Quantification of carbon nanotubes in environmental matrices: Current capabilities, case studies, and future prospects

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ABSTRACT

Carbon nanotubes (CNTs) have numerous exciting potential applications and some that have reached commercialization. As such, quantitative measurements of CNTs in key environmental matrices (water, soil, sediment, and biological tissues) are needed to address concerns about their potential environmental and human health risks and to inform application development. However, standard methods for CNT quantification are not yet available. We systematically and critically review each component of the current methods for CNT quantification including CNT extraction approaches, potential biases, limits of detection, and potential for standardization. This review reveals that many of the techniques with the lowest detection limits require uncommon equipment or expertise, and thus, they are not frequently accessible. Additionally, changes to the CNTs (e.g., agglomeration) after environmental release and matrix effects can cause biases for many of the techniques, and biasing factors vary amongst the techniques. Five case studies are provided to illustrate how to use this information to inform responses to real-world scenarios such as monitoring potential CNT discharge into a river or ecotoxicity testing by a testing laboratory. Overall, substantial progress has been made in improving CNT quantification during the past ten years, but additional work is needed for standardization, development of extraction techniques from complex matrices, and multi-method comparisons of standard samples to reveal the comparability of techniques.
Introduction

The steady increase in potential applications\(^1\) and production\(^{1,2}\) of carbon nanotubes (CNTs) and their inevitable release during the life cycle of products has raised questions regarding their potential impact on humans and the environment.\(^{3,4}\) CNTs can be conceptually understood as rolled up graphitic sheets of hexagonally arranged carbon atoms with \(sp^2\) hybridization. These materials have exceptional mechanical strength as well as thermal and electrical conductivity properties that make them ideal for a myriad of potential applications (e.g. construction, environmental, optical, electronic, and biomedical).\(^5-8\) The annual production capacity of CNTs reached \(2 \times 10^6\) kg (2.25 ktons) yr\(^{-1}\) in 2011 with an estimated production capacity of \(5 \times 10^6\) kg (4.5 ktons) yr\(^{-1}\); this change was a 10-fold increase since 2006.\(^1\) With increasing production volume, it is important to determine the potential for biological exposures to CNT during the production, usage, and disposal of CNT-enabled products. The necessary linchpin to quantifying potential CNT exposure, and any risks from it, is the availability of robust analytical methods for quantifying CNTs in complex environmental matrices.\(^9\) These methods are critical for the assessment of potential CNT exposure, toxicity testing on the potential risks that may occur after exposure, and determination of the environmental fate of CNTs.\(^10\)

Analytical techniques to quantify CNTs usually rely on unique physicochemical properties of CNTs that differentiate them from other compounds in relevant media. These approaches leverage the structural, thermal, and electrical properties of CNTs and include spectroscopic,\(^{11,12,13,14,15}\) optical,\(^{16,17}\) and thermal\(^{16,14,18}\) techniques used individually or in combination.\(^9,15\) Importantly, techniques used for analysis of traditional organic and inorganic toxic chemicals are often not applicable for the following reasons: a) unlike most organic pollutants, CNTs have a distribution of
lengths and diameters rather than a single molecular structure and, therefore, mass spectrometry methods, a key tool in current organic analytical methods, generally cannot be used; the large molecular weight of CNTs could potentially challenge mass spectrometric methods too; b) most techniques cannot distinguish between CNTs and naturally occurring black carbon allotropes (e.g., soot or charcoal), which are present at much higher concentrations in the environment than those modeled for CNTs; c) several other carbon forms are often present in samples (e.g., natural organic matter; NOM) which may interfere with CNT quantification in the sample matrix; and d) the wide range of shapes, sizes, diameters, functional groups, and agglomeration states make it difficult to develop a universal analytical method for quantifying all types of CNTs. In addition, commercially manufactured CNTs may also contain substantial concentrations of metal catalysts, amorphous carbon, and graphitic (non-CNT) nanoparticles (NPs) which may cause biases with some analytical techniques, but are essential for other techniques.\textsuperscript{20-22}

While there have been numerous analytical techniques used to quantify CNTs in various matrices,\textsuperscript{4,14-16,23-38} for each technique there have only been a limited number of studies, often made by a single laboratory, and thus the robustness of the methods is unknown. In particular, relevant experimental parameters including comprehensive characterization of the CNTs and quantities used for testing and calibration procedures are not always reported. Moreover, failed attempts to apply new methods and techniques or to replicate approaches described in previous studies are often not published, and thus, the limitations of each technique such as potential biases for various matrices (e.g., water or soil with natural (NOM) or soil organic matter (SOM)) are often unclear. Overall, while some recent review papers have focused in part on CNT quantification,\textsuperscript{4,39,40} many critical topics (e.g., interferences in key matrices
(environmental, biological, synthetic polymers), and the potential biases with CNT quantification from changes to the CNTs (e.g., oxidation)) related to the development of robust, precise, and reproducible CNT quantification methods have not yet been critically evaluated.

This manuscript reviews CNT quantification techniques and evaluates their applicability for different key matrices (water, soil/sediment, tissue) and different types of CNTs (i.e., single-wall carbon nanotubes (SWCNTs) or multiwall carbon nanotubes (MWCNTs)). We report a critical evaluation and comparison among the advantages and limitations of each technique including biases for relevant matrices, biases from physicochemical changes to CNTs in those matrices (i.e., oxidation/degradation, wrapping with organic molecules, and agglomeration), detection limits in various matrices, the potential for standardization, and the types of CNTs that can be analyzed. In addition, methods for extraction or separation of CNTs from different matrices, which may be necessary for sample preparation for some techniques, are enumerated. These quantification, separation, and extraction techniques may also be relevant for quantifying CNT loading in consumer products but the focus of this paper will be on scenarios relevant for assessing the potential environmental risks and fate of CNTs. For example, potential quantification techniques for representative scenarios related to environmental release and potential ecotoxicological effects are discussed. Future research topics to elucidate and improve the analytical performance of these techniques and CNT quantification in general are also highlighted. This paper is intended to serve as a reference to guide scientists in the area of CNT quantification through the selection of an appropriate technique given a type of CNT, sample matrix, and CNT concentration. Given the substantial literature on physicochemical properties and characterization of CNTs, basic background information on these subjects is not
provided. While CNTs are also widely known to cause artifacts in many nanotoxicology assays such as by adsorbing key reagents, this manuscript will focus on biases related to quantification of CNTs and not biases in the measurements of their potential toxicological effects.

**Extraction and Separation Procedures for CNTs**

Numerous techniques have been investigated to extract or separate CNTs from different matrices to overcome quantification limits in complex biological and environmental media (Table 1). In this manuscript, we define “extraction” as the isolation of analytes from a matrix by their physical transition from one phase into another. In contrast, separation means the isolation of analytes from themselves (e.g. differently sized CNTs), or from a matrix within a given phase (e.g. a mobile phase in chromatography or field flow fractionation). Successful extraction methods usually involve the suspension of CNTs in a specific media in which interfering compounds are less soluble, but the converse approach can also be utilized: removing the matrix while leaving the CNTs. However, most reported separation or extraction methods have only been used by a single research group in one or a small number of studies to partly or fully separate CNTs from an environmental matrix (e.g., asymmetric flow field flow fractionation (AF4), matrix digestion, and sonication with surfactants). Other techniques have not yet been utilized with environmental and biological matrices (e.g., density gradient centrifugation, gel permeation chromatography, capillary electrophoresis, two-polymer phase extraction), but instead have been successfully applied to simpler matrices (e.g., deionized water) or have been used for CNT purification. These techniques may be valuable for use with environmental and biological matrices and are also listed in Table 1. Conversely, there has been more
progress with extraction and analysis of fullerenes, another carbon nanomaterial, from complex matrices.\textsuperscript{50-56}

Currently, many challenges remain in CNT extraction and separation strategies. First, it is unclear to what extent many of these techniques would be applicable for both MWCNTs and SWCNTs given the different properties of these two classes of CNTs, as most methods have only been applied to one or the other. This thought may be extended beyond the number of walls, to include any change in physicochemical properties (e.g., length, internal or external diameter, number of walls, or functional groups). Nevertheless, we expect that separation and extraction techniques may have to be tailored for a specific physicochemical property. For example, a method that can isolate short CNTs from a matrix could be ineffective when used against a population of long, highly entangled CNTs. Second, separation or extraction methods have not yet been applied to CNTs as utilized in potential consumer applications such as in polymer nanocomposite matrices. Given that CNTs will be released into the environment from consumer products, it is important to quantify the release of CNTs from these products after environmental stresses. It may also be important to quantify the concentration of CNTs in the consumer products, such as CNT-containing nanocomposites, to determine the potential quantity that could be released. Given challenges related to collecting and quantifying CNTs released from polymeric nanocomposites, one approach to estimate the quantity of CNTs released is to use a mass balance approach by quantifying the CNT concentration in a product before and after environmentally relevant degradation processes. For example, established methods are needed to extract CNTs from CNT-containing nanocomposites before and after the weathering and degradation processes (e.g., due to UV degradation and abrasion) to enable quantification of CNT concentrations.\textsuperscript{57-60} This will allow scientists to more fully address the complete life
cycle of nano-enabled consumer products. Finally, extraction or separation procedures may change the physicochemical properties of the CNTs, potentially impacting the reliability of results from analytical methods. One such example is the matrix digestion approach described by Doudrick et al.,\textsuperscript{23} which was suitable for subsequent analysis using thermal optical transmittance (TOT), but is potentially unsuitable for spectroscopic quantification by Raman scattering, because of concerns that the Raman spectra (e.g., ratio of D to G band) may be altered by the digestion procedure. Overall, although encouraging results have been obtained for a limited number of studies, the overall development of extraction and separation methods for CNTs from matrices for quantitative analyses is still a relatively new area of research.

**Quantification techniques**

A broad range of techniques have been developed to quantify or identify CNTs in environmentally and biologically relevant matrices (Table 2). In general, the techniques can be sorted into four groups: those that rely on the unique spectroscopic and thermal characteristics of the CNTs (that enable them to be distinguished from the matrix), those that utilize the presence of metal catalyst impurities (associated with the CNTs from the synthesis process), those that require isotopically enriched or depleted CNTs (e.g., with carbon-14 or carbon-13), and finally, microscopic techniques. There are large differences in the sensitivities and applicability of these techniques. Some thermal processes produce detectable gases (CO, CO\textsubscript{2}), while others measure radiative heating of a sample. For example, the microwave method involved irradiating CNT containing samples with microwave radiation, wherein the carbon nanotubes absorb the microwave radiation, and the increase in temperature is proportional to the CNT concentration for a given matrix.\textsuperscript{61,62} When comparing different studies, even those
using the same quantification technique, there is substantial diversity in the characteristics of the CNTs utilized.

It is evident from Figure 1 that, while some instruments used in the CNT quantification techniques are commercially available (e.g., UV/vis/NIR spectroscopy and Raman spectroscopy), most of the techniques require uncommon equipment that need to be partially or wholly custom built (e.g., microwave method, photoacoustic and photothermal imaging) or expertise that is not readily available. The use of uncommon instruments in these techniques also poses challenges for commercial ecotoxicity testing facilities to fulfill guidelines for standard methods related to maintaining a consistent exposure concentration. While some analytical instruments that can be used to quantify CNTs are widely available (e.g., UV/vis spectrophotometry), some of them have significant potential interferences as will be discussed in detail in subsequent sections. To provide one example, challenges related to the use of UV/vis spectrophotometry have recently been described including absorption coefficients dependent on the CNT structure distribution and dispersion method, as well as decreasing absorption coefficients with CNT agglomeration and uncertainty in determining non-CNT from CNT contributions. The lack of robust and widely available analytical methods likely contributes to the exclusive use of nominal concentrations to describe the exposure concentration and the absence of reported changes in CNT concentrations during experiments in many nanoeotoxicology studies.

Microscopic techniques can provide unambiguous identification of the CNTs in a complex matrix (e.g., transmission electron microscopy (TEM) analysis using electron energy loss spectroscopy or high resolution TEM), but low or uneven distributions of CNTs on microscopy samples hamper the conversion of the number of
CNTs detected on (several) images to the number/mass concentration of CNTs in a sample. These limitations can be overcome, for matrices without substantial interferences, by using a centrifugation-based method to capture the CNTs from a known volume onto a microscopy sample holder (e.g. TEM grid). Under these conditions, frequency data (number of CNTs per area) can be converted into particle number and mass concentration metrics.\textsuperscript{67-69} However, when one considers projected environmentally relevant concentrations of CNTs (typically ng to µg kg\textsuperscript{-1} solids),\textsuperscript{70} the likelihood that one captures a CNT onto a microscopy grid with µg-sized environmental samples is exceedingly small. Overall, due to limitations related to the sample preparation issues (low CNT concentration especially compared to other solids, overlapping particles, and uneven distribution of CNTs onto the sample holders), results from electron microscopic techniques remain mainly on a qualitative level, and are currently of limited utility for quantitation.

While electron microscopic techniques are very helpful to confirm the identity of CNTs in a matrix if the CNT loading is sufficiently high, reliable controls of the sample matrix without CNTs, the CNTs alone, the sample holder, and any other interferences are needed to avoid false positive or false negative results, but these controls are rarely available for environmental samples. In addition, the amount of time required for sample preparation depends on the samples matrix and greatly varies among techniques. For example, obtaining TEM images suitable for automated image analyses may require that individual CNTs are evenly distributed on a TEM grid and do not overlap with other particles. This often requires elaborate and tailored extraction, dispersion and deposition techniques that are very time intensive to develop. In contrast, sample preparation for hyperspectral imaging microscopy is usually very fast, as liquid samples can be directly cast onto a microscopy slide and subsequently imaged.
However, the current commercial setup lacks the possibility for automated image acquisition as well as suitable measures to determine the deposited sample volume, which hampers its quantitative capabilities.

Due to the similarities between CNT structure and that of atmospheric soot or carbon black, many analytical techniques that have been used for their extraction or isolation from air, soil, or sediment have been also used to quantify CNTs (e.g., thermal optical transmittance (TOT), chemothermal oxidation at 375 °C (CTO-375), thermogravimetric analysis (TGA), and total organic carbon (TOC)).\textsuperscript{14,16,18,71} While TOT can measure CNTs, custom temperature ramping programs are required for CNTs that differ from standard National Institute for Occupational Safety and Health (NIOSH) methods used for soot analysis on atmospheric samples.\textsuperscript{16} Similar modifications may also help improve CNT quantification by other thermal techniques such as CTO-375. Sampling of soot in air requires separation from the air, and usually involves filters, impactors or centrifugal separation. Airborne CNTs would likely also be captured by these techniques.\textsuperscript{72-76}

All of the quantification techniques are critically assessed in subsequent sections for the potential impact of matrix interferences or interferences from changes that may occur to the CNT in different test systems or the natural environment. For example, the impact of CNT degradation, as has been shown to occur enzymatically and due to interactions with cells and bacteria,\textsuperscript{77-83} and oxidation on the performance of different analytical methods are evaluated. In addition, the limits of detection (LODs) for these techniques in different media are compared and used to assess the potentially relevant techniques for five case study scenarios. The potential for these techniques to be standardized, a critical issue for regulatory agencies, is also discussed.

\textit{Evaluation of potential matrix interferences for quantification procedures}
Perhaps the principal reason that quantification of CNTs in environmentally relevant matrices is challenging is because of matrix interferences, namely difficulties associated with detecting carbon in a carbon background, especially at modeled average environmental CNT concentrations. The matrix characteristics that are most likely to cause interferences are described in detail in Table S1. Overall, natural waters and cell media (e.g., in studies with fish or human cells) have significantly fewer matrix interferences compared to biological tissues, soil/sediment, and released material from nanocomposites. For most spectroscopic measurements, while molecules and suspended particles in natural waters and cell media can potentially scatter incoming/outgoing light thus potentially biasing measurements, methods that account for these effects are generally available; in contrast, separation from the matrix is often needed prior to CNT quantification in tissues, soil/sediment, and fragments released from nanocomposites. For inorganic elemental analysis, having a constant and relatively low background metallic content of the matrix of the same element as the catalyst(s) of the CNTs is most important for all relevant matrices to achieve a low LOD and accuracy. Additionally, the multi-isotopic capability of the inorganic elemental analysis may enable qualitative and/or quantitative isotopic analysis when the isotopic ratios of the catalyst particles differ from those typically observed in the environmental matrix. For single-particle inductively coupled plasma-mass spectrometry (spICP-MS) analysis of CNTs, the background metallic content in nanoparticle form in matrices is similarly important with regards to the accuracy of the measurement, while low background metallic content in dissolved form is necessary for achieving a low LOD. However, spICP-MS instruments operating at microsecond dwell times can only perform nanoparticle isotopic analysis for detection of two elements, a capability which nevertheless can be used to distinguish naturally occurring NPs from their engineered
counterparts. While spICP-time of flight (ToF)-MS has recently shown the capacity for multi-element analysis, the size limit of detection was larger for gold and silver NPs compared to quadrupole-based instruments. Given the expected small amounts of the catalysts associated with individual CNTs and challenges associated with determining the background cut off level for SWCNT analysis using spICP-MS, it is unclear if spICP-ToF-MS will work well for CNT quantification.

Thermal techniques often do not show interferences with natural waters and cell media, although there were technique-specific chemicals in these matrices (e.g., peptone in the media for TGA analysis) that could impact the results. Two key considerations for many of the thermal techniques are whether components in the matrix can change the thermal stability of the CNTs and if there is the potential for overlap in the oxidation temperatures of CNTs and combustible components of the matrix. Thermal techniques could generally work in all matrices but the detection limit will be higher in matrices with more interferences as will be discussed in a subsequent section. Lower LODs may be achievable by first extracting the CNTs or decreasing the bias from other forms of organic carbon.

Quantification of CNTs (and other carbon nanomaterials) via isotopic labelling generally has fewer interferences than the other techniques, but obtaining isotopically enriched CNTs is typically challenging and/or expensive. Furthermore, this approach is only relevant for laboratory studies, not for detecting CNTs released into the environment. A related strategy, labeling CNTs with coatings containing a radioisotope, was used in many early biodistribution studies in the biomedical field, but has not been used in environmental or ecotoxicological studies. The challenge with this approach is that the accuracy of any measurement is contingent upon the radioactive tracer remaining associated with the CNTs.
Natural abundance, stable isotopic measurements (e.g., carbon-13) face similar limitations in that they require a CNT-free sample to which one can compare the isotopic composition in order to deploy the technique quantitatively. In laboratory studies, this is possible and more economically viable than radiolabeling techniques, but one has to carefully select CNT-free controls for quantifying CNTs in environmental samples. Furthermore, while the initial label is more expensive, the analytical techniques required to trace a carbon-14 label (i.e., liquid scintillation counting) are facile compared to the expert preparatory and analytical equipment required to trace natural-abundance isotopes (i.e., much lower levels of either carbon-14 or carbon-13 require accelerator mass spectrometers and isotope ratio mass spectrometers, respectively, and each with closed-tube-combustion preparation upstream). Nevertheless, the carbon source for SWCNTs produced using the high pressure carbon monoxide (HiPco) process is usually biomethane, which has a strong naturally depleted carbon-13 signature, and such CNTs would be good candidates for using natural abundance, stable isotopic measurements.

Evaluation of potential bias from changes to the CNTs

In addition to interferences from different environmentally and biologically relevant matrices, changes that may occur to CNTs while in these matrices can also cause interferences for many of the quantification techniques (Table S2). The extent to which agglomeration, degradation, and wrapping by other molecules occurs depends on the physicochemical properties of the CNTs and of the matrix. It is well known that CNTs will agglomerate in waters with sufficient ionic strength if they are not stabilized through, for example, a surfactant and that CNTs have a large capacity to adsorb natural organic matter. With regards to CNT agglomeration, while most techniques are sensitive to this change (e.g., most thermal techniques, Raman, NIRF, UV/vis/NIR
absorbance, and spICP-MS), some are not impacted by it (e.g., inorganic element analysis) or may even be enhanced (e.g., hyperspectral imaging). Potential interference from CNT agglomeration may result in, for example: a) changes to the intensity or peak wavelengths in the spectrophotometry signals; b) shifts in the thermal stability of the CNTs, which could prevent separation from other components in the matrix, such as black carbon soot; or c) hindering uniform distribution on a filter prior to analysis by TOT. Agglomeration may also increase the heterogeneity and affect representativeness of the subsamples in a matrix, which could lead to increased uncertainty. However, larger subsamples could help lower the uncertainty when feasible.

The literature shows variable results on the degradation of CNTs in environmental matrices. In some studies, degradation of carbon-14 labeled CNTs by enzymes or bacteria has been shown to be slow or not detectable except under specific situations with a special microbial consortium. In contrast, studies assessing the degradation of non-carbon-14 labeled CNTs have often shown substantial degradation. The cause of this discrepancy is unclear. Studies on the photodegradation of CNTs have shown significant modifications to their surface structure or the loss of fluorescence under some experimental conditions. Thus, it is reasonable to assume that some degree of degradation could occur with CNTs in surface waters if they stay suspended for a sufficiently long period. Almost all quantification techniques are sensitive to CNT degradation and oxidation, although the degree of oxidation needed before it impacts quantification varies among techniques. One exception is carbon-14 analysis, which is not impacted by oxidation. In contrast, the degree of oxidation can directly impact CNT thermal properties and potentially the capacity to differentiate between CNTs and other forms of carbon present in the matrix using many of the thermal based techniques.
Wrapping of organic molecules around CNTs, such as proteins or NOM, may also impact most quantification techniques. Many of the potential changes that could cause biases, such as decreased signal intensity of a spectroscopic measurement or a change in the thermal stability of CNTs for thermal measurements, are similar to those discussed for degradation. However, the reason behind these changes is from the impact of the coating on the CNT properties rather than a change to the core CNT material itself as would occur during degradation. One challenge in discussing the potential bias from organic molecules wrapping around CNTs, and also agglomeration and oxidation/degradation, is that the magnitude of the bias relates partly to the degree of agglomeration, oxidation, and the quantity of organic molecules associated with the CNTs. It is possible to foresee examples when these changes in the environmental matrices could have a bias, but it is challenging to quantify the magnitude of the expected bias without information about the sample system (e.g., aqueous phase NOM concentrations can range between 5 mg/L and 50 mg/L) or the extent of oxidation. This information about the sample system or magnitude of likely changes could allow one to account for biases.

Being aware of the potential biases present in a sample from these changes to the CNTs and/or carrier matrix will support researchers in determining to what extent these factors may impact their measurements. However, it might be challenging to get this kind of information from samples with low CNT concentrations when there is a low signal to noise ratio. Environmentally-relevant information on the rate of CNT modifications (e.g., oxidation) by environmental processes is limited, and systematic studies of those processes would be an enormous benefit to parallel efforts to quantify CNTs in the environment. While leaching of metal catalysts from the CNTs in environmental matrices is not explicitly covered in the above changes to the CNTs,
it could dramatically impact analyses using spICP-MS or elemental analysis. The potential for changes in the catalyst particles associated with the CNTs in environmental matrices is the primary reason that these techniques are not more broadly used despite their low LODs.

Detection limits of quantification techniques

The LOD for CNT quantification is one of the most critical performance metrics required to compare the various techniques. However, the definition of the LOD depends partly on how the CNT mass in a given sample is determined. The most common approach is for the whole sample, including CNTs, catalyst particles, and any carbonaceous impurities, to be included in the CNT mass used. It is possible instead to only use the CNTs themselves, at least for SWCNTs where, after purification procedures, the properties are more clearly distinguishable and high quality separation techniques exist. While additional metrics such as number or surface area concentrations are highly desired, the LOD values provided here are for mass concentrations.

There are two different approaches for determining the necessary LOD for quantifying contaminants in the environment. The first requires that the LOD is adequate for quantification of the contaminant at concentrations that may have harmful effects. An alternative requirement is for the analytical techniques to quantify the contaminant at the concentration that it is determined or estimated to be present in the environment. We have compared the LODs for the various analytical techniques using both approaches through comparing the LODs to a species sensitivity distribution for CNT acute toxicity to pelagic organisms (Figure 2) and to modeled environmental concentrations (Figure 3). Several trends are evident from reviewing these figures. First, the LODs in water span several orders of magnitude with some techniques only
capable of quantifying CNTs in samples with concentrations greater than 10 mg/L (e.g., gravimetric measurements), while the most sensitive techniques can detect concentrations between 0.1 µg/L and 1 µg/L (e.g., spICP-MS) (Figure 3). Second, the lowest LOD values are for pristine water samples and increase with higher amounts of potential interferences in the matrix. Higher LODs are observed when NOM is present in waters, and even higher LODs are typically achieved when using CNT quantification techniques in soils, sediments, and biological tissues. Third, multiple techniques appear capable of quantifying CNTs at concentrations relevant for stock suspensions (e.g., 10 mg L\(^{-1}\) to 100 mg L\(^{-1}\)) that could be used for pelagic aquatic toxicity testing (Figure 2). As discussed in more depth in a case study, some techniques could also be used to quantify the initial exposure concentration for ecotoxicity testing and the concentration after the experiment concludes. Fourth, the LODs are often orders of magnitude higher than the average modeled environmental concentration, but some are within the range of modeled sediment concentrations despite the lower LODs for CNT quantification in sediments. This suggests that it may be feasible to quantify CNTs in the environment under certain conditions. Overall, these figures can be used to assess which methods may offer suitable techniques for an intended purpose, as is described in more detail in the case studies. Alternatively, extraction or separation techniques (see above) may be necessary to selectively isolate and concentrate the CNTs prior to analysis.

**Potential for standardization**

There are numerous reference materials (RM; e.g., UV/vis spectroscopy calibration standards) and standard methods that can support the standardization of CNT quantification techniques (Table S3). In addition, there are multiple CNT RMs and representative test materials (Table S4); RMs have assigned values for certain properties, whereas representative test materials are only guaranteed to be stable and...
homogeneous with respect to one or more specified properties but may be used in the
development of test methods which assess properties other than those for which
stability and homogeneity have been shown.\textsuperscript{111} Currently, three RMs are available for
SWCNTs, while MWCNTs are only available as representative test materials. The
careful characterization of the CNT RMs may be useful for the standardization of
numerous techniques, given the wide range of properties that have been certified (i.e.,
the sources of uncertainty are thoroughly understood and the certified values have
meaningful metrological traceability) or for which information values are provided
(i.e., the sources of uncertainty are not fully understood or a limited number of analyses
were performed). Standardized methods are also already available for characterization
of CNTs (e.g., Raman spectroscopy and NIR fluorescence characterization) which
could be modified to develop standard methods for CNT quantitation.\textsuperscript{42,112-118} In
addition, a modified version of a NIOSH standard method for use of TOT for elemental
carbon analysis (NIOSH Method 5040) could potentially be used for CNT
quantification. However, the robustness of this method for CNTs will still need to be
evaluated for different matrices. Extraction and separation procedures also need to be
standardized but are not addressed in this section due to the limited number of studies
on this topic. Research topics that would support the standardization of these techniques
are described in the Future Research Topics section.

**Case studies**

In this section, five case studies will be used to illustrate how the quantitative
methods described in this manuscript could be utilized to address hypothetical
situations requiring CNT quantitation. The scenario for the first two case studies is that
scientists are asked to determine whether the concentration of CNTs in a stream
receiving effluent from a treatment plant where CNTs may be released is above 500 μg
L⁻¹; this concentration was chosen because it is approximately 50 % of the lowest LC₅₀ value of the species sensitivity distribution shown in Figure 2. This scenario will be discussed in the context of whether the CNT characteristics (e.g., SWCNT or MWCNT, catalyst materials, and thermal properties) are known a priori or not. In the third case study, scientists will be trying to measure the exposure concentration to organisms during a laboratory ecotoxicity experiment in a water only system with an organism that has an EC₅₀ value (the concentration at which 50 percent of the organisms are affected) of 10 mg L⁻¹ and the lowest concentration tested is 1 mg L⁻¹. In the fourth case study, CNTs with known characteristics are accidentally released into a lake, and scientists are asked to determine the concentration in the lake sediment. In the fifth case study, “OECD Test 305: Bioaccumulation in fish: aqueous and dietary exposure” is performed using a known type of CNTs and the scientists need to quantify the concentration in the fish tissues.

Case I: CNTs with known characteristics are released into a river

First, identify the techniques that may have LODs better than 500 μg L⁻¹ using Figure 3: UV/vis spectroscopy, inorganic elemental analysis, spICP-MS, NIRF, Raman spectroscopy, TOT, and carbon-14 labeling. Electron microscopy should, in principle, be able to detect CNTs at these concentrations, but it may be challenging to identify CNTs amidst the other particulate matter, and quantification will be challenging as discussed above. Of particle risk is the ability to collect a representative sample where the TEM thin section actually contains a statistically significant number of CNTs. Nevertheless, electron microscopy could be used for a qualitative assessment or to confirm the presence/absence of CNTs based on results from the quantitative analysis. Among the quantitative techniques, the choice of which technique to employ first would depend on numerous factors such as their availability and if the unique properties of the
CNTs of interest may eliminate some of the analytical techniques from consideration (e.g., quality assurance (QA), techniques only applicable for SWCNTs would not be relevant for MWCNT quantification). For example, carbon-14 labeling would not be relevant for field measurements, while NIRF would only be applicable for SWCNTs.\textsuperscript{15}

In addition, Raman spectroscopy analysis would require preconcentration of the sample to yield the desired LOD which may be challenging.\textsuperscript{13} Next, the properties of the river water prior to the discharge location (e.g., thermal profile, elemental composition and organic matter concentration of the water) could be evaluated to assess what biases may be encountered during CNT quantification for various techniques. If it is possible to obtain the CNTs of interest, a next step would be to prepare a CNT dispersion, mix the dispersion with stream water prior to the location of discharge, and then analyze the water using the quantification technique(s) to determine relevant QA/quality control (QC) characteristics such as the LOD, reproducibility, bias, signal to noise ratio, and linearity of calibration curve. It may also be important to test the stability of the CNT in the water prior to the discharge location to assess if agglomeration or oxidation of the CNT could cause a bias; if agglomeration causes a significant bias, it may be possible to disperse the samples such as by adding a surfactant or sonicating the sample. If the QA/QC characteristics are sufficient to provide the needed level of statistical significance for the quantification measurement, the final step would be to analyze the test samples.

**Case II: CNTs with unknown characteristics are released into a river**

The process is substantially more complicated if characteristics of the CNT to be detected are unknown. First, it would be helpful to obtain water samples before and after the point source discharge location. It would then be possible to do some measurements to try to determine if characteristics of the river water reflective of CNT
characteristics are changed. For example, an elemental analysis or spICP-MS analysis of the river waters could be conducted to assess if uncommon elements (e.g., yttrium) or ratios of elements (e.g., cobalt to molybdenum) often used for CNT catalysts are present at different concentrations before and after the location of discharge; measuring these samples before and after filtering could reveal if the metals are associated with particles such as CNTs. One distinct advantage of the metal analysis techniques is that the LODs for many of these elements are orders of magnitude better than the limit of detection needed for the CNTs (Figure 3). This information supported by other characterization techniques (e.g., TEM analysis to assess if SWCNTs or MWCNTs can be identified) could help determine the type of CNT being used. An alternate first step would be to obtain a sample directly at the discharge location and conduct these analyses. The advantage of this approach is that there would not be dilution of the CNTs, but the matrix may be substantially more complex (e.g., wastewater treatment plant effluent). A next step is to spike known concentrations of the specific CNT if identified, or alternatively RM SWCNTs and representative test material MWCNTs, into the river water prior to the discharge location and determine the QA/QC characteristics for the selected techniques and the extent to which agglomeration or oxidation could influence the results. If acceptable results can be obtained with the specific CNT (if identified) or the RM CNTs, then analysis can be conducted on the river sample after the location of discharge.

Case III: Laboratory Ecotoxicity Study

The third case study involves a laboratory ecotoxicity experiment during which the concentration remaining suspended during the experiment needs to be quantified. Depending upon what organism is tested, there may be interferences such as algae or bacteria which remain suspended and have CNTs associated with them. If it is
straightforward to separate the test organisms from the media with suspended CNTs, numerous techniques may be applicable for quantifying the initial CNT concentration in suspension (≥ 1 mg L⁻¹) (see Figure 2). The techniques available to determine the change in concentration during the experiment depend on the LOD needed for these measurements. For example, if it is unlikely that the CNT will settle during the experiment, numerous techniques would enable measurements to show that the concentration remained within 20% of the initial concentration, the desired maximum concentration loss indicated in many OECD tests. However, if substantial settling occurs, it is necessary to determine the lowest detection limit needed (e.g., 0.1 mg L⁻¹ to quantify a loss in concentration of 90% of the initial concentration). When measuring the CNT concentration dispersed in tests with suspended unicellular organisms or small multicellular organisms (e.g., *Tetrahymena thermophila*), the cells themselves may cause biases or require the extraction of the CNTs. It is also unclear if CNTs that are suspended but associated with cells should be counted as part of the total suspended concentration. Nevertheless, many techniques could likely still be used to quantify the total suspended concentration but control experiments to test for potential biases from the cells and the matrix would need to be conducted prior to starting the experiment.

**Case study IV: Quantification of CNTs with known characteristics in lake sediment**

Quantifying CNTs in sediments is substantially more difficult than in water samples. As shown in Figure 3, the LODs for most techniques are at least an order of magnitude higher in soils and sediments compared to in waters. To quantify CNTs in sediments, a first step would be to obtain “clean” sediment from another water body ideally with similar sediment characteristics. Because the CNT type is known in this case study, it is possible to spike this clean sediment with CNTs and then assess the quality of the analytical results (*e.g.*, linearity, LOD, etc.). The suitable techniques for
this analysis will depend upon instrument availability, the type of CNT (e.g., NIRF after
CNT extraction has been shown to be a valuable technique for analysis of SWCNTs in
sediments\textsuperscript{15} but is not applicable to MWCNTs), and the estimated range of probable
CNT concentrations in the sediment. If satisfactory LODs are not available for the
available techniques in the reference sediment, it may be necessary to investigate
extraction or separation methods to decrease the LOD (e.g., \textsuperscript{15,46}). Given the low
detection limits obtained using NIRF after extraction (62 µg/kg)\textsuperscript{15} challenges with
obtaining a better LOD are likely only to be problematic for MWCNTs unless the
SWCNTs are oxidized or modified to the extent that NIRF is not applicable or NIRF is
not available for sample analysis.

\textit{Case study V: Quantification of CNT in fish after a standard toxicity test}

Assessing potential bioaccumulation of chemicals in organisms is an important
component of risk assessment of chemicals. One frequently used test is OECD method
305: Bioaccumulation in fish: aqueous and dietary exposure\textsuperscript{119}. Again, the LODs for
quantifying CNTs in organism tissues are greater than those in water, yet similar to the
LODs for soils and sediments (Figure 3). While the whole fish is usually analyzed in
this method, it may be beneficial to test the CNT biodistribution in addition to the total
concentration in the fish. This is important because CNT translocation across the gut
tract is rarely observed in ecotoxicological studies\textsuperscript{27,31,120,121}. If the biodistribution of
SWCNTs is evaluated, then the technique with the best LOD is NIRF microscopy
which has been reported to detect individual SWCNTs\textsuperscript{31,121}. If this instrument is not
available, Raman microscopy and electron microscopy can be used to assess
biodistribution of CNTs in organisms although it is important to carefully avoid
artifacts\textsuperscript{27,43,122,123}. However, one should note that G/D ratios are strongly influenced by
any $sp^2$ or $sp^3$ hybridized carbons present in the organism for Raman microscopy
analysis. Other microscopic approaches such as photothermal/photoacoustic imaging have also been successfully used to assess the distribution of CNTs in plants, yet are infrequently available (Figure 1).\(^{24}\) To quantify the total concentration of CNTs in the fish, it is possible to use NIRF microscopy for SWCNTs,\(^{31}\) but extraction from the fish tissue will likely be needed for MWCNTs. An extraction procedure has been published for MWCNTs in rat lungs followed by quantification using TOT,\(^{23}\) but this approach has not yet been used in tandem with other quantification techniques or with fish tissues.

If carbon-14 labeled CNTs are available, assessing uptake by and biodistribution in fish through carbon-14 labeling is a viable approach.\(^{124}\) The microwave method has also shown promise for detecting MWCNTs in biological samples (e.g., earthworms) but requires custom built equipment.\(^{62,125}\)

**Future Research Topics**

The analysis that we present here on the current state of the science with regards to quantification of CNTs in matrices relevant for nanotechnology environmental health and safety measurements also reveals several key future research topics to move this field forward. First, most of the quantification techniques developed for aqueous environments will have potential biases or a higher LOD in complex matrices such as soils and biological tissues. Thus, the continued development of CNT extraction and separation procedures for environmental and biological matrices is a critical topic for additional research. Nevertheless, addressing the quality of the CNT separation depends in part on the robustness and precision of the subsequent analytical techniques, which also need to be improved. Second, sensitivity analyses of techniques can provide relevant information regarding the robustness of an experimental procedure to minor changes to a protocol and the contributions of various steps to the total uncertainty of the result. This approach and related approaches such as cause-and-effect analysis can
highlight which steps of a protocol need to be carefully followed to ensure a reliable result and which steps are less critical.\textsuperscript{126} Third, interlaboratory comparisons, where multiple laboratories use the same protocol, are needed to standardize the more mature techniques and extraction and separation procedures. While it is necessary to assess many topics related to analytical precision of a single laboratory (e.g., within and between operator variability, instrument to instrument variability, day-to-day variability, all contributing to the within-laboratory repeatability), interlaboratory comparisons can provide unique information about the comparability of results among laboratories (i.e., between-laboratory reproducibility) and potential factors in the protocols that need to be controlled to standardize the procedure. Such information is needed to provide estimates of the bias and precision of an analytical method. Fourth, analyzing an individual or set of homogenized test samples using multiple techniques will be helpful in highlighting method specific biases and the comparability of results among methods (e.g., similarly to a black carbon quantification ring trial\textsuperscript{127}). This differs from interlaboratory comparisons in that a single sample is analyzed by multiple techniques, as opposed to different laboratories using the same technique and test method. Similar results among orthogonal techniques would lead to greater confidence in the results of the methods while different results could yield insights into biases, strengths, and limitations of different methods. For example, in a recent study on the fate of SWCNTs in a mesocosm, an experimental setup designed to simulate the natural environment that often includes multiple species and which has been used in several nanotoxicity studies,\textsuperscript{128,129} both NIRF and elemental analysis were used on the same samples.\textsuperscript{29} The agreement among these methods suggested that elemental analysis may be a useful approach in these complex matrices if the catalysts used to synthesize the CNTs are of an element with low concentrations in the matrix (e.g., Mo).\textsuperscript{29} A similar
approach could be used to compare among different extraction or separation techniques with a single sample. Fifth, isotopically enriched or depleted CNTs\textsuperscript{21,78} could be used to help develop other orthogonal techniques given that isotopic techniques often have the fewest biases for many of the matrices and changes that could occur to the CNTs in these matrices. Such an approach was used by Schierz et al. to develop the NIRF technique for quantification of SWCNTs in sediments after extraction by also testing the extraction procedure with carbon-14 labeled SWCNTs.\textsuperscript{15} Sixth, using extraction and/or separation techniques in combination such as AF4 followed by capillary electrophoresis could be another promising avenue for future research. Lastly, almost all quantitative techniques require known CNTs to yield information about their characteristic information (e.g., thermal profile, metal catalyst, impurities, NIR spectra, and Raman signature). Additional work is needed to develop techniques for quantification of unknown CNTs in an environmental or biological matrix. Along these lines, the impact of CNT heterogeneities (e.g., different lengths) on their quantification could also be helpful.

**Acknowledgements**

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Supporting Information

Tables describing potential matrix interferences and interferences from changes to the CNTs on selected CNT quantification techniques, standard reference material carbon nanotubes, and standards and references materials related to standardization of carbon nanotube quantification techniques. This information is available free of charge via the Internet at http://pubs.acs.org.

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1192 Monitoring of carboxylic carbon nanotubes in surface water by using


<table>
<thead>
<tr>
<th>Technique</th>
<th>Overview</th>
<th>Strengths</th>
<th>Limitations</th>
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<tbody>
<tr>
<td>Asymmetric Flow Field-Flow Fractionation</td>
<td>Flow-assisted separation technique based on particle diffusion against a hydrodynamic field in the absence of a stationary phase</td>
<td>Enables separation of well dispersed CNTs by length reduces sample polydispersity possibility for online/offline coupling with a variety of analytical techniques can yield complementary information Potential separation of bulk samples of CNTs according to the charge/size ratio length sorting and separation according to bundled/non-bundled can occur high theoretical plate number thus potentially superior resolution power due to the plug-like flow of the electroosmotic flow Potential isolation of CNTs from matrix, either in sediment or supernatant</td>
<td>Time-consuming and laborious separation/method development high sample dilution during the analysis possibility of strong particle-membrane interactions may result in low recoveries separation less efficient low number of theoretical plates than with e.g., capillary electrophoresis Laborious sample preparation for controlled experiments several important challenges still remain including limited sensitivity, non-quantitative recoveries, and reproducibility problems micellar electrokinetic chromatographic cannot be used as CNTs are too large to reside in the intramicellar region Protocol will depend on CNT and matrix further separation of the fraction is challenging without disturbing neighboring fractions Low processing quantity kinetic and transport non-idealities can occur different aggregation states have different buoyant densities It has mainly been used for short single-walled carbon nanotubes; it is unclear if this technique can separate larger SWCNTs or MWCNTs; prefiltration might be needed agglomerates can get trapped within the micellar electrokinetic chromatographic column or the prefiltre; well dispersed suspensions are required only for qualitative analysis; no environmental samples have been tested Different approaches will likely need to be developed for each type of matrix e.g., tissue vs sediment and may need to be developed for different types of tissues Mainly used for CNT suspensions with very little interferences and at low concentrations to avoid clogging the filters it is difficult to regenerate the CNT suspension for further characterization/quantification there might be sample losses and filter interferences Not very reliable in the presence of interfering material or when the oxidation is not complete e.g., coals, very rich organic carbon environments; recoveries might vary between different types of CNTs</td>
</tr>
<tr>
<td>Capillary Electrophoresis</td>
<td>Based on the different electrophoretic mobilities of the species (on the basis of their charge/size ratio) through an electrolyte contained in a fused silica capillary when an electrical field is applied a suspension of CNTs usually in a surfactant is subjected to an electric current</td>
<td>Can enable extraction of specifically modified subpopulations resolves aggregate states</td>
<td></td>
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<tr>
<td>Centrifugation</td>
<td>Large suspended particles are removed first on basis of difference in sedimentation velocities</td>
<td>Can enable extraction of specifically modified subpopulations resolves aggregate states</td>
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<tr>
<td>Density gradient centrifugation</td>
<td>Particles will equilibrate to their isopicynal (equal buoyancy point) in a density gradient at sufficiently high applied acceleration</td>
<td>Can enable extraction of specifically modified subpopulations resolves aggregate states</td>
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<tr>
<td>Size exclusion chromatography</td>
<td>A chromatographic method that separates analytes based on their size and shape by differential exclusion from the pores of the stationary phase no interactions must exist between CNTs and the stationary phase</td>
<td>Relatively simple and inexpensive good size separation for SWCNTs within a certain length limit and shape</td>
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<tr>
<td>Matrix Digestion</td>
<td>Different chemicals or solutions are used to dissolve the matrix e.g., tissues to facilitate subsequent analytical techniques</td>
<td>Lowers detection limits and removes potential biases for many techniques</td>
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<tr>
<td>Micro-nanofiltration</td>
<td>Use of micro and nanopore-sized filters to separate analytes based on their size</td>
<td>Very simple and inexpensive at low CNT concentrations can treat larger volumes than other techniques</td>
<td></td>
</tr>
<tr>
<td>Selective Oxidation</td>
<td>Use of thermal or chemical oxidation to separate more refractory carbon fractions (CNTs) from more labile organic carbon</td>
<td>Allows for a cleaner (and easier) subsequent characterization or quantification</td>
<td></td>
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<tr>
<td>Technique</td>
<td>Description</td>
<td>Example</td>
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<tr>
<td>Sonication with surfactant</td>
<td>Use of a surfactant to create a stable CNT suspension that can then be separated from the remaining non-CNT material that settles down at a different speed</td>
<td>Can extract CNTs with varying surface chemistry from sediment, no special equipment is necessary</td>
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<td></td>
<td>Recovery varies among SWCNTs with no recognizable pattern; repeatability varies, surfactants may interfere with quantitation procedure</td>
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**Table 1: Extraction and separation techniques to isolate CNTs from environmental and biological matrices**
<table>
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<tr>
<th>Method</th>
<th>Overview</th>
<th>Strengths</th>
<th>Limitations</th>
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<tr>
<td><strong>Spectroscopic</strong></td>
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<tr>
<td>Absorbance&lt;sup&gt;43,109,150,151&lt;/sup&gt;</td>
<td>Measures absorbance of aqueous sample; can include ultraviolet, visible, or near infrared wavelengths</td>
<td>Readily available in many environmental laboratories</td>
<td>Interference from other sample components, relatively high detection limit, only applicable for aqueous samples</td>
</tr>
<tr>
<td>Near infrared fluorescence (NIRF) &lt;sup&gt;12,24,31,162&lt;/sup&gt;</td>
<td>A specific emission spectra can be used as an identification tool of SWCNTs; the intensity of the fluorescence signal can be used for quantification of SWCNTs</td>
<td>Quantification/Detection at very low limits of detection</td>
<td>Limited to non-functionalized SWCNTs; semi-conducting SWCNTs but not metallic SWCNTs can be detected</td>
</tr>
<tr>
<td>Raman&lt;sup&gt;13,122,123,153-164&lt;/sup&gt;</td>
<td>Measures radial breathing (SWCNT), G, D and G’ vibrational bands in dry and various solvent suspended samples, tissues</td>
<td>Minimal sample preparation, enables CNT characterization, compatible with in vitro and in vivo samples, can be used with a microscope, low detection limits achieved using resonance Raman conditions</td>
<td>Some matrices may produce interferences, sensitive to laser power, requires calibration for quantitative analysis</td>
</tr>
<tr>
<td><strong>Inorganic Element Analysis&lt;sup&gt;26,32&lt;/sup&gt;</strong></td>
<td>Measures trace catalytic metallic elemental impurities intercalated in the CNT structure (Cr, Co, Cu, Fe, Mo, Ni, Y, Zn), analysis of bulk metal content; the applicability of this approach could be impacted by removal of the metal catalysts by purification but catalysts located within the CNTs often remain after purification processes</td>
<td>Multi-elemental capability and extreme sensitivity of ICP-MS allow an accurate and selective determination of metal impurities of CNT in a wide range of matrices at ngL&lt;sup&gt;-1&lt;/sup&gt; or sub ngL&lt;sup&gt;-1&lt;/sup&gt; levels, the rapid sample throughput of this method is attractive for routine screening</td>
<td>Carbon is generally not detectable with standard ICP-MS methods, quantitative sample dissolution is required prior to analysis; incomplete sample digestion, release of metal ions from the CNTs in the sample matrix, or elemental contamination from the sample digestion steps could lead to an important bias in the bulk metal content determination; the feasibility of using this technique could depend partly on if the metal contents of the CNTs are known a priori</td>
</tr>
<tr>
<td>Single particle inductively coupled plasma-mass spectrometry (spICP-MS)&lt;sup&gt;27&lt;/sup&gt;</td>
<td>Metal catalyst impurities are used as proxies to detect and quantify CNTs; the applicability of this approach could be impacted by removal of the metal catalysts by purification but catalysts located within the CNTs often remain after purification processes</td>
<td>Potential capability for the size, size distribution, and particle number concentration determination of CNT; high selectivity to differentiate CNT at extremely low metal contents from naturally occurring carbon-containing species (i.e. cells, organic detritus, humic acid); very low detection limit</td>
<td>Size/length estimation requires the invalid assumption that metal content is homogeneous among the CNTs, very small particles cannot be separated from the background, leaching of catalysts in the sample matrix prior to spICP-MS analysis can bias the result, only applicable for aqueous samples; the feasibility of using this technique could depend partly on if the metal contents of the CNTs are known a priori</td>
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<tr>
<td><strong>Microscopic</strong></td>
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<tr>
<td>Atomic Force Microscopy&lt;sup&gt;109,165&lt;/sup&gt;</td>
<td>Measure the surface features of a sample by dragging a cantilever over the sample; the length of identifiable tubes can be determined by the movements of the cantilever</td>
<td>Most trusted technique for determining number and length</td>
<td>Deposition bias, measurement bias, and detection errors are all possible in most samples</td>
</tr>
<tr>
<td>Hyperspectral Imaging&lt;sup&gt;166,167&lt;/sup&gt;</td>
<td>Measures reflectance spectra of NPs in a darkfield (visual near infrared / short-wave infrared spectral range), resulting in 2D-optical images with full spectral information that contain a full spectrum (400 nm to 1000 nm or 900 nm to 1700 nm, respectively) in each pixel; CNTs appear bright against a dark background</td>
<td>Easy sample preparation, provides optical (i.e. differentiation between single nanotube and nanotube-agglomerate) and spectral information, allows spatial localization of particles, can provide semi-quantitative information, short-wave infrared spectral range could be applicable for detection of SWCNTs</td>
<td>Currently long analysis times, visual near infrared not specific for CNTs, many potential analysis artifacts</td>
</tr>
</tbody>
</table>
**Photoacoustic (PA)**\(^{14,108-170}\)
PA measures the acoustic response to the rapid volume change resulting from the absorption of an optical pump beam and the transfer of heat to the surrounding environment.

**Photothermal (PT)**\(^{14,108-169}\)
PT measures the optical scattering response of a probe beam to the change in local environment refractive index that results from the absorption of an optical pump beam and the transfer of heat to the surrounding environment.

**Scanning Electron Microscopy and Scanning Transmission Electron Microscopy**
Measures the interaction of a finely focused electron beam with the CNTs; secondary electrons, and transmitted electrons can be used for image formation.

**Transmission Electron Microscopy (TEM)**\(^{27,66}\)
Illuminates a selected sample area (parallel electron beam) and detects the transmitted electron after passing through the samples.

**CTO-375**\(^{18}\)
Quantification of carbon that remains after combustion at 375 °C for 24 h under excess air sample and subsequent chemical oxidation.

**Thermal Gravimetric Analysis (TGA)**\(^{20,171,172}\)
Quantification of mass percentage of phases with distinct thermal stabilities under a variety of reactive atmospheres (usually air) and relatively rapid temperature programs (e.g., heating rates of 5 °C/min to 20 °C/min; room temperature–ca. 950 °C); each sample takes 1 h to 2 h total.

**Thermal Gravimetric Analysis-Mass Spectrometry (TGA-MS)**\(^{14}\)
TGA coupled with mass spectrometric detection of evolved gas fragments, typically in the 2 to 300 m/z range.

**Photoacoustic (PA)**
Suitable for detection in liquids such as water and complex media such as plants, minimal sample preparation, can be quantifiable, excellent penetration depth enables samples > 100 µm, works equally well with metallic and semiconducting SWCNTs and MWCNTs, label free, unaffected by some complex media issues including carbon-on-carbon.

**Photothermal (PT)**
Suitable for detection in liquids such as water and complex media such as plants, minimal sample preparation, can be quantifiable, penetration depth can handle samples up to 10 µm, works equally well with metallic and semiconducting SWCNTs and MWCNTs, label free, unaffected by some complex media issues including carbon-on-carbon, sensitivity down to single particle sensitivity, lower LOD than absorbance-based measurements.

**Scanning Electron Microscopy and Scanning Transmission Electron Microscopy**
Provides detailed morphological properties (length, width, shape) of individual CNTs; individual CNTs can be localized in complex matrices based on morphological criteria.

**Transmission Electron Microscopy (TEM)**
Provides detailed morphological properties (length, width, shape) of individual CNTs; high resolution can be used to distinguish between SWCNTs and MWCNTs; CNTs can be identified in energy filtered TEM images.

**CTO-375**
Particularly good for complex matrices such as soil and sediment.

**Thermal Gravimetric Analysis (TGA)**
A rapid technique that allows the quantification of multiple phases in a single sample, good for complex matrices, no special sample preparation needed.

**Thermal Gravimetric Analysis-Mass Spectrometry (TGA-MS)**
Mass fragments can give insight into the chemical structure of the source material (e.g., C/H/O ratios or unique evolved fragments).

**Photoacoustic (PA)**
Signal is dependent on absorption and heat transfer to material surrounding the CNTs, can be 10x lower sensitivity than PT, medium surrounding CNTs must be transparent to the beams, heating laser must overlap with absorbance of the CNTs, signal scales with size of CNT cluster, non-transparent media may cause detection issues, quantification may require diameter and length distributions.

**Photothermal (PT)**
Same as Photoacoustic plus is limited to thin samples (< 100 µm).

**Scanning Electron Microscopy and Scanning Transmission Electron Microscopy**
Labor intensive, often only qualitative information.

**Transmission Electron Microscopy (TEM)**
Challenging sample preparation for tissues; it may be very hard to detect NPs in complex samples at low concentrations; low contrast (conventional TEM) due to reduced interactions between CNTs at the electron beam at high acceleration voltages.

**CTO-375**
Not fully tested for suspensions, requires high concentrations of CNTs and low concentrations of interferences (e.g., soot interfering with MWCNTs or graphene with SWCNTs).

**Thermal Gravimetric Analysis (TGA)**
Effect of thermal ramp rate and reactive atmospheres on apparent phase distribution is not well understood (and is largely ignored), detection limits are relatively high for solid matrices, potential for interferences between sample matrix (e.g., other carbon nanomaterials, soot, or black carbon) and CNT decomposition temperatures.

**Thermal Gravimetric Analysis-Mass Spectrometry (TGA-MS)**
Current mass spectrometers have poor mass resolution (ca. 1 amu), relatively high detection limits, and low sampling rates relative to the chamber flush rate (i.e., consequently, only a small portion of the evolved mass is transferred to the MS); all reduce identification accuracy and increase detection limit.
<table>
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<th>Total Organic Carbon (TOC) Analysis</th>
<th>TOC analysis can be conducted on water or soil samples by oxidizing (chemical, heated catalyst, UV) carbon to carbon monoxide or dioxide which is detected by infrared or other detectors. TOC analysis of waters has been used to measure CNTs in stock solutions in water.</th>
<th>Very little optimization of temperature or catalytic conditions have been examined; its application to CNT stock solutions have been consistent with prepared masses; any organics, such as natural organic matter, in solution or soils would interfere; this is a non-specific method and thus matrices that contain sufficiently high concentrations of other carbon nanomaterials (e.g., graphene), soot, or black carbons would impact the technique. Too much organic carbon in a sample causes peak overlapping between elemental and organic carbon which affects the accuracy; similar carbonaceous materials such as graphene and fullerene will be counted in the CNT peak if they exist in the sample; unless the peak from CNT is far enough from other carbonaceous material, it is difficult to exclude the other carbonaceous materials but adjusting the temperature program might help to some extent.</th>
</tr>
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<tr>
<td>Thermal Optical Transmittance (TOT)</td>
<td>As the sample is analyzed under programmed temperature, the volatilized and combusted carbon travels to an oxidizing oven, where it is transformed into carbon dioxide (CO₂); the amount of elemental carbon is determined based on the CH₄ signal measured using a flame ionization detector; sample is first heated under inert conditions to remove volatile organic carbon, then oxidizing carrier gas is used for elemental carbon; the portion of TC that is organic carbon or elemental carbon is defined by the method, which determines where the organic carbon-elemental carbon split is placed post-analysis; this split can be automatic on the basis of automatic optical correction; the optical transmittance or reflectance is observed throughout analysis, and the split is placed where the transmittance/reflectance returns to the initial reading; for samples in which optical correction does not work, a manual split defined by the analyst should be used.</td>
<td>Very reliable technique for detecting elemental carbon in environmental matrices, this technique could differentiate between types of CNTs based on their thermal stability.</td>
</tr>
<tr>
<td>Isotopic labeling</td>
<td>A measure of the ratio of $^{13}$C to $^{12}$C, applicable for all CNTs but works best for isotopically enriched or depleted CNTs. Instrumentation is readily available in many environmental laboratories.</td>
<td>Highly dependent on matrix and large variability may be observed for CNTs that are not specifically $^{13}$C enriched.</td>
</tr>
<tr>
<td>Carbon-13 Labelling</td>
<td>Measures beta emissions from carbon-14 emissions, can be used to quantify liquids after mixing with scintillation cocktail or any matrix after combustion in a biological oxidizer, autoradiography can provide spatial distribution of radioactivity. Provides definitive quantification of CNTs in complex matrices, can be used as an orthogonal technique to develop other analytical techniques, can be used to identify degradation products.</td>
<td>High cost to synthesize radioactively labeled CNTs, safety concerns, limited availability of radioactively labeled CNTs.</td>
</tr>
</tbody>
</table>
Other radioactive isotopes\(^{96-98}\) Measures release of emissions from a radioactive isotope that is associated (e.g., attached to a polymer wrapping the CNT) with the CNT. This approach can enable extremely low detection limits, can be used with a range of CNT surface functionalizations, non-destructive sample is possible for gamma emitters. Artifacts are possible if the radioactive isotope becomes separated from the CNT, it may be challenging or impossible to determine if this occurred in complex matrices without orthogonal CNT quantitation techniques.

Additional Techniques

**Analytical Ultracentrifugation (AUC)** \(^{60,165,182-184}\) Measurement of sedimentation velocity distribution, can be used to determine particle density or size/shape distribution. Can measure entire CNT population via absorbance or interference measurement, high resolution, little size bias. Finicky technique that requires well understood and controlled samples for robust analysis.

**Gravimetric**\(^{185}\) The CNT concentration in suspension is estimated by drying a fraction of the suspension and weighing it, or by determining the fraction of CNTs not suspended during the dispersion process (e.g., by sonication) by weighing the mass of CNT particles at the bottom of the container. Uses readily available equipment. Limited to high CNT concentrations, only applicable for aqueous suspensions.

**Microwave Method**\(^{62,125,186,187}\) Measures the temperature rise of a sample at a specific microwave energy within a specific timeframe. Straightforward method for CNT detection and quantification in biological tissue, low cost. Not commercially available; it still remains to be investigated for environmental samples if interferences arise from other carbon allotropes with similar behavior in the microwave field (e.g., carbon black, soot\(^{188}\)).

**aF4-MALS**\(^{46}\) Measures a shape factor \(\rho=radius of gyration/hydrodynamic radius\) of particles present in a complex liquid sample (e.g. surface water, leachate, soil and sediment extract), which is indicative of the particle aspect ratio; comparing these results to a CNT-free sample can then be used for CNT detection. Allows for CNT detection in water, soils, and sediments; may be useful in exposure studies. Need for the baseline of a CNT-free sample, full quantitative use currently not straightforward, often low CNT recoveries for aF4.

### Table 2: Selected techniques for CNT Quantitation

<table>
<thead>
<tr>
<th>Technique</th>
<th>Description</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC</td>
<td>Measurement of sedimentation velocity distribution, can be used to determine particle density or size/shape distribution.</td>
<td>Can measure entire CNT population via absorbance or interference measurement, high resolution, little size bias. Finicky technique that requires well understood and controlled samples for robust analysis.</td>
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<td>Uses readily available equipment. Limited to high CNT concentrations, only applicable for aqueous suspensions.</td>
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</tr>
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<td>Allows for CNT detection in water, soils, and sediments; may be useful in exposure studies. Need for the baseline of a CNT-free sample, full quantitative use currently not straightforward, often low CNT recoveries for aF4.</td>
</tr>
</tbody>
</table>

Abbreviations: Asymmetric flow field flow fractionation with multi-angle light scattering (aF4-MALS), analytical ultracentrifugation (AUC), carbon nanotube (CNT), chemothermal oxidation at 375 °C (CTO-375), inductively coupled plasma-mass spectrometry (ICP-MS), near infrared fluorescence (NIRF), multiwall carbon nanotube (MWCNT), photoacoustic (PA), photothermal (PT), single particle inductively coupled plasma-mass spectrometry (spICP-MS), single-wall carbon nanotube (SWCNT), transmission electron microscopy (TEM), thermal gravimetric analysis (TGA), thermal gravimetric analysis-mass spectrometry (TGA-MS), total organic carbon (TOC), thermal optical transmittance (TOT).
Figure 1: Availability of CNT quantification techniques.

Abbreviations: Asymmetric flow field flow fractionation with multi-angle light scattering (AF4-MALS), analytical ultracentrifugation (AUC), chemothermal oxidation at 375 °C (CTO-375), near infrared fluorescence (NIR), single particle inductively coupled plasma-mass spectrometry (spICP-MS), thermal gravimetric analysis (TGA), thermal gravimetric analysis-mass spectrometry (TGA-MS), total organic carbon (TOC), thermal optical transmittance (TOT), scanning electron microscopy (SEM), transmission electron microscopy (TEM), atomic force microscopy (AFM).
Figure 2: Comparison between detection limits for analytical techniques in a water-only media under optimal conditions juxtaposed with a species sensitivity distribution for CNTs for acute toxicity testing of pelagic organisms. For the species sensitivity distribution, the 95 % confidence for the LC$_{50}$ values is shown by the gray shaded area around the curve. The detection limits for the techniques span a range of one order of magnitude (e.g., 1 mg/L to 10 mg/L). This figure is modified with permission from Garner et al.$^{189}$

Abbreviations: Asymmetric flow field flow fractionation with multi-angle light scattering (aF4-MALS), analytical ultracentrifugation (AUC), chemothermal oxidation at 375 °C (CTO-375), near infrared fluorescence (NIRF), single particle inductively coupled plasma-mass spectrometry (spICP-MS), thermal gravimetric analysis (TGA), thermal gravimetric analysis-mass spectrometry (TGA-MS), total organic carbon (TOC), thermal optical transmittance (TOT).
Figure 3: Detection limits for analytical techniques in various media under optimal conditions and modeled environmental concentrations (1\(^{84}\), 2\(^{85}\), 3\(^{86}\), 4\(^{70}\), 5\(^{19}\)), modeled environmental concentrations are not available for biological matrices. The detection limits for individual techniques span a range of one order of magnitude (e.g., 1 mg/L to 10 mg/L).

Abbreviations: Asymmetric flow field flow fractionation with multi-angle light scattering (aF4-MALS), analytical ultracentrifugation (AUC), chemothermal oxidation at 375 °C (CTO-375), near infrared fluorescence (NIRF), single particle inductively coupled plasma-mass spectrometry (spICP-MS), thermalgravimetric analysis (TGA), thermalgravimetric analysis-mass spectrometry (TGA-MS), total organic carbon (TOC), thermal optical transmittance (TOT).

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<th>Environmental Samples</th>
<th>Biological tissues</th>
<th>Soil/Sediment</th>
<th>Water with NOM</th>
<th>Water without NOM</th>
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<tr>
<td>1</td>
<td>spICP-MS</td>
<td>NIF</td>
<td>Carbon-14</td>
<td>AUC</td>
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<td>2</td>
<td>Inorganic elemental</td>
<td></td>
<td>Carbon-14</td>
<td>TGA</td>
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<tr>
<td>3</td>
<td>Organic elemental</td>
<td></td>
<td>TOT</td>
<td>TGA-MS</td>
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<td></td>
<td>aF4-MALS</td>
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<td>W+4-AMS</td>
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<td></td>
<td>AUC</td>
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<td>MAAMS</td>
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<td></td>
<td>CTO-375</td>
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<td>TGA</td>
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<td>TGA-MS</td>
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</tbody>
</table>

Concentration (mg/L or mg/kg)
Supporting Information
Quantification of carbon nanotubes in environmental matrices: Current capabilities, case studies, and future prospects

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<td>65,709,150,151</td>
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<td>Near infrared</td>
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<td>Raman</td>
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<td>153-164</td>
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<tr>
<td>Humic acid, other Raman active organic contaminants, and suspended particles (e.g., clays) could impact the detection method as could background fluorescence</td>
<td>This matrix may have background fluorescence</td>
<td>This matrix may have auto-fluorescence and may limit light penetration</td>
<td>Light scattering by large particulate material, may require separation prior to Raman analysis</td>
<td>Presence of the aromatic compounds at high concentration could influence the signal as could fluorescence</td>
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<td>Spectrometric</td>
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<td>Inorganic Elemental Analysis</td>
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<td>Single particle</td>
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<td>inductively coupled</td>
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<td>plasma–mass</td>
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<tr>
<td>spectrometry (spICP-MS)</td>
<td>22</td>
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<tr>
<td>Difficult to distinguish CNTs from other particulates containing the same metals, this is most likely for soils/sediments; extraction may be needed first</td>
<td>For all matrices the presence of any other particulates depositing on the measurement substrate will require protocols for selective removal of all non-nanotube components; calculation of length distributions can be hindered by resolution issues (for short nanotubes) and observation bias (undercounting of long nanotubes) and/or the presence of aggregates</td>
<td>For all matrices, soot and other black particles could impact the detection of CNTs</td>
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<tr>
<td>Technique</td>
<td>Description</td>
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<tr>
<td><strong>Photoacoustic (PA) and photothermal (PT)</strong></td>
<td>Water is a good PA/PT solvent, anything else in the sample that absorbs or scatters the beam(s) would decrease signal or increase background. PA/PT works well in tissues transparent to beam(s); PT sensitivity drops in non-transparent tissue. No reports in literature; would be a difficult matrix to detect CNT with a lot of scattering and absorption of the beam(s). A polymer matrix does not inhibit CNT detection; as long as there is a thermal response in the matrix, PT/PA can detect a signal.</td>
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</tr>
<tr>
<td>Scanning Electron Microscopy, Scanning Transmission Electron Microscopy, Transmission Electron Microscopy (TEM)</td>
<td>Biopolymers, low concentration of CNTs compared to other particles leading to overlapping particles on the samples holder. Other fibrillar particles; low contrast between CNTs and biological tissue. Other fibrillar particles; low concentration of CNTs compared to other particles leading to overlapping particles on the samples holder.</td>
<td></td>
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</tr>
<tr>
<td><strong>Thermal CTO-375</strong></td>
<td>Very little interference in this matrix except for high N, organic carbon, or black carbon content waters. Matrices with high N or organic C content can char and form higher stability materials that, together with high BC concentrations, can interfere with the analysis of CNTs; conversely some matrices can produce “catalytic” effects that reduce the oxidation temperature of recalcitrant carbons. Sample specific oxidative strength (protective or catalytic) leading to variable recoveries of spiked CNTs; high organic C can char and high BC content can interfere with the CNT analysis. Some tested polymers (e.g., gamma-poly caprolactone) have lower thermal stability than CNTs which makes this a promising approach.</td>
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<tr>
<td>Thermal Gravimetric Analysis (TGA)</td>
<td>Very little interference expected except for waters with levels of BC that are approximately equal in concentration to the CNTs, NOM can stabilize CNTs. Isolated test materials show little interference, full matrix testing needed, peptone also binds to CNTs and may change oxidation temperature. Interferences are unclear, tested epoxies have overlapping thermal stabilities with CNTs, and seem to influence the...</td>
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</table>
challenge in sample size (typically > 10 mg) and overlap of oxidation temperatures may hinder detection of CNTs

**Thermal Gravimetric Analysis-Mass Spectrometry (TGA-MS)**

- Very little interference; interference with BC can be reduced by MS peak deconvolution
- Interferences with this matrix are unknown, but the unique chemical composition of cell media is promising for lower interferences
- Isolated test materials show little interference and the unique chemical composition of biological tissues suggests low interferences, major challenge in sample size and overlap of oxidation temperatures may hinder detection of CNTs
- Few direct interferences, but can raise background levels and raise the detection limit

The unique chemical compositions of most polymers suggests low interferences, evolved gases should be distinct for CNTs as compared to the polymer matrix

**Total Organic Carbon (TOC) Analysis**

- Interference exists from any organic matter (natural organic matter, soil organic matter, cellular material, serum or other organic compounds, organic polymers, etc.)

Interference exists from any organic matter (natural organic matter, soil organic matter, cellular material, serum or other organic compounds, organic polymers, etc.)
**Thermal Optical Transmittance (TOT)**

Typically little interference

Interferences should be minimal, but may arise if cell material chars into optically absorptive or thermally stable material

Interferences can be minimized by preparatory digestions (demonstrated for mouse lung)

Few direct interferences, unless the soil or sediment has a high non-CNT organic load

Potentially interfering, as many polymers will exhibit poor degradability under the inert atmosphere utilized in the first phase of this method; This could cause charring and confound the measurement of EC once the oxidative atmosphere is introduced

**Isotopic labeling**

<table>
<thead>
<tr>
<th><strong>Carbon-13 Labelling</strong></th>
<th>Very little interference expected</th>
<th>Very little interference expected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Separation of CNTs from tissues is advised as accumulation of CNTs may be tissue dependent; background δ¹³C signatures are necessary for each tissue type</td>
<td>Sulfates may interfere with the preparation of pure CO₂; CNT-free background required for comparative δ¹³C signatures</td>
</tr>
</tbody>
</table>

The potential biases depend on how the carbon-14 is quantified; some compounds may interfere with scintillation cocktails adsorbing beta emissions and could lead to autofluorescence; these issues would not be expected if the sample is combusted using biological oxidation

The potential biases depend on how the carbon-14 is quantified; some compounds may interfere with scintillation cocktails adsorbing beta emissions and could lead to autofluorescence; these issues would not be expected if the sample is combusted using biological oxidation

Interferences have not been observed in previous studies with biological oxidation of the samples; good recovery was also found when sonicating SWCNTs with sodium dodecyl sulfate and using

There may be interference from quenching if the sample is added to liquid scintillation cocktail, but interferences would not be expected for biological oxidation
unlikely with biological oxidation
liquid scintillation counting

Other radioactive isotopes

**Additional Techniques**

Analytical Ultracentrifugation (AUC)

Gravimetric

Microwave Method

aF4-MALS

This would depend to some extent on the radioactive isotope added and quantification used but generally interferences would not be expected for these different matrices; however, the stability of the radioactive tracer may be impacted by the dispersion process in the matrix or metabolic processes in tissues

Measurement of sedimentation requires homogenous dispersions with measureable viscosities and densities; significant light scattering from suspended particles from the matrix will additional likely complicate all but the most rigorous experimental protocols

Measurement of CNTs in these matrices would encounter significant biases depend on the mass of other compounds that would be deposited when drying samples except at very high CNT concentrations

Other carbon forms such as soot may cause interferences; this interference would be most likely for soils and sediments

No known interferences, but theoretically other low density fibre-like/high aspect ratio particles may interfere; if these particles exhibited lower thermal stability compared to the CNTs, oxidation could potentially be used to selectively remove them

**Table S1: Potential Matrix Interferences for Selected Techniques for CNT Quantitation**

Abbreviations: Asymmetric flow field flow fractionation with multi-angle light scattering (aF4-MALS), analytical ultracentrifugation (AUC), black carbon (BC), carbon nanotube (CNT), chemothermal oxidation at 375 °C (CTO-375), inductively coupled plasma-mass spectrometry (ICP-MS), near infrared fluorescence (NIRF), photoacoustic (PA), photothermal (PT), single particle inductively coupled plasma-mass spectrometry (spICP-MS), single-wall carbon nanotube (SWCNT), thermal gravimetric analysis (TGA), thermal gravimetric analysis-mass spectrometry (TGA-MS), total organic carbon (TOC), thermal optical transmittance (TOT).
<table>
<thead>
<tr>
<th>Method</th>
<th>Impact of CNT agglomeration</th>
<th>Impact of CNT Oxidation/Degradation</th>
<th>Wrapping with Organic Molecules (proteins, NOM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spectroscopic</td>
<td>Measured absorbance signal per unit mass typically decreases above a threshold level of nanotube aggregation, especially for the intrinsic nanotube optical transitions, although apparent absorbance in the UV and visible regions may broadly increase due to increased light scattering by larger particles; for intrinsic optical transitions, the transition wavelength will typically red shift and peak intensities will decrease with any reduction from individualized dispersion</td>
<td>Absorbance of intrinsic optical transitions typically decreases monotonically above very low levels</td>
<td>In the absence of changes in agglomeration state, the adsorption of material to the nanotube interface generally will affect the absorbance mostly through red/blue shifts in intrinsic optical transition wavelengths by modification of the local dielectric environment; changes to the surface accessibility of the bulk solvent can also affect optical transition intensities</td>
</tr>
<tr>
<td>Absorbance</td>
<td></td>
<td></td>
<td>Variable</td>
</tr>
<tr>
<td>Near infrared fluorescence (NIRF)</td>
<td>Peak shifts and intensity decrease for SWCNTs could occur for either of these changes</td>
<td></td>
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</tr>
<tr>
<td>Raman</td>
<td>Not a significant factor but G and D band ratio may be sensitive to sample agglomeration</td>
<td>Raman spectra are very sensitive to oxidation or degradation</td>
<td>Vibrational features are sensitive to structural stress which may be caused by wrapping with organic molecules or polymers</td>
</tr>
<tr>
<td>Absorbance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spectrometric</td>
<td>Minimum impact on elemental analysis when a complete sample digestion is performed</td>
<td>Any loss of metals intercalated in CNTs before the elemental analysis would lead to biased results</td>
<td>Minimum impact when the wrapping does not alter the elemental composition of CNT</td>
</tr>
<tr>
<td>Inorganic Elemental Analysis</td>
<td></td>
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</tr>
<tr>
<td>Raman</td>
<td>Severe undercounting effect on actual CNT concentrations since each agglomerate may only be counted as one single pulse depending on the dwell time</td>
<td>Important influence on sizing and counting results because of the increasing contribution of smaller CNTs containing metal masses below instrument detection limit</td>
<td>Wrapping would affect physical transport of the CNT in introduction system, increasing the uncertainty on the size and number concentration determination</td>
</tr>
<tr>
<td>Microscopic</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Atomic Force Microscopy</td>
<td>Agglomeration or oxidation/degradation may impact apparent size distribution and hinder analysis</td>
<td></td>
<td>Variable</td>
</tr>
</tbody>
</table>

S58
<table>
<thead>
<tr>
<th>Hyperspectral Imaging&lt;sup&gt;266,267&lt;/sup&gt;</th>
<th>Better optical visibility due to enhanced scattering from agglomerates</th>
<th>Potential changes in spectral profiles from oxidation/degradation or wrapping with organic molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photoacoustic (PA) and Photothermal (PT)&lt;sup&gt;24, 168-170&lt;/sup&gt;</td>
<td>Will follow the same changes that affect Absorbance; anything that changes the absorption of the CNTs would affect the PT/PA signal causing shifts in peak wavelength and changes in absorption cross-section; degradation would certainly affect the PT/PA signal; the effect of agglomeration, oxidation, and the addition of physisorbed or chemisorbed ligands would be case-by-case</td>
<td>Change in the size distribution, depending on the extent of degradation</td>
</tr>
<tr>
<td>Scanning Electron Microscopy, Scanning Transmission Electron Microscopy, and Transmission Electron Microscopy&lt;sup&gt;27,66&lt;/sup&gt;</td>
<td>CNTs will still be detected if investigated manually, but automated analysis may fail to identify CNT in agglomerates</td>
<td>May reduce the image resolution due to contamination effects (resulting from the volatilization and redeposition of the organic material under the electron beam)</td>
</tr>
</tbody>
</table>

**Thermal**

<table>
<thead>
<tr>
<th>CTO-375&lt;sup&gt;18&lt;/sup&gt;</th>
<th>CNT agglomeration will slightly increase thermal stability, but not to an extent discernable by CTO-375</th>
<th>Oxidation and degradation reduce CNT thermal stability, which would enhance separation from BC but require a different cut off temperature to quantify SWCNTs; MWCNTs will still be interfering with BC</th>
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<tbody>
<tr>
<td></td>
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<td>Organic coatings should be resolved (e.g., more labile than CNTs) by CTO-375 and not affect the measurement; however, proteins can char and cause interference</td>
</tr>
</tbody>
</table>
Thermal Gravimetric Analysis (TGA)  
20,171,172

- CNT thermal stability will increase measurably; SWCNTs may no longer be resolved from BC or soot; MWCNTs should still have higher thermal stability than BC; higher temperature shoulders on oxidation peaks occurs with bundling, changes in oxidation temperature of material when bundled vs not
- Oxidation and degradation of CNTs will reduce the thermal stability, which should help resolve SWCNTs from soot, but will likely not change the MWCNT thermal stability to such an extent that it interferes with BC
- Organic coatings can influence the thermal stability of the CNTs, where lower onset temperatures and broader mass loss events have been observed, increasing potential interferences; proteins can char and cause interference

Thermal Gravimetric Analysis-Mass Spectrometry (TGA-MS)  
14

- CNT thermal stability will increase measurably, no anticipated change in MS signal
- Oxidation of CNTs will reduce the MS-derived advantages, which leverage the low-oxygen content of CNTs; potential for changes in decomposition products
- Organic coatings can change CNT thermal stability and should increase the O and/or N content of the diluting matrix; thus, CNT-derived depletions in O would become easier to observe with organic matter coatings

Total Organic Carbon (TOC) Analysis  
71

- Unlikely to be impacted by aggregation
- Oxidation of CNTs would likely improve detection, as TOC analysis relies upon complete conversion to gaseous carbon mon- or di-oxides
- Any organic surface coating (citrate, amine, etc.) contributes to the carbon detected from the CNT

Thermal Optical Transmittance (TOT)  
16,23

- Sample on the filter won’t be uniform, the split point of organic carbon/elemental carbon needs to be manually chosen instead of by optical information
- Oxidation/degradation decreases the thermal stability and causes peak position shift; no issue with quantifying CNTs unless the sample has huge amount of organic carbon and the peak position of CNT after shifting is getting close to organic carbon
- Having too much organic carbon may affect the thermal stability of CNT, and the signal from organic carbon will overlap that of elemental carbon; organic carbon should be resolved as much as possible

Isotopic labeling

Carbon-13 Labelling  
21,32,78

- Comprehensive oxidation of the CNTs required to prevent isotopic fractionation; agglomeration may affect thermal stability in closed-tube-combustion approaches, and efforts should be made to ensure complete combustion
- Pre-analysis CNT oxidation may have slight impacts on CNT $\delta^{13}$C signature (by virtue of reactive fractionation); these should be small depending on the extent of surface oxidation and/or if that process removes CNT-C from the CNT matrix
- Wrapping with organic molecules will affect $\delta^{13}$C; the effect will depend on the $\delta^{13}$C of the molecule, and measures to separate the coating from the CNT are critical
The impact of agglomeration would depend on the quantification procedure used; interference from self-quenching has been reported in some studies with agglomerates of CNTs, but this would not be expected for quantification using biological oxidation. This is not expected to impact this approach; carbon-14 analyses of released carbon dioxide has been used to quantify CNT degradation. This may impact measurements with liquid scintillation counting of dispersed CNTs but should not impact samples combusted using biological oxidation.

Other radioactive isotopes\(^ {96-98}\) This would not be expected to impact most isotopes unless self-quenching occurs. Oxidation or degradation may render this technique unusable if these processes lead to substantial separation of the radioactive isotope from the CNT. This would not be expected to impact most isotopes unless quenching occurs.

### Additional Techniques

**Analytical Ultracentrifugation (AUC)**

<table>
<thead>
<tr>
<th>Aggregate formation</th>
<th>Interpretation of results will become suspect due to differences in actual sample with respect to expected behavior</th>
<th>Variable effects, will likely bias size analysis</th>
</tr>
</thead>
</table>

Aggregates rather than primary particle would be measured, data analysis potential decreased.

**Gravimetric**\(^ {185}\)

| No impact | No impact unless there is complete degradation to CO\(_2\) | This can limit the accuracy of this approach since the concentration of these organic molecules will need to be assumed to be homogeneously distributed |

**Microwave Method**\(^ {62,125,186,187}\)

| These potential interferences have not been tested for this technique | Modifies CNT interactions with the membrane which can lead to higher or lower losses depending on carrier solution and membrane material |

Enhances material losses on the membrane and hinders accurate shape factor determination; thus agglomeration has to be avoided.

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**Table S2: Potential Interferences for CNT Quantitation from Changes to CNTs for Selected Techniques**

Abbreviations: Asymmetric flow field flow fractionation with multi-angle light scattering (aF4-MALS), analytical ultracentrifugation (AUC), black carbon (BC), carbon nanotube (CNT), chemothermal oxidation at 375 °C (CTO-375), multiwall carbon nanotube (MWCNT), near infrared fluorescence (NIRF), photoacoustic (PA), photothermal (PT), single particle inductively coupled plasma-mass spectrometry (spICP-MS), single-wall carbon nanotube (SWCNT), thermal gravimetric analysis (TGA), thermal gravimetric analysis-mass spectrometry (TGA-MS), total organic carbon (TOC), thermal optical transmittance (TOT).
<table>
<thead>
<tr>
<th>Method</th>
<th>Relevant reference materials or standard methods</th>
<th>Key steps in instrument calibration</th>
<th>Challenges to standardization (i.e., traceability to the SI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spectroscopic</td>
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</tr>
<tr>
<td>Absorbance</td>
<td>Informational values RM8281, ISO/TS 10868:2011</td>
<td>Calibration of wavelength and intensity performed at 100% transmittance. Usable wavelength range should be established by testing the absorbance of a blank sample and considering regions of high absorbance, scattering interference and Beer’s law considerations.</td>
<td>Chemical environment can affect intensity and peak positions. Absorbance of water in NIR wavelengths for cell path lengths &gt; 1 mm.</td>
</tr>
<tr>
<td>Near infrared fluorescence</td>
<td>Informational values RM8281, ISO/TS 10867:2010</td>
<td>Traceable lamp detector train calibration</td>
<td>Chemical environment can affect intensity and peak positions, in-filter effects</td>
</tr>
<tr>
<td>Photoacoustic and photothermal</td>
<td>CNTs with well characterized absorbance of narrow size distribution in a pure solvent could be used for calibration</td>
<td>A standard sample with material similar to the sample CNTs (that absorbs the same wavelength) can be used to tune the setup. Laser power, sensitivity, and time constant can be adjusted for the sample as needed.</td>
<td>No standards published or referenced to date. Short shelf life for samples if in situ CNTs degrade or change over time. Difficult traceability to SI.</td>
</tr>
<tr>
<td>Raman</td>
<td>Frequency (x-axis) calibration standards ASTM E1840, Intensity (Y-axis) E2911, E2529, NIST SRM series 224X</td>
<td>Choose the appropriate standard for frequency and intensity depending on the excitation wavelength. Alternatively, a series of standard solutions (dilution series) of the pure analyte in combination with the internal standard can be used.</td>
<td></td>
</tr>
<tr>
<td>Spectrometric</td>
<td></td>
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<tr>
<td>Inorganic Element Analysis</td>
<td>Dissolved standards of the monitored elements are required to determine the instrument sensitivity for the elemental quantification. Potential influences from residual carbon content and dissolved solids can be accounted for by suitable calibration techniques, including isotope dilution, matrix matched standards and the method of additions. SRM 2483 (single-wall carbon nanotubes [raw soot]) could be used to test instrument performance</td>
<td>ISO/TS 13278:2011E, This Technical Specification provides reference standard methods for the determination of elemental impurities in CNTs using ICP-MS. Results traceable to the SI can be readily achieved using traceable high-purity calibration standards. Calibration is performed with solutions having known concentrations of the metallic analytes of interest and matrix-matched to the composition of the prepared samples.</td>
<td>Lack of control environmental and biological matrices. Guarantee that sample digestion is quantitative prior to elemental analysis.</td>
</tr>
<tr>
<td>Method</td>
<td>Reference Materials</td>
<td>Calibration of ICP-MS</td>
<td>Reference CNT samples with homogeneous size and controlled metal impurities contents are required to address the standardization.</td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td>--------------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------------------------------</td>
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<tr>
<td>Single particle inductively coupled plasma-mass spectrometry (spICP-MS)</td>
<td>Reference Materials: Single element standard solutions available from numerous reference materials producers; NIST RM 8013 Gold Nanoparticle, Nominal 60 nm Diameter; Standards: ISO TS13278 Determination of metal impurities in samples of carbon nanotubes using inductively coupled plasma mass spectrometry</td>
<td>Calibration of ICP-MS instrument sensitivity is performed with solutions having known concentrations of the metallic analytes of interest and matrix-matched to the composition of the prepared samples. Calibration of sample transport efficiency is performed using metallic nanoparticles having known size (metal does not need to be the same as the trace metal analytes). Sample transport efficiency may also be calibrated using the waste collection method, but this method is generally less reliable.</td>
<td>Reference CNT samples with homogeneous size and controlled metal impurities contents are required to address the standardization.</td>
</tr>
<tr>
<td><strong>Microscopic</strong></td>
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<tr>
<td>Atomic Force Microscopy</td>
<td>ASTM E2859-11 Standard guide for size measurement of nanoparticles Using atomic force microscopy, NIST RM8281, NRC Canada SWCNT-1</td>
<td>In-plane resolution, i.e. distance/pixel should be selected to enable identification of smallest expected particles of interest.</td>
<td>Surface roughness of deposition substrates varies significantly with preparation methodology. Polydisperse samples may require measurements at multiple resolutions to identify small particles, and to locate larger particles. Unspecific absorption in the VNIR spectral range</td>
</tr>
<tr>
<td>Hyperspectral Imaging</td>
<td>A representative spectral library is generated from the parent material. The spectral library is then used to detect the same material in a sample (e.g. cell) using a mapping algorithm</td>
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<tr>
<td>Scanning Electron Microscopy</td>
<td>ISO/TS 10798:2011 Nanotechnologies -- Characterization of single-wall carbon nanotubes using scanning electron microscopy and energy dispersive X-ray spectrometry analysis</td>
<td>Reference materials (regarding CNT's number concentrations) must be used to evaluate instrumental losses during sample preparation</td>
<td>Reference CNT suspensions with certified number concentrations must be developed; the shelf life of these suspensions maybe limited due to CNT agglomeration.</td>
</tr>
<tr>
<td>Transmission Electron Microscopy</td>
<td>ISO/TS 10797:2012 Nanotechnologies -- Characterization of single-wall carbon nanotubes using transmission electron microscopy</td>
<td>Reference materials (regarding CNT's number concentrations) must be used to evaluate instrumental losses during sample preparation</td>
<td>Reference CNT suspensions with certified number concentrations must be developed. The shelf life of these suspensions maybe limited due to CNT aggregation.</td>
</tr>
<tr>
<td><strong>Thermal</strong></td>
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<tr>
<td>CTO-375</td>
<td></td>
<td>Temperature and mass calibration required</td>
<td>No reference materials for temperature calibration; traceable mass standards available</td>
</tr>
<tr>
<td>Thermal Gravimetric Analysis-Mass Spectrometry</td>
<td></td>
<td>Temperature and mass calibration required. MS peak identification database needed.</td>
<td>No reference materials for temperature calibration; traceable mass standards available</td>
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<tr>
<td>Total Organic Carbon Analysis</td>
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<tr>
<td>Thermal Optical Transmittance</td>
<td>NIOSH, Elemental Carbon (Diesel Particulate): Method 5040. In NIOSH, Manual of Analytical Methods, 4th ed., 2003.</td>
<td>Sucrose solution and methane gas carbon standards are often used for mass calibration</td>
<td>Each CNT has slightly different peak position depending on defect, purity, functional group etc.; also it differs by the temperature program.</td>
</tr>
</tbody>
</table>

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Isotopic labeling

**Carbon-13 Labelling**
Standards include calcium carbonate (commonly used Vienna PeeDee Belemnite and NBS $^{13}$C standard), barium carbonate

Ensuring accuracy of standards is key to reliable measurements. Also, running standards throughout sample analysis is required to understand measurement drift. Samples containing sulfate cause contamination in the final product. Small samples may not release enough gas for the analysis. Nanotubes will differ in their $^{13}$C ratios based on original source of C.

**Carbon-14 Labelling**
Standards include NIST 4222C (carbon-14 hexadecane radioactivity standard solution)

Calibration depends on the method (liquid scintillation counting, autoradiography, biological oxidation) used to quantify the radioactivity. For all methods, it may be necessary to calibrate the instrument using other radioactive carbon-14 chemicals or elements.

**Other isotopes**
Multiple radioactivity standards are available from NIST (e.g., 4915F cobalt-60 radioactivity standard solution) and from other organizations

Calibration depends on the instrument used to measure the radioactivity

One principal challenge is the stability of the radioactive isotope onto the carbon nanotube.

Additional techniques

**Analytical Ultracentrifugation**
SRM under development for ensuring radial measurement precision; sedimentation of Bovine Serum Albumin (BSA) frequently used as an unofficial standard

External evaluation of temperature calibration and bulk solution viscosity and density properties are critical for correct measurements.

Requires unique absorbance or refractive index signals from solute differentiable from media.

**Gravimetric**
A broad range of mass RMs and protocols are available for gravimetric measurements

Balances can be calibrated using device-specific procedures, reference masses are readily available

Works only for a limited number of conditions and matrices

**Microwave Method**
CNT material used in the exposure experiment, (reference) control material (e.g., CNT-free biological tissue such as NIST SRM 1573 Tomato leaves)

The very same CNT material that is to be quantified must be used to calibrate the instrument; a calibration curve is generated using the thermal response as a function of known CNT amounts spiked into tissue samples

A main limitation to standardization is that the instrument used to make these measurements is not readily available

**Field flow fraction/asymmetric flow field flow fraction/multi-angle light scattering**
Certified polystyrene (PS) beads (Single or mix, available from NIST or other sources), Bovine serum albumin (BSA), Any other certified particle standard (e.g., Au, Ag, and SiO2) that can be dispersed in the carrier solution

PS beads dispersed in the used carrier solution are used for retention time calibration (hydrodynamic diameter). An isotropic scatterer is used for normalization of the MALS detector angles (e.g., 20nm PS beads). BSA is used for molecular weight calibration of the MALS detector

Reference CNT samples with homogeneous size and controlled particle impurities (e.g., soot) would be required for aF4-MALS quality assurance. Changes in the chemical environment of the CNTs as well as changes of the CNTs themselves (e.g., surface functionalizations, length distributions) can affect retention time in aF4; standardized methods must include extensive methodological details to ensure reproducibility

Table S3: Standards and calibration of selected carbon nanotube quantification techniques
<table>
<thead>
<tr>
<th>Material</th>
<th>NIST SRM 2483 Single-wall carbon nanotube soot</th>
<th>NIST RM8281 Single-wall carbon nanotubes (dispersed, three length-resolved populations)</th>
<th>NRC Canada: SWCNT-1 Single-wall carbon nanotube certified reference material</th>
<th>JRC Multiwall carbon nanotube representative test materials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y/N</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
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</table>

*Are certified, reference, or information values provided for these characteristics?*

<table>
<thead>
<tr>
<th>Characterization</th>
<th>AFM imaging</th>
<th>Elemental composition</th>
<th>NIR fluorescence spectra</th>
<th>Raman ratio</th>
<th>Raman spectra</th>
<th>SEM imaging</th>
<th>Specific surface area</th>
<th>TEM imaging</th>
<th>Thermogravimetric analysis (residual mass and oxidation temperature)</th>
<th>UV-vis-NIR absorbance spectra</th>
<th>X-ray diffraction</th>
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<tr>
<td></td>
<td>No</td>
<td>Yes</td>
<td>Information values</td>
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</tbody>
</table>

**Table S4:** Characterization of carbon nanotube reference materials, standard reference materials, and representative test material.