Environmental Science Nano

PAPER

View Article Online
View Journal | View Issue

Cite this: *Environ. Sci.: Nano*, 2019, **6**, 380

Emerging investigator series: quantification of multiwall carbon nanotubes in plant tissues with spectroscopic analysis†

Kamol K. Das,[a] Valeria Nava,[a] Che-Wei Chang,[b] James W. Chan,[b] Baoshan Xing [c] and Yu Yang *[a]

Received 7th November 2018,
Accepted 11th December 2018

DOI: 10.1039/c8en01252k

rsc.li/es-nano

If agricultural plants are exposed to carbon nanotubes (CNTs), they can potentially take up the CNTs from growth media and translocate them to their different tissues. In addition, agricultural application of CNTs recently attracted increasing attention, as they could promote germination, enhance crop yield, and exhibit other benefits. For evaluating the environmental effects of CNTs and optimizing their agricultural application, it is essential to quantify CNTs in plant tissues. In this study, pristine (p-) and carboxyl-functionalized (c-) multiwall CNTs (MWCNTs) were extracted from plant tissues by a sequential digestion with nitric acid (HNO_3) and sulfuric acid (H_2SO_4). The extracted MWCNTs were stabilized with nonionic surfactant Triton X-100 and analyzed with ultraviolet-visible (UV-vis) spectroscopic analysis to measure the concentration of the MWCNTs in plant (lettuce) tissues. The MWCNT concentration was linearly correlated with the absorbance at 800 nm. The detection limit for p- and c-MWCNTs was achieved at 0.10–0.12, 0.070–0.081, 0.019–0.18 μg mg^{-1} for leaf, stem, and root tissues, respectively. The developed method was applied for lettuce (*Lactuca sativa*, cv. black seeded Simpson) hydroponically grown with 5, 10, 20 mg L^{-1} of p-MWCNTs and c-MWCNTs in the culture solution. We detected 0.21 ± 0.05–4.57 ± 0.39 μg mg^{-1} p-MWCNTs and 0.20 ± 0.17–0.75 ± 0.25 μg mg^{-1} c-MWCNTs in the lettuce roots, positively correlated with the dose of CNTs in solution. We have developed a method for rapid quantification of CNTs in plant tissues using a widely-accessible technique, which can enable reliable analysis of CNTs in plant tissues and provide critical information for evaluating the environmental implications and managing agricultural application of CNTs.

Environmental significance

The increasing production and application of carbon nanotubes (CNTs) for industrial and consumer products will lead to continuous accumulation of CNTs in soils, which can reach a concentration that is concerning regarding plant uptake and human exposure in the future. On the other hand, several studies demonstrated positive effects of CNTs on plant growth, with a great potential for agricultural application. Managing the environmental risks and application of CNTs requires information about their concentration in environmental media, such as agricultural plants. We have developed a method for the rapid quantification of CNTs in agricultural plants by coupling digestion with ultraviolet-visible (UV-vis) spectroscopy. The method was efficient to quantify both pristine (p-) and carboxyl-functionalized (c-) multiwall CNTs (p/c-MWCNTs) in the leaf, stem, and root tissues of lettuce. This rapid quantification method will be useful for understanding the fate and transport of carbonaceous nanomaterials in environmental media and managing their application to secure sustainable nanotechnology.

Introduction

The wide application of carbon nanotubes (CNTs) in consumer products, composite materials, and biomedical usage has led to their rapidly increasing production.[1] The global CNT market is expected to reach 8.1 billion dollars by 2024.[2] As CNTs are presumably persistent, these carbonaceous nanomaterials will be accumulated in water and soil upon the release from manufactured products during all the stages of their life cycles. Agricultural plants can potentially take up

[a] *Department of Civil and Environmental Engineering, University of Nevada-Reno, 1664 N. Virginia Street, Reno, NV 89557, USA. E-mail: yuy@unr.edu*
[b] *Center for Biophotonics, University of California-Davis Medical Center, Sacramento, California 95817, USA*
[c] *Stockbridge School of Agriculture, University of Massachusetts-Amherst, 410 Paige Laboratory, Amherst, MA 01003, USA*

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c8en01252k

and translocate the CNTs from soil to their different tissues, *e.g.* leaves, flowers, and fruits,[3–5] which raised concerns about the ecological and human health risks caused by CNTs. On the other hand, CNTs can be applied in agriculture to enhance the growth of agricultural crops and promote the delivery of pesticides/fertilizers. For instance, the application of CNTs in soils enhanced the flower and fruit production of tomatoes.[3,6,7] Information about the CNT concentration in plants is crucial for understanding the ecological and human health risks caused by CNTs and improving their agricultural application. However, the quantitative information about CNT uptake and translocation in plants is sparse mainly due to technical difficulties for quantifying CNTs in biological tissues.

For quantifying CNTs in biological tissues, previous studies have examined various methods, including programmed thermal analysis (PTA), near-infrared spectroscopy, Raman spectroscopy, inductively coupled plasma mass spectroscopy (ICP-MS), thermogravimetric analysis (TGA), microwave-induced heating methods, and application of ^{14}C-labelled CNTs.[5,7–11] Interferences from the background biological tissues and low concentrations of CNTs make their quantification challenging.[11] The removal of interfering background biological tissues requires prolonged digestion and extensive purification of samples.[8,9] Spectroscopic analysis has been used to quantify aqueous phase concentrations of multiwall CNTs (MWCNTs) using the absorbance at wavelengths of 500, 530, 550, 600, and 800 nm.[12] A linear relationship was observed between the applied CNT concentrations and UV-vis absorbance obtained at these wavelengths.[13–16] Extinction coefficients were similar for CNTs with different diameters or structures.[16] The potential application of the spectroscopic analysis for the quantification of CNTs in biological samples can be attractive, as the instruments are widely accessible and easy to operate and the analysis is rapid, although the removal of interference from background materials can be challenging. In addition, the formation of CNT aggregates regulated by their surface properties and aqueous chemical conditions can influence the quantification of CNTs in the aqueous phase, and it is crucial to suspend CNTs homogeneously.[10,17]

In this study, spectroscopic analysis was developed for the quantification of pristine (p-) and carboxyl-functionalized (c-) MWCNTs in lettuce (*Lactuca sativa*, Bionda Ricciolina) tissues. The interference of background tissues were minimized by a sequential digestion, and the detection limit of CNTs in plant tissues was determined. The rapid extraction and analysis of the MWCNTs were conducted by reducing the digestion time and using an optimized preparation process for analyzing the samples in the aqueous phase. Finally, the developed method was applied to quantify the MWCNTs in lettuce hydroponically grown with CNTs in the culture solution. We have performed the quantitative analysis of CNTs in plant tissues with programmed thermal analysis (PTA),[18] however, it requires special equipment for PTA, which is not widely accessible and could limit its application. In this work, optical analysis coupled with digestion was developed for the quantification of CNTs, which is widely accessible and can potentially enable the rapid quantification of CNTs in environmental matrices.

Materials and methods

Materials

Research-grade p- and c-MWCNTs were purchased from Nanocyl (http://www.nanocyl.com/product/). The average diameter and length of the studied MWCNTs are 9.5 nm and 1.0 μm, respectively. More information about the MWCNTs can be found in previous publications, and their major physicochemical properties are listed in Table S1 (ESI†).[19,20] Concentrated nitric acid (HNO_3) (15.8 M) and sulfuric acid (H_2SO_4) (18.4 M) were purchased from EMD Millipore (Boston, MA) and VWR (Wayne, PA). Nonionic surfactant Triton X-100 (TX-100) was purchased from VWR (Wayne, PA).

Preparation of the MWCNT suspension

For the spectroscopic analysis of the original and digested CNTs as well as the spiking of lettuce tissues, a suspension of the MWCNTs was prepared by adding 2.0 mg MWCNTs to 2.0 mL of 2.0 mg mL^{-1} TX-100 solution (made with double deionized water (DDW) (18.3 MΩ cm)) and sonicating the solution for 30 minutes (Branson Ultrasonic 2510, 100 W at 40 kHz).

Digestion and extraction of the c/p-MWCNTs in the plant tissues

Eight-week-old lettuce (*Lactuca sativa*, Bionda Ricciolina) plants were purchased from a local nursery (Sparks, Nevada). The plants were washed with DDW and separated into leaves, stems, and roots, and dried in an oven at 80 °C for 12 hours. The dried plant tissues were ground and sieved with a 60 mesh (<0.25 mm) sieve. Partial samples were spiked with the MWCNTs by adding a pre-determined amount of the MWCNT suspension to the dried lettuce tissue powders to achieve the concentration of 125–600 μg MWCNTs per g lettuce tissues. The lettuce tissues with the MWCNTs were subject to the sequential digestion, developed in our recent study.[18] In brief, an aliquot (1 mL) of HNO_3 (15.8 M) was added to ~20.0 mg of the leaf, stem, or root tissues in 15.0 mL Corex glass centrifuge tubes. The centrifuge tube was placed inside the Corex digestion tube containing 15.0 mL DDW in a digestion chamber for 5 hours of digestion at 60 °C. After the digestion, 5.0 mL DDW was added to the digested samples, and the samples were centrifuged at 3000 rpm for 10 minutes. The precipitates were subject to secondary digestion, for which 0.3 mL H_2SO_4 (18.4 M) was added to the residues from HNO_3 digestion, and the samples were set for 3 hours of digestion at 60 °C. As soon as digestion was finished, 5.0 mL of DDW was added to the extract and centrifuged at 3000 rpm for 10 minutes. The supernatant was discarded from the tubes leaving 0.5 mL in the tubes with the precipitates. After the slurry was neutralized with 0.2–0.3

mL concentrated NH_4OH, it was centrifuged at 3000 rpm for additional 10 minutes, and the supernatant was discarded. 1.0 mL of the nonionic surfactant was added into the precipitate and vortexed for 1–5 seconds to obtain the homogeneous suspension of the digested c/p-MWCNTs and/or lettuce tissue residues, and the suspension was analyzed immediately with UV-Vis spectroscopy. To analyze the impact of digestion on the analysis of the original MWCNTs and the background contribution of the lettuce tissues, the MWCNT suspension and lettuce materials without the MWCNTs were also subject to the same digestion.

Spectroscopic analysis

For spectroscopic analysis, the suspension of the MWCNTs prepared with TX-100 or the suspension obtained after the digestion was analyzed with an Evolution 260 BIO UV-visible spectrophotometer (Thermo Fisher Scientific, Waltham, MA USA). The absorbance spectra of the aqueous phase suspension (1.0 mL) of the original p-MWCNTs and c-MWCNTs were obtained. The full scan spectra of the aqueous phase of TX-100 (2.0 mg mL^{-1}) and the TX-100-aided suspension of the p-MWCNTs and c-MWCNTs were obtained at 200–1000 nm in quartz cuvettes (ESI,† Fig. S1 and S2). The featureless spectra of the p-MWCNTs and c-MWCNTs were observed for the TX-100 aided suspension of both MWCNTs. Previous studies have used the absorbance at 800 nm for the quantification of CNTs in the aqueous phase.[13,14] The final absorbance of the prepared CNT suspension or digestion suspension was measured at 800 nm. To keep the absorbance value at 0.2–1, the suspension was diluted with TX-100 (2.0 mg mL^{-1}) solution.[15]

Plant cultivation and application of the developed method

Lettuce (*Lactuca sativa*, cv. black seeded Simpson) was grown hydroponically with the MWCNTs in the culture solution, and the plants were harvested after three weeks to quantify the MWCNTs in the leaf, stem, and root tissues. The plants were treated with the p-MWCNTs or c-MWCNTs of 5, 10, 20 mg L^{-1}. Briefly, the lettuce seedlings were grown in a greenhouse under natural conditions (30/15 °C (day/night), 15–63% daily relative humidity, natural light) for four weeks. The four-week-old healthy seedlings of similar size were used in the exposure experiments with the TX-100 suspended p-MWCNTs and c-MWCNTs. An amount of 10% Hoagland solution (Sigma-Aldrich Hoagland No. 2) was used as the medium (pH adjusted to 6.2–6.5) containing either the p-MWCNT or c-MWCNT at 0, 5, 10, or 20 mg L^{-1} in amber vials. An air pump was used to continuously aerate the solution, and the Hoagland solution was added as needed to compensate for the evapotranspiration loss.

After three weeks of culture, the plants were harvested and separated into leaf, stem, and root tissues. The plants were rinsed three to five times with DDW upon harvest. The root tissues were sonicated for five minutes in DDW to remove the external MWCNTs sorbed on the root surface. The tissues were dried in an oven at 80 °C for 12 hours and stored at 4 °C. Using the method developed in this study, the dried tissues were digested and analyzed with UV-vis spectroscopy for the quantification of the uptake and translocation of the MWCNTs.

Results and discussion

Stability of the MWCNT suspension and calibration curve

An absorption peak for TX-100 (2.0 mg mL^{-1}) was observed at 276 nm, followed by the featureless spectra at 300–900 nm (ESI,† Fig. S1). In comparison, both p-MWCNT and c-MWCNT (12 μg mL^{-1}) suspensions with TX-100 did not show any additional peaks at 200–900 nm (ESI,† Fig. S2). Similarly, the featureless spectra of the surfactant- and humic substance-stabilized single wall CNTs (SWCNTs) and MWCNTs at 200–1200 nm were observed by previous studies.[11,17] Following the published methods,[21,22] we have made a power-law regression for the wavelength-dependent absorption of the MWCNTs using eqn (1) (ESI,† Fig. S3):

$$A = C\lambda^{-AAE} \quad (1)$$

where A is the absorption, C is a constant, λ is the wavelength, AAE is the Ångstrom exponent. The Ångstrom exponent for absorption was 0.60 and 0.68 for the p- and c-MWCNTs, indicating that their optical properties are similar to those of natural black carbon.[21–23]

For the quantification of the MWCNTs, following published research, the absorbance at 800 nm was used for the quantification of the concentrations of the p-MWCNTs and c-MWCNTs prepared with TX-100 solution.[13,14] A linear relationship was obtained between the surfactant-calibrated absorbance at 800 nm and the concentrations of the p-MWCNTs ($y = 0.014x + 0.0044$, $R^2 = 0.99$, $p < 0.01$; or $Abs_{800} = 0.014\ C_{CNT} + 0.0044$, C_{CNT} is the concentration of the p-MWCNT, Abs_{800} is the absorbance at 800 nm) and c-MWCNTs ($y = 0.015x + 0.0037$, $R^2 = 0.99$, $p < 0.01$; or $Abs_{800} = 0.015C_{CNT} + 0.0037$, C_{CNT} is the concentration of the c-MWCNT) (Fig. 1). This result implied that the absorbance at 800 nm can be used for the quantification of the p- and c-MWCNTs suspended with TX-100. Hyung *et al.*[13] suspended the MWCNTs with dissolved organic matter, and they found a linear relationship between the absorbance at 800 nm and the concentration of the MWCNTs (1.0–7.0 μg mL^{-1}) in the suspension. The absorbance of CNTs has been attributed to the π electrons present in the benzene rings of CNTs.[16] The extinction coefficient of the p-MWCNTs and c-MWCNTs was calculated to be 0.0035 and 0.0038 mL μg^{-1} cm^{-1}, comparable to the reported values of 0.0046–0.0054 mL μg^{-1} cm^{-1}.[16]

Digestion of the plant tissues and spectroscopic analysis

The lettuce tissues were digested sequentially with HNO_3 and H_2SO_4 to minimize the influences of plant biomass on the spectroscopic analysis of the MWCNTs. The spectra for all the digested plant tissues showed that the absorbance gradually decreased from 300 nm to 700 nm, reaching a stable baseline at 700–800 nm with a minimum absorbance of

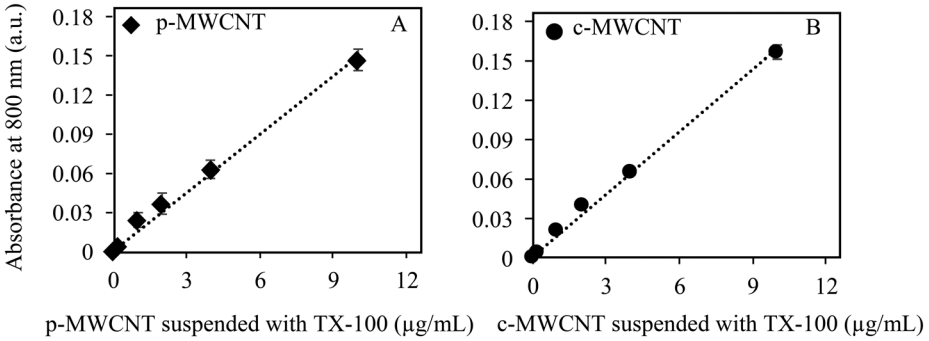

Fig. 1 Calibration curves obtained for the p-MWCNTs (2.5–12.0 μg μL^{-1}) (A) and c-MWCNTs (B) (2.5–12.0 μg μL^{-1}) prepared with nonionic surfactant Triton X-100 (TX-100) (2.0 mg mL^{-1}). A linear relationship has been found for the p-MWCNTs (R^2 = 0.99, $p < 0.01$) and c-MWCNTs (R^2 = 0.99, $p < 0.01$) for the concentrations of 0.2 to 10.0 μg mL^{-1}. The error bars showed the replicates of 3 samples at each point.

0.006–0.005 (ESI,† Fig. S4). This observation was similar to the other reported spectra for lignin extracted from various plants.[24,25] The extracted lignin showed a peak absorption at 340 nm, and a baseline at 500–1100 nm.[25] As another important component of the plant tissues, the extracted hemicellulose and cellulose have an even lower absorption compared to lignin.[26] The absorbance at 800 nm for the digested plant tissues followed the order leaf ≥ root > stem (Fig. 2). There was no significant difference between the absorbance of the leaf and root tissues for most samples ($p > 0.05$). In comparison, the absorbance of the stem tissues was significantly lower than that of the leaves and roots ($p < 0.05$). The absorbance obtained for ~20.0 mg samples was 0.022 ± 0.0080, 0.0080 ± 0.0020, and 0.015 ± 0.0060 for the digested leaf, stem, and root tissues, respectively.

The sequential digestion of the lettuce tissues with HNO_3 and H_2SO_4 facilitated the decomposition and removal of biomass. Our recent studies showed that the digestion of the plant tissues with HNO_3 reduced the biomass of the leaf, stem, and root to 1–2% residual.[20] Further digestion with H_2SO_4 decreased the residual biomass to 0.02% of the original values. The variation in the absorbance for the residual materials of the leaf, stem, and root could be due to their different contents of cellulose, lignin, proteins, and other compositions.[27] Lignin was more resistant to digestion and had higher absorption compared to cellulose and other components. The leaf and root in the lettuce have a higher content of lignin than the stem, which can partially explain their relatively higher absorption after digestion.

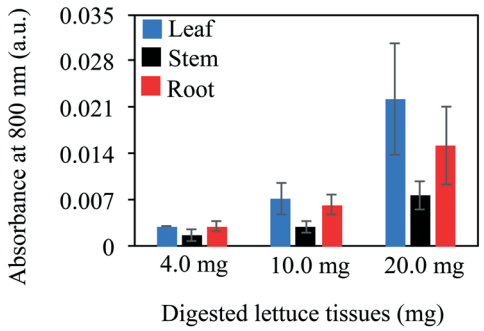

Fig. 2 Spectroscopic absorbance of the digestion residue of the lettuce leaf, stem, and root. The residue was suspended with nonionic surfactant TX-100 (2.0 mg mL^{-1}), and the absorbance was measured at 800 nm. The error bars showed the standard deviations obtained from the replicated samples. Three replicates were used for 4.0 mg and 10.0 mg, and six replicates were used for 20 mg.

Digestion and recovery of the MWCNTs

The influence of sequential digestion on the spectroscopic analysis of the p-MWCNTs and c-MWCNTs was examined. After the digestion, the absorption spectra of the MWCNTs were similar to those the original, with an absorption peak at 276 nm presumably from TX-100 and the featureless spectra after 300 nm (ESI,† Fig. S5). A linear relationship was obtained between the amount of the p-MWCNTs (R^2 = 0.98)/c-MWCNTs (R^2 = 0.99) (2.5–12.0 μg mL^{-1}) and the absorbance at 800 nm (Fig. 3). Based on the linear regression for the original MWCNTs, the recoveries of the MWCNTs were calculated following eqn (2) and (3):

$$C_{obs} = k\mathrm{Abs}_{800} + b \quad (2)$$

$$R = \frac{C_{obs}}{C_{dig}} \quad (3)$$

where C_{obs} is the observed concentration of the MWCNTs, Abs_{800} is the observed absorption at 800 nm, k and b are the regression parameters obtained for the original MWCNTs (Fig. 1), R is the recovery, C_{dig} is the concentration of the MWCNTs used for digestion. The recoveries of the p-MWCNTs (55.2 ± 10.3%) are greater than those of the c-MWCNTs (46.1 ± 6.2%) ($p < 0.05$). These recovery values for the c-MWCNTs are comparable to those of the MWCNTs after H_2SO_4 digestion in a previous study.[9] Doudrick *et al.*[9] observed that the recoveries of the pristine MWCNTs were higher than those of the functionalized MWCNTs. The relatively lower recoveries of the c-MWCNTs compared to the p-MWCNTs could be due to their higher degradation during the digestion with HNO_3 and H_2SO_4.[28] The recovery of CNTs can potentially be improved by alternative digestion using

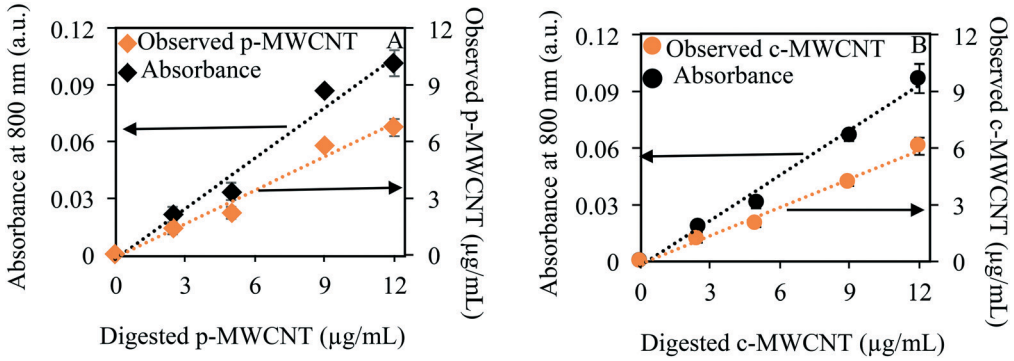

Fig. 3 Calibration curves for the digested p-MWCNT (A) and c-MWCNT (B) based on the absorbance at 800 nm. The MWCNTs were digested sequentially with HNO_3 (for five hours at 60 °C) and H_2SO_4 (three hours at 60 °C). A linear relationship with the absorbance was found for the concentrations of the p-MWCNTs (R^2 = 0.98, $p < 0.01$) and c-MWCNTs (R^2 = 0.99, $p < 0.01$). The expected p-MWCNTs (A) and c-MWCNTs (B) were calculated based on the linear regression for the original MWCNTs (eqn (1) and (2), Fig. 1). The error bars represent the standard deviation from triplicate samples.

specific enzymes for plant tissue removal, which is in our current research. Our results suggest that the UV-vis absorbance can be applied to quantify the p-MWCNTs and c-MWCNTs after the digestion. The final suspension for UV-vis spectroscopy was prepared by sequential digestion with HNO_3 (for 5 hours) and H_2SO_4 (for 3 hours) at 60 °C, followed by neutralization with NH_4OH and suspended with TX-100 (ESI,† Fig. S6).

Digestion and quantification of the MWCNTs in the MWCNT-spiked lettuce tissues

The spectra of the digested MWCNT-spiked lettuce tissues, showing the absorption peak at 276 nm and featureless spectra at 300–900 nm, were similar to those of the digested tissues without the MWCNTs (ESI,† Fig. S7). Upon subtraction of the background absorption from the digestion residue of the plant tissues, linear relationships were obtained between the absorbance at 800 nm and concentration of the MWCNTs in the final digestion solution (Fig. 4, ESI,† Tables S2 and S3, $R^2 > 0.94$). Consequently, there was also a close regression between the absorbance at 800 nm and the spiked p-MWCNT concentrations in the leaf (R^2 = 0.99), stem (R^2 = 0.99), and root (R^2 = 0.95). Based on the calculation using eqn (1) and (2), the recoveries of the p-MWCNTs from the leaf, stem, and root were 64.8 ± 17.0%, 39.2 ± 9.4%, and 68.6 ± 18.6%, respectively. The recoveries of the p-MWCNTs from the leaf and root are not significantly different ($p > 0.05$), when the value for the stem was significantly lower than that for the leaf and root ($p < 0.05$). The recoveries of the p-MWCNTs in the presence of the leaf and root tissues were higher than those for the p-MWCNTs digested without the plant tissues ($p < 0.05$). The digestion residue of the plant tissues may protect the MWCNTs from oxidation by the strong acid or affect the aggregation/precipitation of the CNTs in the case of the spiked leaf and root tissues.

Linear relationships were also found between the absorbance at 800 nm and spiked concentration of the c-MWCNTs (0.12–0.59 μg mg^{-1}) in the leaf (R^2 = 0.98), stem (R^2 = 0.97), and root (R^2 = 0.94). The recoveries of the c-MWCNTs were 51.9 ± 13.9%, 54.9 ± 10.7%, and 38.8 ± 12.7% for the leaf, stem, and root, respectively. There was no significant difference between the recoveries of the c-MWCNTs from the leaf and stem tissues ($p > 0.05$). In comparison, the c-MWCNTs recovered from the root tissues are lower than those from the leaf and stem, and the control c-MWCNTs ($p < 0.05$). The recoveries of the c-MWCNTs in the leaf and root were much lower than the p-MWCNTs; however, the value was higher for the c-MWCNTs in the stem than the p-MWCNTs. Previous studies showed that the recovery of CNTs during the digestion depended on their ability to form aggregates.[8] The negatively charged c-MWCNTs likely had less efficiency to form stable aggregation due to the electrostatic repulsion between the CNTs.

Previous work showed that the concentration of CNTs linearly governed their spectroscopic absorption, due to π electrons.[15,16] In this study, our work demonstrated linear relationships between the concentration of the digested p- and c-MWCNTs in the lettuce leaf, stem, and root tissues and absorbance at 800 nm. After the sequential digestion to remove plant biomass, the absorbance derived from the π electrons in graphene sheets still obeyed a linear response to the concentration of the MWCNTs. The intactness of the MWCNTs following strong chemical digestions has been shown by other studies using thermal and Raman analyses.[9,20] As the dispersion and aggregation of the MWCNTs can influence their absorption coefficient,[15,30] the addition of the nonionic surfactant can facilitate the homogenous suspension and efficient quantification of the digested MWCNTs. The recoveries of the MWCNTs could also be influenced by the presence of functional groups on the MWCNTs and the types of the plant tissues. In general, the p-MWCNTs had higher recoveries than the negatively charged c-MWCNTs.

Considering the background absorption from the residual plant tissues and recoveries of the MWCNTs after the sequential digestion, the detection limits of the p-MWCNTs in the

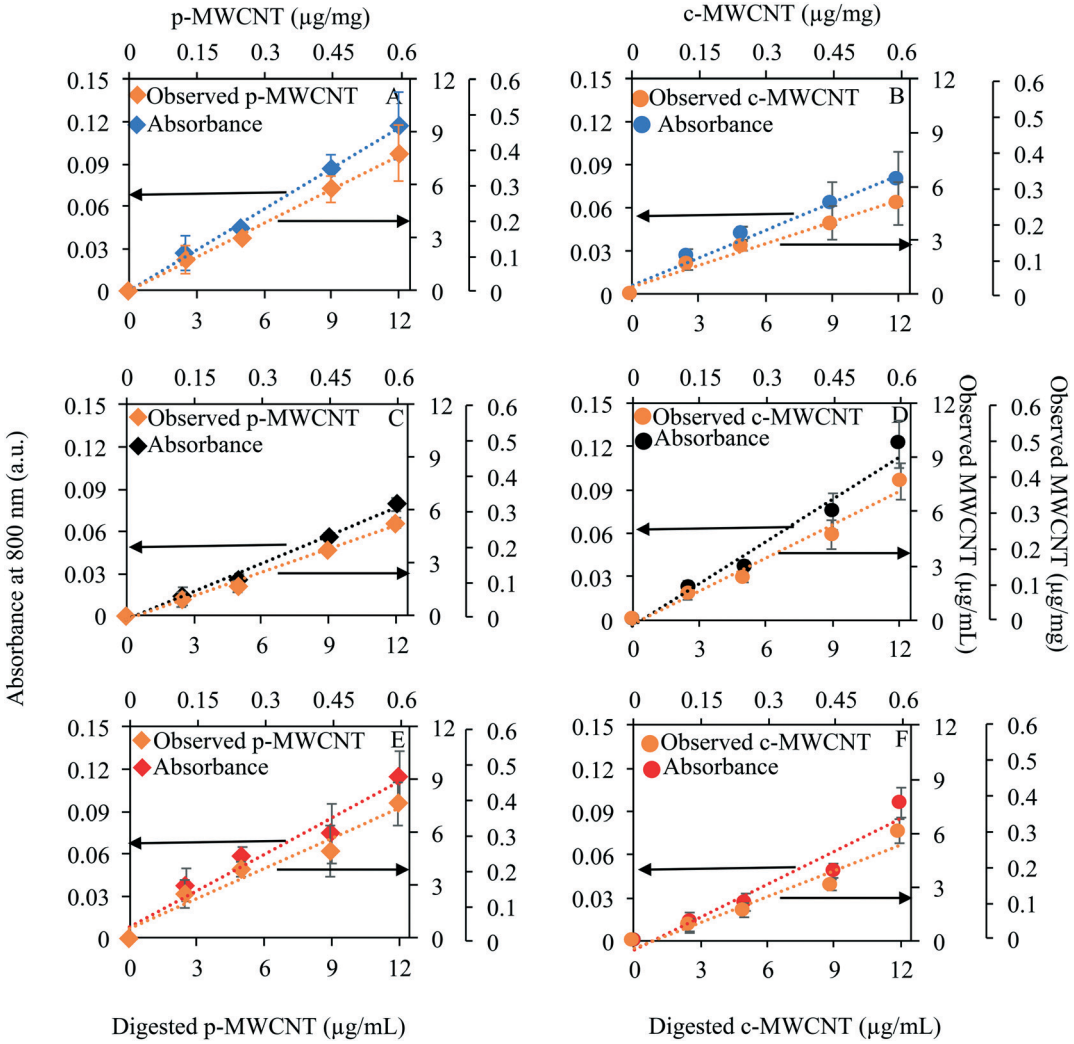

Fig. 4 Calibration curves for the digested p-MWCNT- (A, C and E) and c-MWCNT- (B, D and F) spiked lettuce tissues based on the absorbance at 800 nm. The c/p-MWCNT-spiked lettuce tissues were digested sequentially with HNO_3 and H_2SO_4. The residue was suspended with TX-100 (2.0 mg mL^{-1}). Regressions ($R^2 > 0.94$, $p < 0.01$) were obtained for the lettuce tissues spiked with the p-MWCNTs and c-MWCNTs (ESI,† Tables S2 and S3). The expected concentrations of the p-MWCNTs (A, C and E) and c-MWCNTs (B, D and F) were calculated based on the regression for the original CNTs. The error bars represent the standard deviations derived from triplicate samples.

leaf, stem, and root were determined to be 0.12, 0.081, and 0.019 μg p-MWCNT per mg plant tissues (ESI,† text, Table S4), respectively. The detection limit of the c-MWCNTs was similar to values of 0.10, 0.070, and 0.18 μg c-MWCNT per mg of the leaf, stem, and root tissues, respectively. The detection limit of the MWCNTs with a value of 19–180 μg g^{-1} plant tissues was comparable to those of the other methods such as TGA-MS, and PTA,[9,29,31] but higher than those obtained with microwave induced heating analysis.[6] The spectroscopic procedure developed in this study has an additional advantage of wide accessibility and rapid analysis. The analysis of the MWCNTs with the UV-vis absorption at 800 nm will facilitate quick and easy detection and quantification of CNTs varying in surface chemistry. As previous studies showed that the extinction coefficients of CNTs are not interfered with by the structure and diameter of the CNTs,[16] our method can also be potentially applicable to a broader range of CNTs with varied sizes, diameters and structures. Raman spectroscopy, a complementary method to UV-vis spectroscopy, could be applied for improving the detection limit of CNTs in plant tissues. The digestion of the plant tissues by a single digestion step (HNO_3 digestion) was efficient to remove the interferences in the Raman signals from the plant tissues.[20] Using this single step digestion and Raman spectroscopy could help to avoid additional digestion steps and enhance CNT recovery.

Last but not least, the stable homogeneous suspension of the MWNCTs was essential for their quantification (Fig. 3 and 4). In previous studies, the homogeneous suspensions of the MWCNTs were achieved by treating the CNTs with anionic, cationic, and nonionic surfactants and polyethylene glycol (PEG) and centrifugation at high speed for several hours (~6 h) (Han *et al.*, 2008;[14] Liu *et al.*, 2009[10]). Digestion with strong oxidative reagents could possibly increase the

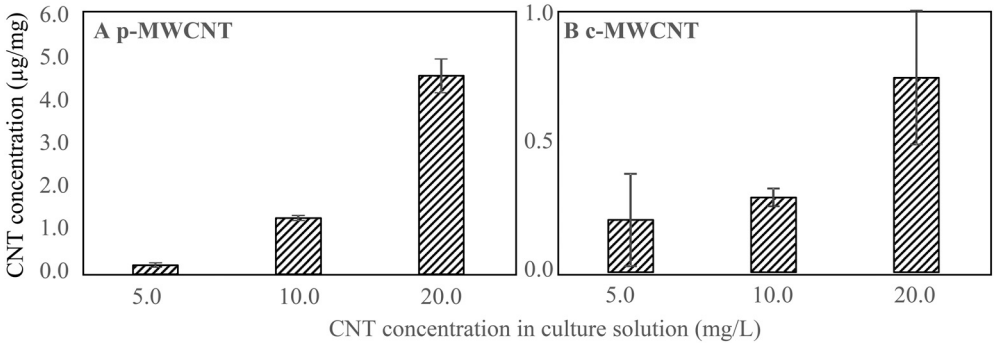

Fig. 5 Concentrations of the p-MWCNT (A) and c-MWCNT (B) in the lettuce roots quantified by digestion coupled with the analysis of UV-vis absorbance at 800 nm. The plants were grown in a greenhouse using hydroponic systems containing 5, 10, and 20 mg L^{-1} CNT solutions.

functionalization of the MWCNTs and affect the suspension stability. However, the application of TX-100 with the original and digested MWCNTs (original and spiked in the plant tissues) showed the versatility of this nonionic surfactant for preparing the homogeneous suspension, providing linear calibration curves for the CNT concentration varying in orders of magnitude (Fig. 3 and 4). This method can be useful for suspending CNTs or other carbonaceous nanomaterials for other analysis.

Analysis of the MWCNTs in the hydroponically cultured lettuce tissues

We have applied our method for the quantification of the multiwall carbon nanotubes (MWCNTs) in lettuce (*L. sativa,* cv. black seeded Simpson) hydroponically cultured with 5, 10, 20 mg L^{-1} pristine (p-) and carboxyl-functionalized (c-) MWCNTs. We detected 0.21 ± 0.05–4.57 ± 0.39 μg mg^{-1} p-MWCNTs and 0.20 ± 0.17–0.75 ± 0.25 μg mg^{-1} c-MWCNTs in the lettuce roots, positively correlated with the dose of CNTs in solution (Pearson correlation coefficient r = 0.98, $p < 0.05$) (Fig. 5). The bioconcentration factor for the root (C_{root}/C_{water}, with C_{root} and C_{water} representing concentrations in the root and culture solution, respectively) ranged from 0.042–0.23 and 0.028–0.040 L g^{-1} for the p-MWCNT and c-MWCNTs, respectively. In addition, the concentration of the p-MWCNTs in the leaf (0–0.014 μg mg^{-1}) was also much higher than that of the c-MWNCTs (below background), although it is still below the detection limit (ESI,† Fig. S8).

In other culture experiments, the concentrations of the CNTs in the plant tissues ranged from 0.001–0.085 μg mg^{-1} plant tissues.[7] Using the ^{14}C-labeled MWCNTs, Zhao *et al.*[5] captured 0.001–0.077 μg MWCNTs per mg plant tissues in *Arabidopsis*, rice, maize, and soybean grown under hydroponic conditions containing 2.5 mg L^{-1} MWCNTs. The bioconcentration factor ranged from 0.0004–0.031 L g^{-1}. Another study showed that the accumulation of single wall CNTs (SWCNTs) was 0–0.024 μg mg^{-1} in corn, grown in SWCNT-applied soil.[7] Our method can be applied to unambiguously determine the concentration of CNTs in such culture experiments, without the need of radio-labelled materials and special equipment set-up. The accumulation of CNTs quantified in the lettuce roots was similar to the other reported values for CNTs in the plant tissues, although we did not determine unambiguous translocation.

Based on the emission data, it was estimated that the concentration of CNTs in soils ranged from 23–46 ng kg^{-1}, and for the biosolid-applied soils, the concentration of CNTs can range up to 11 μg kg^{-1}.[32] Using the biological uptake factor determined in the recent studies,[7] the concentration of CNTs in agricultural plants can range up to 5 μg kg^{-1}, which was much lower than the detection limit of our method. The spectroscopic analysis can potentially be used to quantify the concentration of CNTs in the cultured samples and provide critical information for evaluating their environmental effects and managing their application, although further investigations are needed to validate its application for the natural samples and improve the detection limit.

Conclusions

A method using UV-vis spectroscopic analysis coupled with sequential digestion has been developed for the quantification of p-MWCNTs and c-MWCNTs in lettuce leaf, stem, and root tissues. The digestion removed the plant biomass and facilitated the extraction of the MWCNTs. Using this method, the detection limits of the p-MWCNTs and c-MWCNTs were achieved as 0.10–0.12, 0.070–0.081, and 0.019–0.18 μg mg^{-1} for the leaf, stem, and root, respectively. Based on the experiments for the spiked lettuce tissues, the recovery of the p-MWCNTs and c-MWCNTs ranged from 39.2–68.6% and 38.8–54.9%, respectively, which can be potentially improved by alternative enzymatic digestion. This method is rapid and widely-accessible compared to other technologies such as programmed thermal analysis, and potentially can enable reliable quantification of CNTs in a larger amount of environmental samples. Using this method, we have quantified the concentration of the p-MWCNTs and c-MWCNTs to be 0.21 ± 0.05–4.57 ± 0.39 and 0.20 ± 0.17–0.75 ± 0.25 μg mg^{-1} in the root tissues of the lettuce hydroponically cultured with the CNT-spiked culture solution, respectively. The method can also be potentially used for the quantification of the MWCNTs in other environmental media to determine the environmental risk of CNTs and optimize their application.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was supported by the USDA (Grant No. 2015-67018-23120), the DOE (Grant No. DE-SC0014275), the University of Nevada, Reno (a startup fund to Y. Yang), and the National Natural Science Foundation of China (NSFC Grant No. 41629101). This work was also partially supported by the USDA grant 2017-69007-26309 and NSF grant 1808372.

References

1 M. F. L. De Volder, S. H. Tawfick, R. H. Baughman and A. J. Hart, *Science*, 2013, **339**, 535–539.
2 G. M. Insights, *Carbon Nanotubes Market to hit USD 8.1bn by 2024*, Global Market Insights, Inc., 2016.
3 M. V. Khodakovskaya, K. de Silva, D. A. Nedosekin, E. Dervishi, A. S. Biris, E. V. Shashkov, E. I. Galanzha and V. P. Zharov, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**, 1028–1033.
4 G. S. Zhai, S. M. Gutowski, K. S. Walters, B. Yan and J. L. Schnoor, *Environ. Sci. Technol.*, 2015, **49**, 7380–7390.
5 Q. Zhao, C. Ma, J. C. White, O. P. Dhankher, X. Zhang, S. Zhang and B. Xing, *Carbon*, 2017, **114**, 661–670.
6 M. V. Khodakovskaya, K. de Silva, A. S. Biris, E. Dervishi and H. Villagarcia, *ACS Nano*, 2012, **6**, 2128–2135.
7 A. M. Cano, K. Kohl, S. Deleon, P. Payton, F. Irin, M. Saed, S. A. Shah, M. J. Green and J. E. Canas-Carrell, *Chemosphere*, 2016, **152**, 117–122.
8 E. Wild and K. C. Jones, *Environ. Sci. Technol.*, 2009, **43**, 5290–5294.
9 K. Doudrick, N. Corson, G. Oberdorster, A. C. Eder, P. Herckes, R. U. Halden and P. Westerhoff, *ACS Nano*, 2013, **7**, 8849–8856.
10 Z. Liu, C. Davis, W. B. Cai, L. He, X. Y. Chen and H. J. Dai, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 1410–1415.
11 E. J. Petersen, D. X. Flores-Cervantes, T. D. Bucheli, L. C. C. Elliott, J. A. Fagan, A. Gogos, S. Hanna, R. Kagi, E. Mansfield, A. R. M. Bustos, D. L. Plata, V. Reipa, P. Westerhoff and M. R. Winchester, *Environ. Sci. Technol.*, 2016, **50**, 4587–4605.
12 C. Cerrillo, G. Barandika, A. Igartua, O. Areitioaurtena, A. Marcaide and G. Mendoza, *Environ. Toxicol. Chem.*, 2015, **34**, 1854–1862.
13 H. Hyung, J. D. Fortner, J. B. Hughes and J. H. Kim, *Environ. Sci. Technol.*, 2007, **41**, 179–184.
14 Z. T. Han, F. W. Zhang, D. H. Lin and B. S. Xing, *Environ. Sci. Technol.*, 2008, **42**, 6869–6875.
15 S. Attal, R. Thiruvengadathan and O. Regev, *Anal. Chem.*, 2006, **78**, 8098–8104.
16 G. A. Rance, D. H. Marsh, R. J. Nicholas and A. N. Khlobystov, *Chem. Phys. Lett.*, 2010, **493**, 19–23.
17 N. B. Saleh, L. D. Pfefferle and M. Elimelech, *Environ. Sci. Technol.*, 2008, **42**, 7963–7969.
18 K. K. Das, L. Bancroft, X. L. Wang, J. C. Chow, B. S. Xing and Y. Yang, *Environ. Sci. Technol. Lett.*, 2018, **5**, 442–447.
19 Y. You, K. K. Das, H. Guo, C.-W. Chang, M. Navas-Moreno, J. W. Chan, P. Verburg, S. R. Poulson, X. Wang, B. Xing and Y. Yang, *Environ. Sci. Technol.*, 2017, **51**, 2068–2076.
20 K. K. Das, Y. You, M. Torres, F. Barrios-Masias, X. Wang, S. Tao, B. Xing and Y. Yang, *Environ. Sci.: Nano*, 2018, 659–668.
21 J. C. Chow, J. G. Watson, M. C. Green, X. L. Wang, L. W. A. Chen, D. L. Trimble, P. M. Cropper, S. D. Kohl and S. B. Gronstal, *J. Air Waste Manage. Assoc.*, 2018, **68**, 494–510.
22 H. Moosmueller, R. K. Chakrabarty and W. P. Arnott, *J. Quant. Spectrosc. Radiat. Transfer*, 2009, **110**, 844–878.
23 T. C. Bond, Spectral dependence of visible light absorption by carbonaceous particles emitted from coal combustion, *Geophys. Res. Lett.*, 2001, **28**, 4075–4078.
24 A. Hambardzumyan, L. Foulon, B. Chabbert and V. Aguie-Beghin, *Biomacromolecules*, 2012, **13**, 4081–4088.
25 L. M. Kline, D. G. Hayes, A. R. Womac and N. Labbe, *BioResources*, 2010, **5**, 1366–1383.
26 T. Bikova and A. Treimanis, *Carbohydr. Polym.*, 2004, **55**, 315–322.
27 D. Mishima, M. Tateda, M. Ike and M. Fujita, *Bioresour. Technol.*, 2006, **97**, 2166–2172.
28 V. Datsyuk, M. Kalyva, K. Papagelis, J. Parthenios, D. Tasis, A. Siokou, I. Kallitsis and C. Galiotis, *Carbon*, 2008, **46**, 833–840.
29 K. Doudrick, P. Herckes and P. Westerhoff, *Environ. Sci. Technol.*, 2012, **46**, 12246–12253.
30 C. Cerrillo, G. Barandika, A. Igartua, O. Areitioaurtena, N. Uranga and G. Mendoza, *Environ. Toxicol. Chem.*, 2016, **35**, 74–83.
31 D. L. Plata, C. M. Reddy and P. M. Gschwend, *Environ. Sci. Technol.*, 2012, **46**, 12254–12261.
32 T. Y. Sun, N. A. Bornhoft, K. Hungerbuhler and B. Nowack, *Environ. Sci. Technol.*, 2016, **50**, 4701–4711.