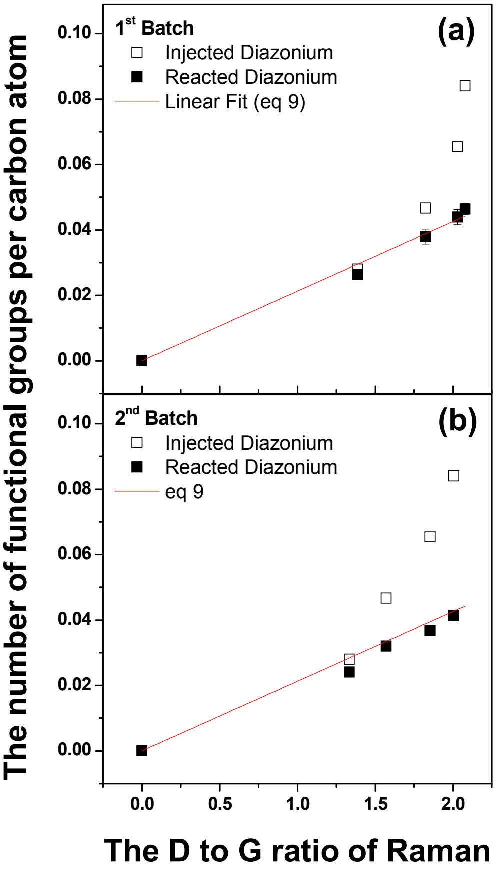

**Figure 4.** The relationship between the D to G area ratio and the number of 4-nitrophenyl functional groups per carbon atom for $1^{st}$ Batch (a) and $2^{nd}$ Batch (b) of functionalized SWNT samples.

## Conclusions

We present a simple and reliable estimation scheme that can quantitatively count the number of functional groups per carbon atom in functionalized SWNTs by independently measuring the densities of various functionalized SWNT samples having different chemical conversions and their D to G area ratios using Raman spectroscopy. We quantitatively show that injected reagents do not completely attach to SWNT surfaces, especially when more than approximately 0.0263 functional groups per carbon atom are attached to SWNT surfaces (i.e., 2.354 functional groups per nm (6,5) SWNT), therefore above this value, the number of functional groups per carbon atom calculated from the injected concentrations of reagents cannot be linearly related to the area ratio of D to G peaks of Raman spectrum, which has been extensively used to measure the extent of SWNT functionalization. However, the number of functional groups per carbon atom estimated using the analytical method developed in this study linearly depends on the area ratio of D to G peaks of Raman spectrum and this estimation scheme is valid irrespective of SWNT batches and SWNT diameters, i.e., SWNT production schemes, therefore, the actual number of functional groups attached to SWNT surfaces can be accurately estimated using simple and fast Raman measurements. This would lay the foundation for rigorous analysis and applications such as SWNT chemistry, composite fabrication, biosensors, drugs and gene deliveries.

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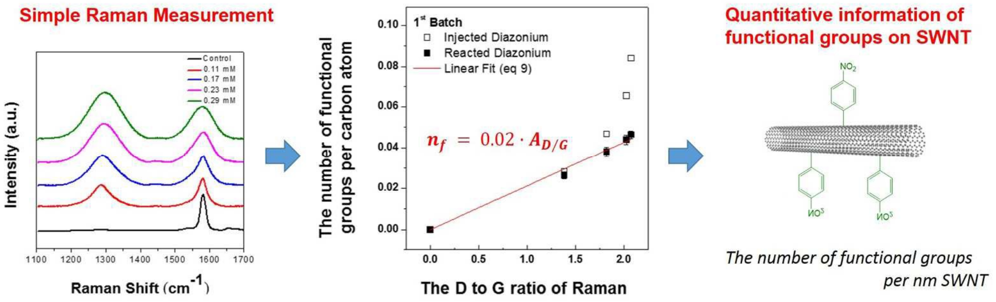

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