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## Enriched surface acidity for surfactant-free suspensions of carboxylated carbon nanotubes purified by centrifugation

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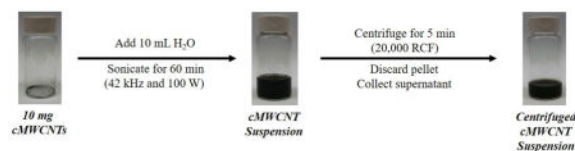
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### Abstract

It is well known that surfactant-suspended carbon nanotube (CNT) samples can be purified by centrifugation to decrease agglomerates and increase individually-dispersed CNTs. However, centrifugation is not always part of protocols to prepare CNT samples used in biomedical applications. Herein, using carboxylated multi-walled CNTs (cMWCNTs) suspended in water without a surfactant, we developed a Boehm titrimetric method for the analysis of centrifuged cMWCNT suspensions and used it to show that the surface acidity of oxidized carbon materials in aqueous cMWCNT suspensions was enriched by ~40% by a single low-speed centrifugation step. This significant difference in surface acidity between un-centrifuged and centrifuged cMWCNT suspensions has not been previously appreciated and is important because the degree of surface acidity is known to affect the interactions of cMWCNTs with biological systems.

### Graphical Abstract



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#### Conflict of interest

The authors have no conflicts of interest to declare.

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## Keywords

Carbon nanomaterial sample preparation; Oxidized multi-walled carbon nanotube; Boehm titration; Nanotoxicology; Nanomedicine

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## 1. Introduction

Carbon nanotubes (CNTs) have unique physiochemical properties that make them useful for the potential diagnosis and treatment of a number of diseases, especially cancer.[1–6] An important consideration for these and other biomedical applications of CNTs is how CNT samples are prepared.[7] In brief, almost all commercial CNT products are supplied as powdered soot that contains some degree of metallic and carbonaceous impurities in addition to CNTs. To prepare samples of pristine, non-functionalized CNTs for biomedical applications, the first step typically involves sonicating a known mass of CNT soot in an aqueous solution of a surfactant (e.g., a biocompatible polymer, protein, or serum) to yield a CNT suspension,[8] and a second optional step involves centrifugation of the CNT suspension to remove heavier metal-containing CNTs, bundles, and other agglomerated materials.[9] To prepare oxidized or carboxylated CNT (cCNT) samples for biomedical applications, two methods are commonplace that do not involve the use of surfactants. One method reported by several groups is to sonicate cCNT soot in deionized water before adding the resulting aqueous cCNT suspension to cell culture medium,[10–14] while others have reported an additional centrifugation step to purify the aqueous cCNT suspension (herewith called a centrifuged cCNT suspension) before adding this material to cell culture medium or blood.[15–18] Another important consideration for biomedical applications of CNTs is therefore a thorough physiochemical characterization of the exact CNT sample that is presented to living cells or intact organisms.[19–28] At a minimum, this involves some measure of CNT structures, amounts, dimensions, porosities, impurities, and in the case of cCNTs, a measure of surface acidity, defined as the acidic groups covalently attached to cCNT surfaces and potential acidic carbonaceous substances adsorbed to cCNT surfaces (i.e., oxidative debris).[13,29–36]

The acidic surface properties of cCNTs primarily stem from the presence of carboxyl, lactonic, hydroxyl, and phenolic groups, which are generated through reaction of CNTs with acidic liquid oxidants or high-temperature oxygen.[37–39] Acidic groups on cCNT surfaces have been assessed qualitatively using thermogravimetric analysis (TGA),[40–43] semi-quantitatively using Fourier transform-infrared (FT-IR) spectroscopy,[24,44–46] and quantitatively using either fluorescence spectroscopy and dye-derivatized cCNTs,[47–49] x-ray photoelectron spectroscopy (XPS),[24,43–45,50,51] or acid-base titrimetry.[52–55] While these and other approaches have their advantages and limitations, a survey of the literature reveals that they have been used overwhelmingly to assess the surface acidity of un-centrifuged cCNT suspensions, and rarely to assess the surface acidity of cCNT suspensions purified by centrifugation. This is notable because centrifuging an aqueous cCNT suspension without a surfactant should facilitate the removal of hydrophobic, non-oxidized soot components from the supernatant, as opposed to when a surfactant is used where both oxidized and non-oxidized components would be coated by surfactant and

suspended in the supernatant. In other words, the process of centrifuging an aqueous suspension of cCNT soot could selectively enrich the centrifuged sample with oxidized carbon material.

Herein, a Boehm titrimetric method for the analysis of surfactant-free suspensions of cCNTs was developed and was used to show that the surface acidities of aqueous suspensions of multi-walled cCNTs (cMWCNTs) and centrifuged cMWCNT suspensions are not equivalent. Specifically, the surface acidities of aqueous cMWCNT suspensions and centrifuged cMWCNT suspensions were  $7.46 \pm 0.41$  and  $19.09 \pm 0.52$  mmols/g, respectively – a result of an increase in suspended oxidized carbon material and a decrease in agglomerated materials in the centrifuged cMWCNT suspensions. This significant difference in surface acidity between un-centrifuged and centrifuged cMWCNT suspensions has not been previously appreciated and is important because the degree of surface acidity is known to affect the biodegradation rates of cCNT samples,[56,57] as well as, the *in vitro* and *in vivo* toxicity profiles of cCNT samples.[58–61]

## 2. Experimental

### 2.1. Materials and solutions

Carboxylated MWCNT (cMWCNT) soot (product SC-M10; lot 1256YJF-070510) was purchased from Nanostructured & Amorphous Materials, Inc. (Houston, TX, USA) and its properties are described in Table 1. Caution, a fine-particulates respirator and other personal protective equipment (PPE) should be worn when handling cCNT soot.[62] Sodium hydroxide and standard buffer solutions of pH  $4.00 \pm 0.01$ ,  $7.00 \pm 0.01$ , and  $10.00 \pm 0.02$  were purchased from Fisher Scientific (Houston, TX, USA). HCl concentrate (0.01 mol), potassium hydrogen phthalate (KHP), and phenolphthalein were purchased from Sigma-Aldrich (St. Louis, MO, USA).

### 2.2. Preparation and standardization of sodium hydroxide

Approximately 200 mg of NaOH was weighed and transferred to a 500.0-mL volumetric flask, dissolved, brought to volume, and stored in a 500-mL polyethylene bottle. All ~0.01 M NaOH solutions were standardized against KHP using phenolphthalein as the indicator. In brief, ~50 mg of KHP was weighed, transferred to a 125-mL Erlenmeyer flask, and dissolved in 25.0 mL of 18.2 M $\Omega$ -cm deionized water with five drops of phenolphthalein. Using a 50.00-mL buret, NaOH solutions were titrated to endpoint with KHP. This process was repeated four times to determine the mean concentration and standard deviation (SD) of the standardized NaOH. NaOH solutions were used within seven days of being standardized.

### 2.3. Preparation of cMWCNT suspensions and centrifuged cMWCNT suspensions

The preparation of cMWCNT suspensions and centrifuged cMWCNT suspensions started with the addition of 10.0 mL of 18.2 M $\Omega$ -cm deionized water to 10.0 mg of as-received cMWCNT soot that was weighed into a pre-cleaned, 20-mL scintillation vial (Figure 1). The mixture was sonicated for 60 min using a Branson model 2510 bath sonifier (100 W, 42 kHz) with the bath water being changed every 30 min to maintain the temperature <15 °C; bath sonication was chosen so as to avoid nanotube perturbations induced by intense probe

sonication. The purification of aqueous cMWCNT suspensions involved a single low-speed centrifugation step. To accommodate the use of a benchtop centrifuge (Eppendorf, model 5424), 10.0-mL suspensions were divided by transferring 1-mL aliquots into ten 1.5-mL centrifuge tubes, which were each centrifuged for 5 min at 20,000 RCF. The top ~900  $\mu\text{L}$  from each supernatant was collected carefully using a micropipette so as to not disturb the pellet and combined in a pre-cleaned scintillation vial to afford a ~9-mL sample of a centrifuged cMWCNT suspension.

#### 2.4. Raman spectroscopy

Raman spectra were acquired using a Jobin Yvon Horiba HR800 high-resolution LabRam Raman microscope system equipped with a 250- $\mu\text{m}$  entrance slit and an 1100- $\mu\text{m}$  pinhole. The 633-nm laser excitation was provided by a Spectra-Physics model 127 helium-neon laser operating at 20 mW. A 50 $\times$ /0.5 NA LM-Plan objective was used with a neutral density filter of 1.0. Spectral acquisition was performed with a 1.0-s integration time, a minimum overlap of 50, and a 3-subpixel average; each spectrum was presented as an average of 2 scans. Wavenumber calibration was performed using the 520.5  $\text{cm}^{-1}$  line of a crystalline Si wafer. A 15- $\mu\text{L}$  aliquot of either a cMWCNT suspension or centrifuged cMWCNT suspension was deposited on to a crystalline Si wafer and dried at room temperature; spectra were acquired from at least five different regions of dried material across the wafer.

#### 2.5. UV-VIS-NIR spectrophotometry

All UV-VIS-NIR spectrophotometric analyses were performed using a Shimadzu UV-161PC spectrophotometer. All spectra were obtained as a single scan that was background corrected against a deionized water reference using a medium scan speed with 0.5-nm intervals and 1.0-cm quartz cuvettes. Spectra of cMWCNT suspensions and centrifuged cMWCNT suspensions were acquired after dilution with deionized water.

#### 2.6. Dynamic light scattering (DLS)

The particle size distribution of cMWCNT suspensions and centrifuged cMWCNT suspensions diluted 1:10 with deionized water were analyzed by DLS using a 633-nm laser and a backscatter measurement angle of 173 $^\circ$  (Zetasizer Nano-ZS 3600, Malvern Instruments, Worcestershire, UK). Ten consecutive 30-s runs were taken per measurement at 25  $^\circ\text{C}$  and the instrument was calibrated with polybead standards (Polysciences, Warrington, PA, USA). The particle size, in terms of hydrodynamic diameter, was calculated using a viscosity and refractive index of 0.8872 cP and 1.330, respectively for deionized water, and an absorption and refractive index of 0.010 and 1.891, respectively, for cMWCNTs.

#### 2.7. Acidimetric Boehm titration of cMWCNT suspensions

Boehm titrations work on the principle that carbon surface oxygen groups have different acidities that can be neutralized by bases of different strengths; for example, weak bases such as sodium bicarbonate can be used to neutralize carboxylic acids and strong bases such as sodium hydroxide can be used to neutralize all Brønsted acids.[37–39] While the Boehm method has been applied to the analysis of oxidized carbon materials for over fifty years, [37–39,63–69] a standard protocol has rarely been followed for the analyses of cCNTs.

[24,29,32,35,36,43,49,50,52,54,55,70–75] For example, there are different routines to determine endpoints, differences in base treatment times and agitation methods, differences in methods to address dissolved carbon dioxide, and differences in determining the mass of carbon material titrated. Herein, the first major parameter considered was the agitation method used to expose carbon materials to the chosen base – a process that can damage a carbon lattice and create new oxygen surface groups. The method of Oickle and co-workers was employed since they showed that shaking was a less destructive method of agitation relative to rigorous stirring and sonication.[72] Specifically, 4 mL of a 1.0-mg/mL cMWCNT suspension and 25.00 mL of standardized NaOH were combined in a 50-mL plastic vial and then shaken for 24 h to afford a NaOH-treated cMWCNT suspension. The second major factor to consider in a Boehm titration is how to minimize CO<sub>2</sub> effects, which can distort neutralization points associated with the acidic groups present on a carbon material.[69,71,74] The method of Kim and co-workers was employed whereby re-acidification of the deprotonated sample was followed by a back-titration with NaOH.[69] Specifically, 10.00 mL of the NaOH-treated cMWCNT suspension and 20.00 mL of ~0.01 M HCl standard were combined in a 150-mL beaker that was covered using parafilm. Finally, to determine the surface acidity (i.e., the mols of acidic groups per mass of carbon material analyzed), a magnetic stir bar and a calibrated Accumet model 13–620–299A pH electrode were placed in the reaction mixture, which was titrated at room temperature (22 ± 2°C) by recording the pH after the delivery of each 0.10-mL aliquot of standardized NaOH from a 50.00-mL buret.

## 2.8. Acidimetric Boehm titration of centrifuged cMWCNT suspensions

The same titration protocol described above for cMWCNT suspensions was performed on centrifuged cMWCNT suspensions except for the method to determine the amount of carbon material titrated. This is because the mass of soot determined using an analytical balance during the preparation of a cMWCNT suspension can no longer be applied to a centrifuged cMWCNT suspension since a large portion of the original mass (i.e., the centrifugation pellet) is discarded. Instead, the amount of carbon material titrated in centrifuged cMWCNT suspensions was determined using UV-VIS-NIR spectroscopy and a calibration curve prepared from the  $\pi$ -plasmon absorbance of aqueous standards of non-centrifuged suspensions of cMWCNT soot containing known amounts of carbon material.[76,77] The accuracy of this method was assessed by a gravimetric analysis of dried centrifuged cMWCNT suspensions; cMWCNT concentrations determined using the spectrophotometric method were within 2% of the values determined using the gravimetric method.

## 3. Results and discussion

### 3.1. Elemental analysis of the as-received cMWCNT soot

Almost all CNT manufacturing processes use a carbon feedstock, metal catalysts, and heat to yield a heterogeneous CNT soot that contains metallic and carbonaceous impurities in addition to CNTs. The cMWCNT soot chosen for this work represents one of the most common commercial methods of CNT synthesis, a metal-catalyzed chemical vapor deposition (CVD) process, and a common combination of liquid oxidants, sulfuric acid and potassium permanganate, for carboxylation of pristine CNT surfaces. As shown in Table 1,

this soot is reported to have a carbon content of >95% by weight, a metal content of <3.7%, and a chloride content of <1.0%. To assess the composition of the as-received material, sub-samples of the cMWCNT soot were subjected to our carbon nanomaterial elemental analysis protocol.[78] As shown in Table 2, the major findings were that the 94.37% carbon content was within 1% of the value reported by the manufacturer, the oxygen content was 4.12%, the hydrogen content was <1%, sulfur and nitrogen were not detected, and the sum of the C, H, and O weight percentages was 98.91%, indicating that the Fe/Co/Ni-catalyzed CVD cMWCNT soot was essentially halide- and metal-free.

### 3.2. Spectroscopic analysis of cMWCNT suspensions and centrifuged cMWCNT suspensions

Aqueous cMWCNT suspensions and centrifuged cMWCNT suspensions (Figure 1) were characterized by Raman and UV-VIS-NIR spectroscopies. Representative 633-nm Raman spectra of a cMWCNT suspension and a centrifuged cMWCNT suspension are shown in Figure 2. Prominent Raman peaks characteristic of MWCNTs such as the disorder-induced mode (D-band) at  $\sim 1300\text{ cm}^{-1}$ , the tangential stretching mode (G-band) at  $\sim 1590\text{ cm}^{-1}$ , and the second-order  $G'$ -band at  $\sim 2600\text{ cm}^{-1}$  were observed in both spectra. There were no significant differences between the D/G-ratios ( $\sim 1.4$ ), the full width at half maximum (FWHM) peak widths of the D-bands ( $\sim 60\text{ cm}^{-1}$ ), and the FWHM peak widths of the G-bands ( $\sim 74\text{ cm}^{-1}$ ), indicating that the spectral properties of nanotubes in cMWCNT suspensions and centrifuged cMWCNT suspensions were similar.

Representative UV-VIS-NIR spectra of a 20- $\mu\text{g/mL}$  cMWCNT suspension and a 20- $\mu\text{g/mL}$  centrifuged cMWCNT suspension are shown in Figure 3. Two main absorption features are observed in each spectrum. The first is the prominent  $\pi$ -plasmon absorption peak at  $\sim 240\text{ nm}$  resulting from the collective excitation of the  $\pi$ -electron systems from all  $\text{sp}^2$ -hybridized carbonaceous material in the soot.[79] The absorbance of this UV peak can be used to qualitatively evaluate the degree of CNT exfoliation in aqueous CNT samples,[80,81] where an increase in the amount of individually-dispersed MWCNTs results in an increase in the peak area.[81] The second main spectral feature is a non-resonant background comprising contributions from both cMWCNTs and other materials.[82,83] Comparison of the spectra in Figure 3 reveals (i) that the area of the  $\pi$ -plasmon absorption peak for the cMWCNT suspension was  $\sim 54\%$  less than that of the centrifuged cMWCNT suspension, indicative of increased agglomeration of cMWCNTs and carbonaceous material in a non-centrifuged suspension, and (ii) that the background absorbance of the cMWCNT suspension at 800 nm was  $\sim 40\%$  greater than that of the centrifuged cMWCNT suspension, similar to the increased background absorbances observed for non-centrifuged CNT suspensions relative to centrifuged CNT suspensions.[84,85] These spectral observations were also corroborated by DLS measurements which revealed a decrease in particle size following centrifugation. As shown in Figure 4, cMWCNT suspensions displayed two distinct populations of particles, one with a hydrodynamic diameter of  $\sim 277\text{ nm}$  and the other with a hydrodynamic diameter of  $\sim 109\text{ nm}$ , while centrifuged cMWCNT suspensions displayed only one population of particles with a hydrodynamic diameter of  $\sim 115\text{ nm}$ , indicating that larger agglomerates were removed by low-speed centrifugation.[33]



The oxidation of CNTs can generate oxidative debris in the form of particulate matter such as oxidized carbon fragments (OCFs),[25] and/or molecular debris such as polyaromatic hydrocarbons (PAHs).[55] If present, both types of debris remain stably bound to cCNT surfaces at acidic and neutral pH but can be desorbed by strong base. To determine if oxidative debris was present in cMWCNT suspensions purified by centrifugation, centrifuged cMWCNT suspensions that were treated with NaOH were passed through a 0.1- $\mu\text{m}$  polyvinylidene fluoride (PVDF) membrane filter followed by analysis of the filtrate using Raman and UV-VIS-NIR spectroscopies. Raman D-, G-, and G'-bands were not detected (data not shown), indicating that short cMWCNTs and OCF debris were not present in the filtrate. With the UV-VIS-NIR spectra of filtrates, no absorbance at 240 nm was observed and the background absorbance between 375 – 1100 nm was negligible ( $<0.01$  a.u., data not shown), indicating that short cMWCNTs and OCF debris were not present in the filtrate. However, a single small peak at 289 nm with an absorbance of 0.13 a.u. was observed in suspensions of centrifuged cMWCNTs indicating a trace amount of a PAH.[55]

### 3.3. Titration of cMWCNT suspensions

As described in the Experimental section, cMWCNT suspensions were first exposed to a known amount of NaOH to deprotonate potential acidic groups, then a known amount of HCl was added to re-acidify the suspensions, followed by back titration with NaOH to quantify acidic surface groups. A representative titration curve and first derivative plot for a cMWCNT suspension are shown in Figure 5a. As NaOH is added, the excess HCl is neutralized to an equivalence point of pH 7. Since the amount of excess HCl is directly proportional to the amount of excess NaOH that was present in the NaOH-treated cMWCNT suspension, the determined amount of excess NaOH can be subtracted from the amount of initial NaOH added to quantitate the amount of acidic groups that were present on the cMWCNTs. Specifically, the mols of acidic groups for cMWCNT suspensions were calculated using the following equation,

$$n_{AG} = [B]V_B - ([HCl]V_{HCl} - [NaOH]V_{NaOH}) \frac{V_B}{V_a} \quad (1)$$

where  $n_{AG}$  denotes the mols of acidic groups for the cMWCNT suspension,  $[B]$  and  $V_B$  are the concentration and volume of NaOH used to treat the cMWCNT suspension,  $[HCl]$  and  $V_{HCl}$  are the concentration and volume of HCl added to re-acidify the reaction mixture,  $[NaOH]$  and  $V_{NaOH}$  are the concentration and volume of titrant needed to reach the equivalence point, and  $V_a$  is the volume of NaOH-treated cMWCNT suspension titrated.[71] As shown in Table 3, the mean mols of acidic groups calculated for cMWCNT suspensions was  $2.96 \times 10^{-5}$ .

The mass of material titrated in cMWCNT suspensions was determined using the following equation,

$$m = \frac{(D * V_t)}{10^6} \quad (2)$$

where  $m$  is the weighted mass of material analyzed in grams,  $V_t$  is the volume of cMWCNT suspension treated with NaOH, and  $D$  is the initial concentration of carbon material in the suspension. As shown in Table 3, the mass of material titrated in cMWCNT suspensions was  $3.96 \times 10^{-3}$  g. Subsequently, the mmols of acidic groups per gram of carbon material in cMWCNT suspensions was calculated using the following equation,

$$\left( \frac{mmol}{g} \right) = \frac{n_{AG}}{m} \times 10^3 \quad (3)$$

where  $n_{AG}$  is the mmols of acidic groups calculated using Equation 1, and  $m$  is the mass of carbon material analyzed calculated using Equation 2. As shown in Table 3, the mean surface acidity of cMWCNT suspensions, defined as the mmols of acidic groups per gram of material analyzed, was  $7.46 \pm 0.41$  mmol/g.

### 3.4. Titration of centrifuged cMWCNT suspensions

To our knowledge, Boehm titrimetric methods have been performed exclusively on aqueous suspensions of as-synthesized and purified cCNT soot, and not on centrifuged cCNT suspensions. One reason for this could be the additional experimentation required to determine the mass of material titrated. In brief, when a soot product is analyzed as-received, or when a soot purification step is performed before the preparation of a cCNT suspension (e.g., a Soxhlet extraction or low-temperature heat treatment[29,31]), the mass of soot can be determined easily using an analytical balance. However, when a purification step follows the preparation of a soot suspension (as is the case with centrifuged cMWCNT suspensions), the mass of carbon material is no longer known since large portions of material can be pelleted and discarded following consecutive centrifugation steps.[86] Therefore, the same titration protocol described above for cMWCNT suspensions was performed on centrifuged cMWCNT suspensions with the exception that the amount of carbon material titrated in centrifuged cMWCNT suspensions was determined from a calibration curve prepared from the  $\pi$ -plasmon absorbance of aqueous standards of non-centrifuged suspensions of cMWCNT soot (Figure 6).

A representative titration curve and first derivative plot for a centrifuged cMWCNT suspension are shown in Figure 5b, and as shown in Table 3, the mean mols of acidic groups determined for centrifuged cMWCNT suspensions using Equation 1 was  $2.79 \times 10^{-5}$ . Next, the mass of material analyzed was determined using the following equation,

$$y = 0.030x + 0.016 \quad (4)$$



where  $y$  is the  $\pi$ -plasmon absorbance of centrifuged cMWCNT suspensions from the standard curve, and  $x$  is the concentration of carbon material in a centrifuged cMWCNT suspension in  $\mu\text{g/mL}$ . Next, the resulting concentration was used in Equation 2 to calculate the mass of carbon material analyzed in centrifuged cMWCNT suspensions (e.g.,  $1.46 \times 10^{-3}$  g). Finally, the mean surface acidity of centrifuged cMWCNT suspensions was calculated using Equation 3 to be  $19.09 \pm 0.52$  mmol/g. We know of only one other work to quantify the acidic surface groups of carbon material in centrifuged cCNT suspensions; [49] in brief, using fluorescence spectroscopy and 1-aminopyrene dye-treated samples, it was determined that MWCNTs oxidized for 24 h with nitric and sulfuric acids were 15 – 22% oxidized, which is in accordance with the 23% level of oxidation calculated for our centrifuged cMWCNT suspensions.

### 3.5. Enriched surface acidity of centrifuged cMWCNT suspensions

The ~40% enrichment of surface acidity for centrifuged cMWCNT suspensions relative to non-centrifuged cMWCNT suspensions can be explained as follows. Starting with a single lot of high-purity cMWCNT soot which was bath sonicated in deionized water without surfactant, a dark suspension was afforded that was observed to be free of precipitants, and that displayed carbon material concentrations on the order of  $990 \mu\text{g/mL}$ . Samples of the cMWCNT suspensions were centrifuged at 20,000 RCF for 5 min to afford a small dark pellet of agglomerated material and a homogeneous dark-gray supernatant (i.e., a centrifuged cMWCNT suspension) that displayed carbon material concentrations on the order of  $365 \mu\text{g/mL}$ . Raman spectroscopic analysis indicated that both suspensions and centrifuged suspensions possessed cMWCNTs of similar quality. UV-VIS-NIR spectroscopic analysis indicated an increase in suspended oxidized carbon material and a decrease in agglomerated material in centrifuged cMWCNT suspensions, evidence that centrifuged cMWCNT suspensions were enriched with oxidized carbon material. Additional evidence that cMWCNT suspensions were purified by centrifugation was provided by DLS measurements which showed that a population of larger particles were removed by centrifugation. Aqueous cMWCNT suspensions and centrifuged cMWCNT suspensions were analyzed by a Boehm titrimetric method, and interestingly, both samples displayed similar equivalence point volumes (Figure 5) and mols of surface acidic groups (Table 3). The resulting ~40% difference in the measured surface acidity ( $7.46 \pm 0.41$  vs.  $19.09 \pm 0.52$  mmols/g) is therefore consistent with the ~37% difference in the masses of materials titrated and the hypothesis that centrifuging an aqueous cMWCNT suspension without a surfactant would remove hydrophobic, non-oxidized soot components.

## 4. Conclusion

The literature contains many different protocols for preparing samples of cCNTs for biomedical applications. One reason for a lack of standardized sample-preparation protocols is due to the variety of cCNT investigations. For example, if the research is concerned with a toxicity assessment of as-received cCNT soot, then the cCNT suspensions are not purified by design. Conversely, if the research requires a suspension of individually-dispersed cCNTs, an additional purification step such as centrifugation and/or filtration can be undertaken. A second reason for differing protocols is the variety in the quality of

commercial cCNT soot products with additional purification steps being required for soot products containing high levels of metallic and/or carbonaceous impurities. Herein, using Boehm titrimetry and cMWCNTs suspended in water without a surfactant, the surface acidity of oxidized carbon material in aqueous cMWCNT suspensions was shown to be enriched by ~40% by a single low-speed centrifugation step. In conclusion, an important consideration for biomedical applications of cCNTs is a thorough physiochemical characterization of the exact cCNT sample that is presented to living cells or organisms. Therefore, determining if a cCNT sample-preparation protocol enriches surface acidity is critical with respect to making accurate assessments concerning the interactions of cCNTs with biological systems.

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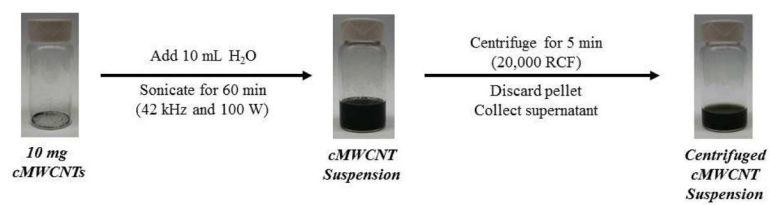
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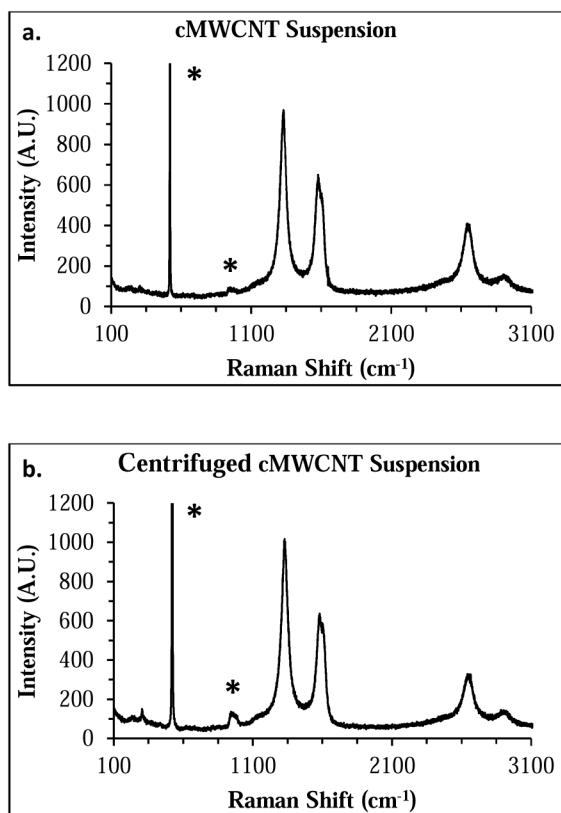


**HIGHLIGHTS**

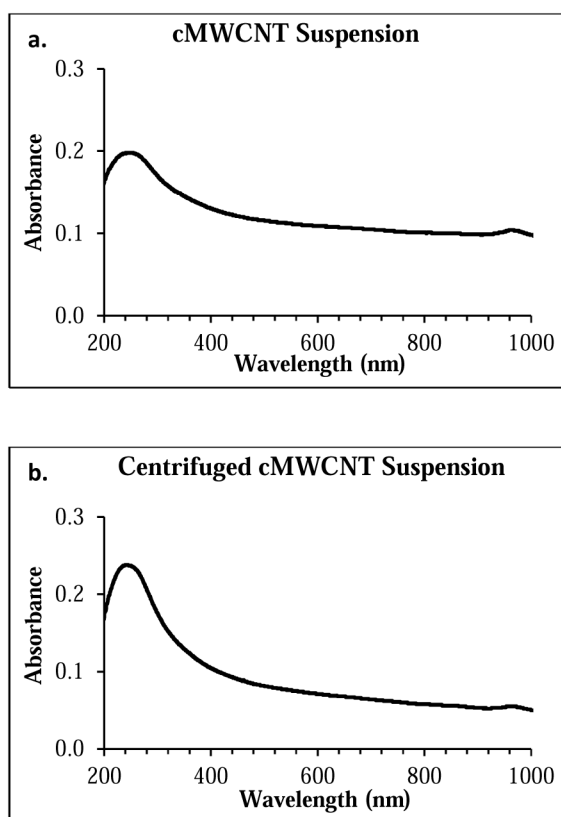
- Boehm titrimetric method for oxidized CNT suspensions purified by centrifugation
- Surface acidity affects CNT biodegradation rates and CNT toxicity profiles
- Centrifugation of surfactant-free samples of oxidized CNTs enriches surface acidity



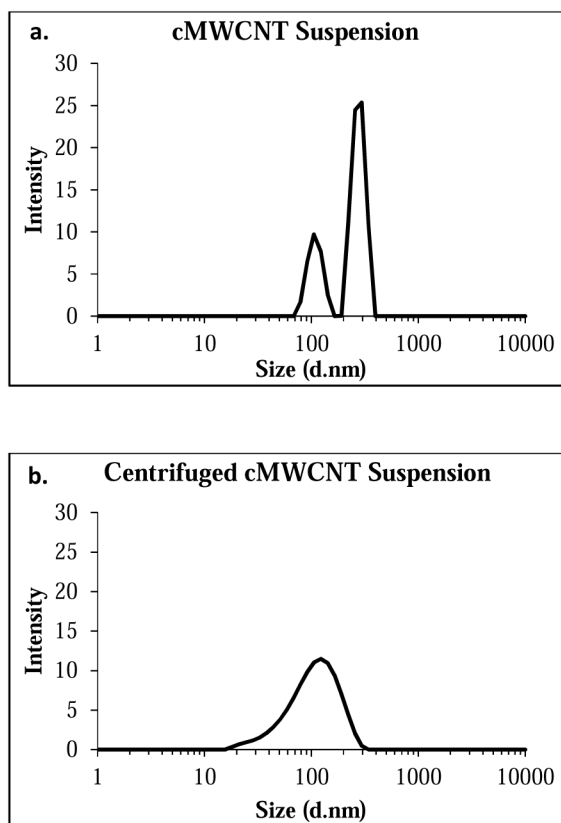
**Fig. 1.** Overview of the procedure to prepare cMWCNT suspensions and centrifuged cMWCNT suspensions.



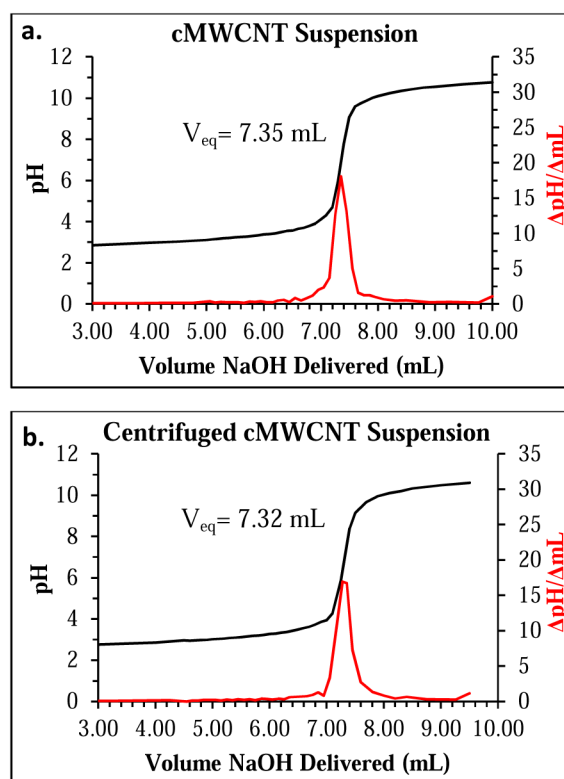
**Fig. 2.** Representative 633-nm Raman spectra of a cMWCNT suspension (a) and a centrifuged cMWCNT suspension (b). Characteristic carbon nanomaterial Raman bands (e.g., D-bands at  $\sim 1300\text{ cm}^{-1}$ , G-bands at  $\sim 1600\text{ cm}^{-1}$ , and G'-bands at  $\sim 2600\text{ cm}^{-1}$ ) were observed in both spectra. Peaks denoted by an asterisk originate from the Si-wafer substrate.



**Fig. 3.** Representative background-corrected UV-VIS-NIR absorption spectra of a 20- $\mu\text{g}/\text{mL}$  cMWCNT suspension (a) and a 20- $\mu\text{g}/\text{mL}$  centrifuged cMWCNT suspension (b).

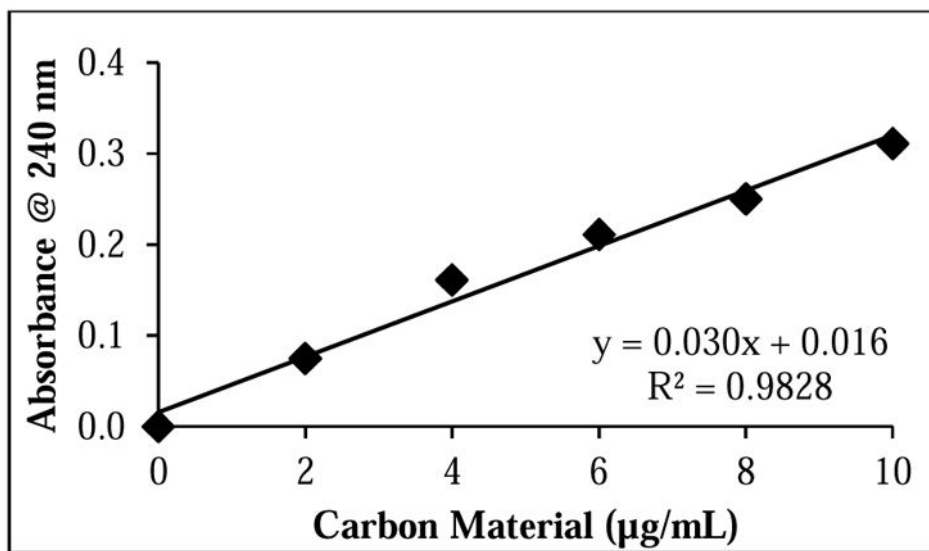


**Fig. 4.** Representative DLS particle size distribution of a diluted cMWCNT suspension (a) and a diluted centrifuged cMWCNT suspension (b).



**Fig. 5.** Representative titration (black) and first derivative (red) curves of a NaOH-treated cMWCNT aqueous suspension (a), and a NaOH-treated, centrifuged cMWCNT suspension (b). The equivalence point volumes ( $V_{eq}$ ) for each sample ( $7.39 \pm 0.05$  and  $7.33 \pm 0.02$ , respectively) were calculated using a second derivative (not shown).





**Fig. 6.** Calibration curve of carbon material in standard cMWCNT suspensions used to determine the concentration of carbon material in centrifuged cMWCNT suspensions.

**Table 1**

Properties of SC-M10 cMWCNT soot reported by the manufacturer.

<b>Synthetic Method</b>	CVD
<b>Catalytic Metals</b>	Fe, Co, Ni
<b>% Carbon Purity</b>	>95
<b>% Metals</b>	<3.7
<b>% Chloride</b>	<1.0
<b>Inner Diameter (nm)</b>	5 – 10
<b>Outer Diameter (nm)</b>	10 – 20
<b>Length (µm)</b>	0.5 – 2
<b>Oxidizing Agents</b>	H <sub>2</sub> SO <sub>4</sub> & KMnO <sub>4</sub>
<b>% COOH</b>	1.9 – 2.1

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**Table 2**

Elemental percentages of as-received SC-M10 cMWCNT soot.

	cMWCNT soot (%) <sup>a</sup>
<b>C</b>	94.37 ± 0.32
<b>H</b>	0.43 ± 0.24
<b>N</b>	0.00 ± 0.00
<b>S</b>	0.00 ± 0.00
<b>O</b>	4.12 ± 0.93

<sup>a</sup>CHNS/O analyses were performed as described previously,[78] with the exception that the cMWCNT soot was vacuum dried at 100 °C for 4 h prior to analysis. Each elemental percentage is from n = 2 independent analyses (± SD).

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**Table 3**

Acidimetric titration results for cMWCNT suspensions and centrifuged cMWCNT suspensions.

	cMWCNT Suspensions	Centrifuged cMWCNT Suspensions
Acidic groups (mol) <sup>a</sup>	$2.96 \times 10^{-5}$	$2.79 \times 10^{-5}$
Mass of carbon material analyzed (g) <sup>b</sup>	$3.96 \times 10^{-3}$	$1.46 \times 10^{-3}$
Acidic groups (mmol/g) <sup>c</sup>	$7.46 \pm 0.41$	$19.09 \pm 0.52$

<sup>a</sup>Determined by acidimetric titrations (Figure 5) and Equation 1.

<sup>b</sup>For cMWCNT suspensions, masses were determined using an analytical balance and Equation 2; for centrifuged cMWCNT suspensions, masses were determined using a UV-VIS calibration curve (Figure 6) and Equation 4.

<sup>c</sup>Determined from three independent measurements ( $\pm$  SD) using Equation 3.