Detection and Quantification of Graphene Family Nanomaterials in the Environment

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ABSTRACT

An increase in production of commercial products containing graphene-family nanomaterials (GFNs) has led to concern over their release into the environment. The fate and potential ecotoxicological effects of GFNs in the environment are currently unclear, partially due to the limited analytical methods for GFN measurements. In this review, the unique properties of GFNs that are useful for their detection and quantification are discussed. The capacity of several classes of techniques to identify and/or quantify GFNs in different environmental matrices (water, soil, sediment, and organisms), after environmental transformations, and after release from a polymer matrix of a product is evaluated. Extraction and strategies to combine methods for more accurate discrimination of GFNs from environmental interferences as well as from other carbonaceous nanomaterials are recommended. Overall, a comprehensive review of the techniques available to detect and quantify GFNs are systematically presented to inform the state of the science, guide researchers in their selection of the best technique for the system under investigation, and enable further development of GFN metrology in environmental matrices. Two case studies are described to provide practical examples of choosing which techniques to utilize for detection or quantification of GFNs in specific scenarios. Since the available quantitative techniques are somewhat limited, more research is required to distinguish GFNs from other carbonaceous materials and improve the accuracy and detection limits of GFNs at more environmentally relevant concentrations.

48 **TOC Artwork**

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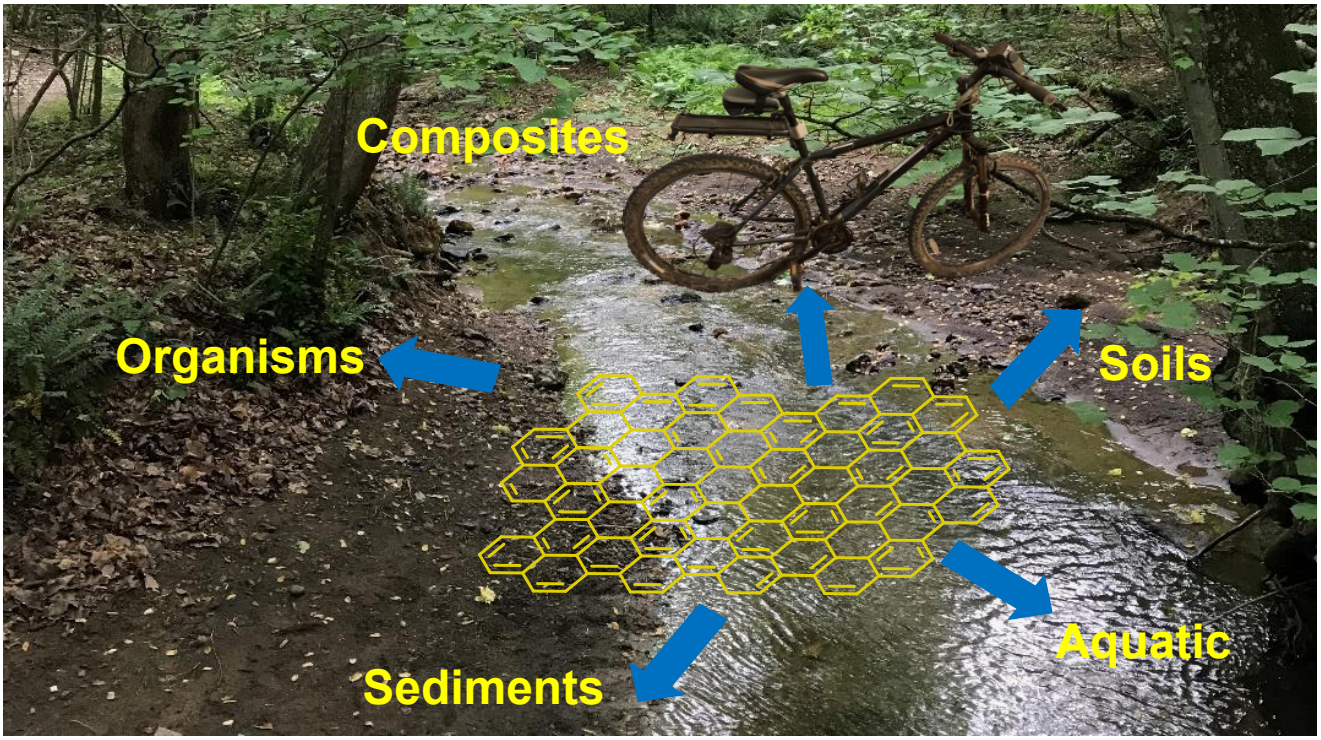

INTRODUCTION

Graphene family nanomaterials (GFNs) are a class of carbonaceous nanomaterials, similar in chemical structure to graphite, but with a thickness on the order of nanometers and lateral dimensions typically in the micron range. GFNs contain an sp^2-hybridized network of fused benzene rings existing as a single sheet or a few layers of sheets. There are many categories of GFNs; definitions provided by Bianco et al. will be used throughout this paper.[1] Graphene, the most widely known type of GFN, is a fully graphenic, single-layer sheet of sp^2 hybridized carbon. Graphene is typically prepared by chemical vapor deposition, micromechanical cleavage of graphite, or reduction of graphene oxide.[2] Graphene oxide (GO) is similarly composed of a single sheet of graphenic carbon that contains areas of disrupted aromaticity where carbon atoms are oxidized. Oxygen functional groups can include epoxide, hydroxyl, carbonyl, and carboxyl groups, which can reside along the basal plane or the edges of the graphenic structure.[3, 4] Generally, GO has high C/O ratios around 2.0 and sometimes as high as 3.0. GO is typically prepared by the oxidation of graphite in strong acids and other oxidants followed by sonication.[5] Reduced graphene oxide (rGO) is GO in a form that contains fewer oxygen functional groups and a greater proportion of graphenic carbon; rGO can be prepared by exposure of GO to thermal,[6, 7] ultraviolet (UV),[8, 9] biodegradation,[10] and chemical processes.[4] Few-layer graphene (FLG) are composed of several graphene layers, typically 2 to 5. Graphene quantum dots (GQDs) are similar to graphene, but have lateral dimensions on the nanometer-scale, rather than the micron-scale. They are often produced for biomedical imaging, photonic devices, electronic devices, and catalysis applications and are tuned for their fluorescence properties.[11, 12] Unlike fullerenes but similar to carbon nanotubes, GFNs typically exist as a distribution of particles with varying defects, sizes, thicknesses, and oxidation levels.[4]

Graphene family nanomaterials (GFNs) have novel properties that include high electrical and thermal conductivity, and tensile strength as high as 130,000 MPa compared to 300 MPa to 440 MPa for low carbon steel.[13, 14] As a result, GFNs have the potential for use in a broad range of fields and commercial applications.[15, 16] Globally, over 26,000 graphene-related patents have been filed since graphene was first isolated in 2003.[17] Overall, the total annual sales of graphene was $12 million in 2013 and was projected to reach $20 million in sales by 2016.[17, 18] In 2027, the production volume of graphene is expected to reach 3800 metric tons with total annual sales of $300 million.[19] GFNs are being developed for use in functional coatings, anti-corrosion applications, antifouling and antibacterial applications, membranes, conductive inks, supercapacitors, optoelectronics, and touch screens.[20-22] Bendable phones containing graphene are also in development.[23] On the market, a range of products are readily available from pre-mixed graphene/epoxy resins and graphene-modified polymer masterbatches (pre-mixed granular pellets) to graphene scratch-resistant and heat-cooling coatings, graphene conductivity agents, inks, bike helmets, tennis and badminton rackets, and batteries.[24-29]

With the increased production and use of GFNs in consumer products and their potential for release into the environment and exposure to humans, it is critical to understand their

environmental fate and potential health and ecological risks.[30, 31] In terms of GFN fate, the ranges of critical coagulation concentrations (CCC) reported for GO in aqueous media are 38 mmol/L to 200 mmol/L of NaCl and 0.9 mmol/L to 2.6 mmol/L of $CaCl_2$, depending on lateral size, number of layers, initial GO concentration, and solution pH.[32-36] Graphene and rGO have lesser or no functionalization (compared to GO), and are thus less stable than GO in aqueous media. In a recent study, the CCC in NaCl decreased from 200 mmol/L for pristine GO to 35 mmol/L and 30 mmol/L upon Solvothermal reduction of pristine GO for 1 h and 2 h, respectively.[36] The CCC in NaCl for graphene is about 1.6 mmol/L to 10 mmol/L, depending on initial concentration and lateral size.[37] Based on these colloidal stability behaviors, GFNs may be unstable in some surface waters and groundwater,[32, 36-38] and may result in exposure of organisms in the pelagic zone initially, and then organisms in the benthic zones as the nanomaterials agglomerate and settle out. Organisms in terrestrial environments will also be exposed, for instance, if biosolids containing GFNs are applied to farmlands.[38] Most of the studies on the environmental persistence and fate of GFNs have been conducted in simple environmental media (e.g., water with natural organic matter (NOM) but not soil or sediment media).[39-44] While the concentration of GFNs in natural waters has not yet been modeled or measured, useful estimates for the expected range can be based on the average concentrations for CNTs and fullerenes, which have been modeled to be in the low ng/L range or less, concentrations orders of magnitude lower than those for current GFN detection/quantification methods.[45] In addition, studies on the ecotoxicity and fate of GFNs have almost exclusively focused on the GFNs as produced by the manufacturer and not on the particles released from consumer products containing GFNs such as polymer nanocomposites, due in part, to a lack of methods for quantifying GFNs in the presence of other carbonaceous materials. Therefore, methods for quantification of GFNs at low concentrations and in complex environmental media and consumer product-relevant matrices are urgently needed.

Organisms are likely to come into contact with GFNs that have been released into the environment, and it is important to understand the implications of these exposures. Numerous studies on the potential environmental impacts of GFNs have focused on trophic transfer of GFNs,[46, 47] bioaccumulation of GFNs, or toxicological effects to bacteria,[48-53] pelagic (e.g., fish, zooplankton, etc.),[37, 41, 42, 54-58] soil (e.g., earthworms,[57] plants[59, 60]), and benthic organisms (e.g., organisms that burrow in sediments).[57] Similar to carbon nanotubes (CNTs), GFNs show varying degrees of toxicity that depend on oxidation level, dispersion quality, size, surface area, orientation or alignment, and organism type,[52, 53, 61-65] and have shown the capacity to impact the toxicity of organic and inorganic co-contaminants.[66, 67] Concentrations as low as 0.01 mg/L GO have caused elevated β-galactosidase biosynthesis in zebrafish embryos.[68] Conversely, *Artemia* larvae showed no effects with GO levels as high as 100 mg/L.[69, 70] Bacterial effects generally occurred at GFN concentrations ranging from 5 to 100 mg/L.[52, 71, 72] A bacterial community from a wastewater treatment plant (WWTP) showed effects at GO concentrations less than 10 mg/L.[73] Graphene and GO inhibited algal growth at ≥ 0.675 mg/L and ≥ 1.25 mg/L, respectively.[74] In-vitro cell exposure shows effects of GO and rGO between 2 mg/L and 25 mg/L for blue mussel

hemocytes,[75] zebrafish gill cells,[76] and mouse fibroblasts.[77] This four orders of magnitude difference is not surprising based on the variety of organisms, endpoints, types of material tested, and different exposure durations and conditions. Although the concentration of GFNs in the environment are expected to be lower than the toxicity thresholds reported in most current studies, different endpoints may be required to determine molecular level effects (such as DNA damage, metabolism interference), effects on sensitive populations , and long-term effects of low (ug/L to ng/L) concentrations.[78] Furthermore, most of these studies did not provide measurements of the GFN body burden, a measurement which may be more predictive of the toxic effects observed as compared to the exposure concentration, and typically the exposure concentration was not measured after the exposure period. More robust measurements of the GFN in the exposure media and in the organisms tested can reduce uncertainties in assessing the potential ecotoxicological risks of GFNs.

While insights can be drawn from quantitative procedures used for other carbon nanomaterials (CNMs), the unique properties of GFNs indicate that new or modified procedures may be needed for detection and quantification of these materials. For example, many chromatographic techniques (e.g., liquid chromatography-mass spectrometry) have been utilized to accurately quantify fullerene particles, but this approach will likely not work for GFNs since these materials possess higher polydispersity than individually-dispersed fullerene particles that have controlled stoichiometry.[79-81] In addition, GFNs do not have the same near-infrared fluorescence patterns that have been used for quantification of individually dispersed single-wall CNTs (SWCNTs);[82-84] the reason that GFNs cannot be quantified using near-infrared fluorescence spectroscopy is that they are not composed of varying conformations or chiralities as are SWCNTs. Carbon nanotubes also sometimes contain residual metal catalysts from the manufacturing process,[85] which can be used as a proxy to measure CNT concentration with single particle-inductively coupled mass spectrometry (spICP-MS)[86, 87] and total inorganic elemental analysis using, for example, ICP-atomic emission spectroscopy (ICP-AES).[88] Unlike some methods of CNT synthesis, graphene is not typically manufactured with metal nanoparticles that can be used for detection. However, other methods used for the detection and quantification of carbon nanotubes and fullerenes may be similarly applied to GFNs. While reviews have been conducted on quantitative methods for the analysis of CNTs and fullerenes,[80, 89-91] the applicability of many of these methods for GFNs is still unclear.

This paper provides a comprehensive review of analytical methods for detection and quantification of GFNs in various environmental media, such as water, soil, sediments, and organisms. Measurements of GFNs in these media are critical for studies assessing the environmental fate and potential ecotoxicological effects of GFNs. Given that GFNs will likely be released into the environment after use in consumer products, quantification techniques for the assessment of GFN release from polymer nanocomposites will also be evaluated. The unique properties of GFNs that can be useful for quantification and identification in environmental media and consumer-relevant matrices will be discussed. Potential biases and detection limits,

when available, will be provided for relevant techniques in each type of environmental medium, as well as the current ability to differentiate GFNs from other carbonaceous nanomaterials. Key topics for future work will also be described which include the importance of GFN extraction, a process necessary in many cases to separate GFNs from interfering compounds and concentrate GFNs to reach detection limit requirements. Extraction will be considered in the context of current studies and future research needs. Furthermore, case studies will be provided to apply the techniques described to two different environmentally important scenarios.

UNIQUE PROPERTIES OF GFNS THAT ALLOW FOR DETECTION/QUANTIFICATION

The detection and quantification of GFNs in simple and complex systems requires measurements that are specific to the unique properties of GFNs. These properties can include the interaction of GFNs with light, the graphitic and electronic structure, and the two-dimensional shape and size of GFNs.[3, 4, 16, 92] Figure 1 provides an overview of selected techniques grouped by spectroscopic, spectrometric, microscopic, thermal, and labeling categories for GFN measurements and Table 1 provides technique descriptions with strengths and limitations. Table S1 summarizes the detection limits of GFNs for the few techniques for which this information has been provided in the literature.

The GFN size and oxidation level can significantly alter the measurement obtained from a given technique. GFNs tend to be composed of a heterogeneous distribution of sizes, amorphous impurities, and levels of exfoliation which adds complexity to their quantification. Currently, there is information about the impact of lateral size and GFN agglomeration state on quantification methods, but information about the impact of GFN thickness on quantification is not yet readily available.[32, 37, 40] In terms of lateral size, the ratio of edge defects to graphitic regions decreases with GFN lateral size, changing the electronic properties. Oxidation generally leads to a change in the chemical and electronic structure of the GFN. Oxidation leads to an increasing number of defect sites containing oxygen functional groups (e.g., epoxides, carboxylic acids, alcohols, carbonyls), which disrupt the aromaticity of the graphitic structure and, generally, decrease the electrical conductivity.[4] These oxygen functional groups often serve as anchor points for derivatization or metal ion tagging, which can enable GFN detection and quantification.[40, 41] In comparison to graphene, GO has the advantage of being readily dispersible in water.[4] This facilitates detection and quantification of GO in aqueous systems, since only minor agglomeration occurs except in waters with high ionic strength.[32] Graphene, on the other hand, agglomerates readily and requires extensive exfoliation processes and addition of surfactants to be suspended in water. This presents a challenge for detection, since graphene can exist in many different agglomeration states from system to system. However, this is not as substantial of an issue with thermal, isotopic, or radioactive labeling methods. Environmentally relevant processes such as ultraviolet (UV), chemical, and biological degradation have shown the capacity to transform GFNs through oxidation to CO_2, reduction of GO, and GFN fragmentation.[40, 41, 72, 93-95] The large variations in GFN structure observed as a function of

oxidation level and material size as a result of these environmental processes presents challenges for quantification. Nevertheless, a combination of techniques can usually be employed to identify the presence of GFNs and sometimes quantify them.[40]

The measurement limitations presented must also be considered in the context of the media and systems in which GFNs will be detected and quantified. These can include aqueous and complex environmental media such as soils and sediments, polymer fragments containing GFNs released from products, and biological systems such as cells and tissues (Figure S1). The main challenge with all of these systems is that detection of CNMs must often take place in a matrix containing high amounts of carbon.[96] As a result, there are several potential ways that the media, matrix, or system can cause interferences such as absorbance overlap in the same region of the UV-Visible (UV-Vis) spectrum, thermal profile overlap with NOM, and obscuration of the two-dimensional GFN shape in the presence of other materials using microscopy (Figure S1). Table S2 describes the methods presented in Table 1 as applied to different matrices with information on what has been previously studied in these systems, when extraction is or might be required, and the potential biases associated with these matrices. In the following sections, these matrices described and considered in the context of the classes of techniques used for measurement of GFNs that are subsequently described.

RELEVANT MATRICES

One key factor related to GFN detection and/or quantification is that various environmentally and biologically relevant matrices may impact the type of techniques used. In the following sections, general details will be provided about the potential impact of matrix on GFN quantification. Then, in the **Classes of Techniques Used for Detection and/or Quantification of GFNs** section, different classes of techniques and their use with different matrices will be discussed in depth.

Measurement of pristine GFNs in aqueous systems

Over the course of their life cycle, GFNs are likely to end up in aqueous systems such as freshwater, wastewater and marine water bodies including bottom sediments.[30, 38] A large majority of GFN measurements that have been made in a laboratory setting involve suspensions of GFNs prepared in purified (i.e., deionized (DI) water) aqueous systems or synthetic media (e.g., EPA hard water) rather than in natural water.[32, 36, 97-99] For example, GFNs have been measured in purified water using UV-Vis spectroscopy,[16, 100] Raman spectroscopy, [101, 102] and fluorimetry.[103] More complex natural waters typically contain NOM, microorganisms, inorganic species, suspended particles, and pollutants, all of which have the potential to interfere with GFN detection and/or quantification (Table 1).

Measurement of GFNs in soils/sediments

Soils and sediments are extremely complex, and they constitute some of the largest sinks for engineered nanomaterials.[104, 105] There is currently no study measuring GFNs in soils and sediments without carbon-14 labeling, and the complexity of these matrices will most likely require extraction of GFNs prior to detection and quantification.[57]

Measurement of GFNs in cells/organism tissues

Detection and quantification of GFNs in biological matrices is important for understanding the fate, bioavailability, bioaccumulation, and potential adverse effects of the GFNs on organisms. Analytical techniques for detection and/ or quantification of GFNs in carbon based biological matrices present similar challenges to detection and/or quantification of GFNs in soils and sediments. These techniques also will require that the GFNs be extracted from biological systems prior to measurements, while only a few techniques (e.g. using labeled GFNs) can be used to analyze GFNs *in situ*.[42, 57, 58, 106]

Measurement of released GFNs from consumer products such as polymer nanocomposites

A large fraction of GFNs will be used as additives in consumer products. Many of these consumer products will use GFNs embedded in polymer matrices to enhance material properties. For example, GFNs can enhance mechanical strength, electrical properties, and barrier properties of a polymer.[107, 108] As these GFN/polymer nanocomposites go through their life cycle, GFNs can potentially be released from the consumer product into the environment via mechanical wear, thermal, UV, and other weathering conditions.[109, 110] GFN release from polymer matrices is not a simple process and can generate different types of released particles that include freely released GFNs, GFN(s) partly encapsulated in polymer fragments, and GFN(s) fully encapsulated in polymer fragments (Figure 2). Therefore, the polymer matrix can interfere with GFN detection and quantification. This has previously been shown with abraded CNT/polymer nanocomposites during simulated wear experiments.[111, 112] Methods to detect the heterogenous mixture of particles released from polymer nanocomposites as well as methods to remove polymer interferences are needed. Furthermore, the detection of GFNs becomes even more challenging when a polymer matrix and environmental matrix, such as natural water and soils/sediments, are combined.

CLASSES OF TECHNIQUES USED FOR DETECTION AND/OR QUANTIFICATION OF GFNS

Spectroscopic Techniques

Spectroscopically, the interaction of GFNs with light can enable GFN-specific measurements. In this case, oxidation level, lateral size, and agglomeration state must be considered since they change the interaction of GFNs with light. The spectroscopic techniques considered in this review include UV-Vis spectroscopy, fluorescence spectroscopy, Raman spectroscopy, X-ray photoelectron spectroscopy, and a few other specialized techniques.

UV-Vis Spectroscopy

UV-Vis spectroscopy (absorbance mode) is the most commonly used method for quantifying GFNs in purified, synthetic, and natural waters due to the ease of use, low cost, and availability of spectrophotometers in environmental laboratories.[32, 40, 44, 92, 113-116] The absorbance of a GFN can be related to its mass concentration in suspension using the Beer-Lambert law, but the particles must be well-dispersed.[117, 118] The absorbance of GFNs is typically measured at wavelengths around 220 nm to 300 nm. The absorption peak of graphene around 265 nm is due to $\pi \rightarrow \pi^*$ transitions, which shifts to shorter wavelengths (around 230 nm) when graphene is oxidized to GO.[92, 119, 120] For graphene, surfactants are often required for colloidal stability in water so that consistent UV-Vis measurements can be obtained. It is often challenging to prevent the surfactant from absorbing in the same region of the spectrum as graphene.[121] Alternatively, organic solvents can sometimes be used to suspend graphene.[122] UV-Vis measurements of GO around 300 nm targets the peak (shoulder) originating from the $n \rightarrow \pi^*$ transitions of the oxygen functional groups.[120, 123, 124] In natural and synthetic waters, it is typically challenging to detect and/or quantify GFNs via UV-Vis spectroscopy because of the complexity of the medium, non-specificity of the technique, and potential for agglomeration of the GFNs.[32, 36, 38, 96, 114] For instance, several constituents of natural or synthetic waters such as salts, nutrients, NOM, and suspended solids absorb light in the UV region, making it impossible to use UV-Vis spectroscopy to quantify GFNs in these media without extracting the nanomaterials. For these reasons, it is useful to have a reference spectrum of the GFN material in purified water whenever possible. It is also important to have measurements of the natural/synthetic water without GFNs and of the natural/synthetic water after adding a known amount of GFN to determine if measurements can be made without significant interference using a specified technique. This same approach has been taken in biological systems where CNTs were quantified in cells by lysing the cells and determining the absorbance of the lysate spiked with known amounts of CNTs to develop a calibration curve.[125] Another approach used for CNT suspensions has been to measure absorbance increases at longer wavelengths from light-scattering by the suspended particles, which is proportional to CNT mass concentration, but this approach has not yet been shown to be effective with GFNs.[126] In addition, the low expected average environmental concentrations of GFNs (i.e., average in the low ng/L range if the concentrations are similar to those modeled for CNTs) makes UV-Vis spectroscopy, with detection limits estimated to be in the tens of µg/L to mg/L range for GFNs (Table S1), likely unsuitable for quantifying GFNs in natural surface waters.[96] In laboratory studies where challenges arising from matrix effects and high detection limits are overcome, biases may still arise from GFN size distribution, method of dispersion, and agglomeration state, all of which may influence the absorption coefficients.

UV-Vis measurements of GFNs in other matrices (e.g., polymer fragments, soil/sediment, cells or tissue components) can prove to be even more complex. These measurements must be performed in a liquid medium, usually water, that is part of or surrounding the matrix (e.g., released particles from a GFN polymer nanocomposite suspended

in water). Spectroscopically, the interaction of the matrix (e.g. polymer fragment, soil/sediment, cells or tissue components) with electromagnetic radiation must be sufficiently different from that of the GFN to avoid overlap in the GFN spectrum. This is challenging because many polymers, inorganic particles from soils/sediments, and biological materials absorb light around the wavelength of GFN absorption (200 nm to 300 nm range).[117, 127, 128] Another approach is to make use of analytical ultracentrifugation with UV detection, as has been performed with CNTs to separate various CNT structures by size prior to detection.[127-129] Overall, the UV-Vis approach is likely to work for quantifying GFNs in matrices when they are well-defined and do not have significant interferences at the wavelength used for GFN quantification.

Fluorescence

The ability of GFNs to fluoresce is sometimes useful for GFN characterization in purified and synthetic waters.[103, 113, 123] Both GO and rGO are detectable by instruments capable of measuring near-infrared (NIR), visible (vis) and ultraviolet (UV) fluorescence.[103, 130] Also, graphene quantum dots, or graphene fragments with a lateral dimension on the nanoscale (rather than micron scale), are designed specifically for their unique fluorescence 'tunability' but are still challenging to prepare synthetically in terms of size, surface chemistry, and photoluminescence properties.[11] Pristine graphene, on the other hand, is not readily fluorescent because it has a zero band gap.[3, 131] In general, the fluorescence properties of GFNs will vary as the result of changes to the electronic structure caused by alterations in size and oxidation level, which can happen via transformation processes in the environment.[103] Thus, fluorimetry is not used as widely as UV-Vis spectroscopy to quantify GFNs in laboratory studies conducted in aqueous media. This may also be due, in part, to the non-linear relationship between fluorescence intensity and the concentration of GFNs in aqueous media—making the technique mostly useful for semi-quantitative analysis.[113]

Similar to UV-Vis spectroscopy, the applicability of fluorescence in detecting GFNs in natural waters, soils/sediment, polymer fragments, and cells/tissues is limited. Fluorimetry requires well-dispersed particles and is non-specific, making it impossible to use the technique for *in situ* quantification of GFNs in natural waters containing other fluorescent materials. In addition, the interactions of salts and NOM with GFNs can interfere with GFN fluorescence. In biological matrices, the intrinsic photoluminescence of GO can ideally be used to trace GO. However, the emission efficiency of GO is low,[132, 133] and may be affected by interference from cellular components. For GFN/polymer nanocomposites, degraded or highly oxidized polymer fragments generated during polymer degradation processes often fluoresce strongly and will likely interfere with GFN detection.[134] Therefore, it is highly unlikely that fluorescence will be utilized to detect GFNs in most environmental matrices since 1) GFN structures are not homogenous and may change in the environment, which leads to a changing fluorescence spectra

and 2) many components in environmental matrices and polymer fragments will interfere since they are also fluorescent.

Raman Spectroscopy

Raman spectroscopy offers better specificity than UV-Vis spectroscopy and fluorimetry for identifying graphitic forms of carbon such as GFNs. In Raman spectroscopy, GFNs can be detected using the signature defective (D, ~1350 cm^{-1}) and graphitic (G, ~1580 cm^{-1}) bands representative of the sp^2 hybridized network of carbon disrupted by edges and defects along the basal plane.[101, 102] With Raman spectroscopy, higher oxygen functional group levels increase the D band intensity and decrease the G band intensity, leading to higher D/G band ratios for GO than for graphene. A decrease in lateral size also increases the number of defect sites relative to the graphitic carbon regions, increasing the D/G ratio.[40, 135] The intensity of the D and G bands can be used to quantify the GFN concentration in a consistent Raman configuration or by measuring the intensity of the D or G band relative to a reference peak.[136, 137] The G' band (~2650 cm^{-1}) can also be used with pristine graphene, but decreases in intensity occur much more readily than in the G band with an increasing number of defects in the graphitic structure, which are likely to form in the environment.[101]

Raman instruments are configured differently depending on their use for dry or liquid samples and the choice of which configuration to use can depend on the GFN form and matrix under investigation. For example, it is more appropriate to measure GFNs in powder form with a Raman microscope while it is more appropriate to measure GFNs in an aqueous suspension with a Raman system built to hold cuvettes. Raman instruments are also widely availability at universities but less available in environmental testing laboratories.

Raman spectroscopy is commonly used for detection of GFNs in purified aqueous systems and some synthetic media, but not in natural waters, which contain other types of graphitic carbons (such as humic acid, clays, black carbon, and other graphitic carbon) that can have overlapping D and G bands.[138, 139] When GFNs are analyzed in water with Raman spectroscopy, the G band of GFNs overlaps with the H-O-H bending transition of water band (1640 cm^{-1}), which has previously been shown to limit the quantification of CNTs, at least with respect to the G band.[140]

The characteristic nature of the D and G bands of GFNs allows for the use of Raman spectroscopy in detecting and quantifying GFNs in biological matrices and polymer fragments provided there is a reference peak to use for normalization.[137, 141] Raman spectroscopy has low throughput, however, which makes it challenging to use for probing large sample areas in dried-down polymer fragments and determining the detection limit in tissue matrices. Another challenge is that degraded or highly oxidized polymer fragments generated during environmental weathering processes, often fluoresce strongly and can interfere with the D and G bands in the

Raman spectrum, either through a rising background or development of overlapping bands from fluorescent byproducts. Nevertheless, the D and G bands of GFNs in Raman spectroscopy are fairly unique and can be used to distinguish the GFNs from the polymer matrix if polymer byproduct peaks do not overlap and the fluorescent background is adequately corrected.[141] Thus, it is likely that Raman spectroscopy will be used and continually developed for GFN detection in biological matrices and polymer fragments. In contrast, soils and sediments contain numerous forms of carbonaceous substances (including graphitic forms), making it impossible to utilize Raman spectroscopy except after an extraction procedure is performed.[142, 143]

X-ray Photoelectron Spectroscopy (XPS)

X-ray photoelectron spectroscopy (XPS) analyses are widely used in studies for characterizing and, at times, detecting GFNs present in purified and synthetic waters.[72, 93, 113, 115, 144-148] It is important to note that for XPS, samples must be deposited on a substrate and any water present must be evaporated prior to measurement. XPS is subject to interferences from the abundant, naturally-occurring carbon constituents in natural waters, sediments and biological matrices, and is therefore not very useful for quantifying GFNs in these matrices. However, this may change if sophisticated extraction techniques are developed. Nevertheless, the carbon content of GFNs can be quantified relative to another element in the absence of other carbonaceous species or the presence of a less conductive carbonaceous material (e.g., a polymer).[63, 149] When one carbonaceous material such as a polymer is less conductive than the GFN, the charge neutralizer of the XPS system can be turned off, and the polymer component of the C(1s) peak can differentially charge or shift away from the GFN component, thus allowing (hypothetically) for GFN component deconvolution, integration, and semi-quantification. This has been demonstrated with CNTs and is yet to be demonstrated with GFNs.[63, 149] It is only likely to be successful with graphene or rGO, since they are conductive; in contrast, GO is not conductive with a graphenic structure disrupted by oxygen functional groups. Another important point is that XPS is a surface sensitive technique that can probe only the top ~10 nm of a material so it cannot be used for reliable bulk measurements of larger polymer fragments.[150] Furthermore, GFNs must be homogeneously distributed within the sample since the spot size covers an area on the order of microns and in terms of sample amount, a few milligrams of material are needed for analysis. Other disadvantages include the high cost of XPS and the fact that samples are prone to contamination through adventitious carbon adsorbed onto sample surfaces.[151] Overall, it is unlikely that XPS will be used to detect GFNs in environmental matrices, since all matrices contain carbonaceous species. However, XPS may be useful to detect GFNs in small polymer fragments released from polymeric nanocomposites into pure aqueous systems.

Other Spectroscopic Approaches

Transient absorption spectroscopy, a specialized technique, has also been shown to provide fast visualization and quantitation of GFNs within living cells but the accuracy of quantitation is dependent on the dispersion state of the nanomaterials.[133] This technique is only likely to be applied in specialized laboratories due to its high cost and complexity.

GFNs also have unique X-ray diffraction patterns, which may make their detection possible with X-ray diffraction (XRD) techniques. However, a large amount of material 10 mg to 100 mg is required.[152, 153] Furthermore, a GFN reference is necessary to distinguish a particular GFN from the matrix it resides in. Consequently, XRD may be most useful for evaluating released GFN/polymer nanocomposite fragments in mg quantities.[152] Furthermore, XRD instrumentation is generally expensive and not always available to environmental testing laboratories.

Microscopic Techniques

The two-dimensional shape and lateral size of GFNs (tens of nanometers to several micrometers) enables their detection with a variety of microscopic techniques such as atomic force microscopy (AFM), scanning electron microscopy (SEM), transmission electron microscopy (TEM), laser scanning confocal microscopy (LSCM), and hyperspectral imaging.[16, 154] When compared to CNTs which have cylindrical structures, the 2D morphology of GFNs is less distinct, which may make GFN identification in natural matrices via microscopy very challenging. GFN quantification is possible with microscopic techniques but it may be overly time-consuming and as a result, is often impractical or infeasible in complex matrices.[155] For electron microscopy, limitations may include the choice of dilution factor when drying down GFNs so that particles do not overlap, assessing consistency in and determination of thicknesses and agglomeration state, and evaluating the degree to which the GFNs wrinkle, which could make GFN counting a challenging task. There are no reports, to our knowledge, where the researchers counted the number of graphene layers and the number of graphene particles present, especially in an environmental sample. In addition, these instruments are often fairly expensive, and accessibility is limited to universities and other user facilities rather than environmental testing laboratories.

For microscopic techniques, any water present must be completely evaporated, except when using techniques such as environmental SEM (ESEM), cryo SEM (CSEM), AFM, or low vacuum SEM (LVSEM).[113, 115, 137, 147, 156-158] Drying of samples for microscopy may introduce artifacts, but this can often be avoided with careful sample preparation. For instance, salts left after evaporating synthetic media or natural water can deposit onto or even mask GFNs (depending on the salinity of the synthetic media or natural water), but an ultrafiltration step prior to drying can substantially reduce the salt concentration present. In general, the amount of

GFNs expected to be present in natural waters is very low compared to the amount of other particle types (e.g., clay), which may make the detection of GFNs in natural waters challenging via microscopy. With proper dilution, microscopic techniques such as energy-filtered TEM (EFTEM) and hyperspectral imaging may be used to identify GFNs in some environmental matrices based on unique GFN interactions with electrons and the electromagnetic spectrum.[156] With proper dilution, semi-quantitative analysis of GFNs may be possible with microscopic techniques such as TEM (e.g. by using software programs such as ImageJ),[159-161] laser scanning confocal microscopy (LSCM),[159] and hyperspectral imaging.[156] Due to the complexity of soils and sediments, GFNs would have to be extracted from these matrices prior to identifying them using microscopy.

In polymer nanocomposite fragments, GFNs can be observed with techniques such as SEM and TEM if GFNs are close to the polymer surface (within 10 nm to 100 nm).[162] Some light microscopy techniques, such as laser scanning confocal microscopy (LSCM), may also be employed depending on the size of the particles with respect to the diffraction limit of light used in the microscope. However, detection and quantification of different fragment types and freely released GFNs is time-consuming and often impractical since a high number of images are required for robust statistical inferences to be made. In general, microscopy will continue to be a useful tool for GFN detection, and sometimes quantification. However, efforts are needed to decrease the time it takes to prepare and image samples, and the improvements that can be made will likely only be incremental. Nevertheless, microscopy will continue to be useful as a supplementary characterization technique.

Thermal Techniques

The graphitic structure of graphene also leads to high thermal stabilities which decrease with increasing GFN oxidation level.[96] The high thermal stability of graphene permits its detection at much higher temperatures than GO.[96, 163, 164] Figure 3 reports the temperature range at which GFNs show the most change during thermal decomposition under inert conditions. Areas of overlap with the different media, matrices, and systems presented in this text are shown (Figure 3) and illustrations of thermal gravimetric analysis (TGA) profiles for different GFNs and polymer matrices are shown in Figure S2a and S2b, respectively. Unlike for graphene, the decreased thermal stability of GO causes its thermal profile to overlap with many carbonaceous species, thus hindering its quantification using thermal methods.

Analytical techniques that leverage the unique thermal properties of GFNs such as thermal gravimetric analysis (TGA), total organic carbon (TOC) analysis,[145] and programmed thermal analysis (PTA)[96] are useful both for characterizing and quantifying CNMs, by drying down an aliquot from aqueous media. Since graphene is thermally stable, there is not much interference when using thermal techniques in purified, synthetic, and natural waters which

contain mostly labile forms of carbon. However, interference is typically higher in natural waters that contain large amounts of suspended particles or in complex media such as untreated wastewater.[96] In such systems, the thermal profiles of GFNs, the matrix (e.g., natural water) without GFN if available, and GFNs mixed into the matrix should be characterized to assess any matrix interferences and determine if the matrix impacts the thermal stability of the GFNs. Overlaps between the thermal profiles of GFNs and the matrix can be easily accounted for when they are not substantial.

Unlike graphene, GO and sometimes rGO have a very high oxygen content and number of defect sites, which make them less thermally stable.[165] The application of thermal techniques, such as PTA, to quantify GO (without further modifications) may be restricted to purified or synthetic waters without added NOM if there is an overlap between the GO and NOM thermal profiles. For aqueous media containing NOM or natural waters, it may be necessary to chemically reduce (using hydrazine, sodium borohydride, ascorbic acid, etc.[92, 96, 165-167]) GO in order to increase its thermal stability relative to that of the organic carbon in the matrix.[96] In addition, other carbonaceous (nano)materials such as soot, CNTs, and fullerenes may be present with GFNs in natural waters (e.g., wastewater), which may make the detection and quantification of GFNs via thermal techniques more complicated. One possible solution is to selectively extract GFNs from the matrix while excluding other carbonaceous materials like soot and CNTs, but there are currently very few methods for achieving such a selective extraction and all extraction procedures result in some loss of the analyte.[168] Alternatively, it may be possible to add known quantities of the GFN material of interest (or a GFN with a similar thermal profile) to the water matrix to quantify the amount of background carbonaceous materials interfering with GFN quantification.[96] Another probable challenge with using thermal techniques for quantifying GFNs in natural waters is the potentially high detection limit of some thermal instruments relative to the amount of GFNs expected in natural waters. A detection limit of 1.7 µg was reported for GO in pure water using PTA while other thermal techniques such as TOC analysis and TGA may have even higher detection limits.[96] More so, the detection limit of these instruments may increase substantially when GFNs are mixed into complex natural waters depending upon the overlap in the thermal profile of the matrix and GFN. Overall, none of the techniques described so far can conclusively detect GFNs without measurement of a reference GFN, the matrix without added GFN, and/or extraction.

Analytical methods relying on the thermal properties of GFNs may be applicable to quantify the nanomaterials in biological matrices without extraction if there is not substantial interference between the thermal profiles of the nanomaterials and matrices. Given the small amount of GFNs expected to be internalized in cells and tissues, even slight interferences from the matrix can overshadow the GFN signals. However, high concentrations may be present in the gut tract of organisms such as *Daphnia magna*; therefore, if voiding of the gut tract is not performed, measurements of total body burden will be dominated by the GFN mass in the gut tract, yet, unlike bioaccumulation measurements for dissolved chemicals, these values will not

reflect the GFN mass adsorbed across epithelial surfaces. [41, 58] Similar to other matrices, the thermal instability of GO compared to graphene, may make it difficult to detect GO in biological matrices via thermal techniques due to substantial interference. Also, changes in the chemical and thermal properties of intracellular/internalized GFNs (relative to their pristine forms) are currently unknown, and may interfere with analyses. Quantification of GFNs in biological matrices via thermal techniques may be less challenging if the nanomaterials are extracted by digestion or lysis of the cells/tissues either chemically or mechanically.[96] For example, PTA has been used to quantify CNTs in rat lungs[169] and GFNs in wastewater biomass after extraction.[96, 118] Care has to be taken to ensure that the chemicals used for digestion do not degrade or oxidize GFNs if the analysis technique can be impacted by the GFN oxidation state.

Studies have not yet been conducted to investigate the ability to detect and quantify GFNs in soils and sediments using thermal techniques, but studies have shown that CNTs can be measured in these matrices using thermal techniques albeit with varying levels of success.[170-172] The major challenge of using thermal techniques for characterizing GFNs in soils and sediments is that these matrices contain thermally stable elemental carbon (e.g., soot), which can substantially interfere with GFNs, as was the case with CNTs.[170, 171] However, the ion ratios of gases evolved upon thermal degradation of GFNs may be substantially different from the ion ratios of gases originating from soil and sediments, which provides an opportunity to quantify the nanomaterials in these matrices.[172] The required instrumentation for this type of analysis is relatively expensive and uncommon in environmental science laboratories, as the thermal instrument has to be coupled with a mass spectrometer to analyze the gases evolved. Thus, there is a need for methods that are more readily available for practical detection and quantification of GFNs in soils and sediments.

Graphene can be easily distinguished from a polymer matrix using thermal analytical techniques since the thermal stability of polymers tends to be below 400 °C, well below the thermal stability of graphene (Figure S2a). In contrast, the thermal profile of GO can overlap with the thermal profile of many polymers (Figure S2b). Furthermore, the mass loss of GO and rGO is gradual over a large temperature range (Figure 3) in TGA, making it challenging to differentiate the polymer from the GFN. Experimentation with conditions such as a switchover from inert gas to air flow at different temperatures may be useful, in some cases, for differentiation of polymer from GFN. Small differences in thermograms, such as first derivative shifts in TGA or slight shifts in glass transition temperatures (T_g) with differential scanning calorimetry (DSC), can be employed to measure the mass fraction of GFNs in polymer fragments or polymer matrices.[173-176] These approaches, however, require a reference polymer material and a calibration curve of similarly dispersed GFNs in the polymer at varied concentrations. If lower mass fractions (< 1 %) of GFNs are incorporated into polymers, their signal must be discernible from the polymer background and from polymer charring. This approach is not practical in every application.

Labeling Techniques

Metal ion labeling, isotopic labeling, and fluorescence labeling of GFNs provides a unique opportunity for the detection and quantification of GFNs that avoids some of the interferences observed with other methods. However, these approaches are typically only applicable for laboratory studies given that GFNs in the environment will not be labeled. Labeling of GFNs with materials that are (or have properties that are) not intrinsically found in environmental matrices provides an opportunity for detecting and quantifying the nanomaterials within these complex matrices.

In aqueous systems, especially natural water, there are very few interferences (with well-designed labels) compared to most other methods. Similar to CNTs, metal ions can potentially be coordinated to GO or incorporated into a GFN structure for use as a GFN proxy.[141, 177] Inorganic elemental analysis using techniques such as ICP-MS can then be employed to detect and quantify the GFN concentration.[141, 155] The metal ion used must be properly chosen so that the metal is not present in the natural water at sufficiently high concentrations to bias the measurements. Furthermore, the coordination of the metal to the GO oxygen functional groups must remain unchanged throughout the experiment or proper controls must be run to measure the percentage of coordinated metal ion loss during any environmental transformation.[155]

Isotopic labeling of graphitic carbon can also be used as a means for detection and quantification.[42] ^{14}C-isotopes are stable, and techniques based on their detection and quantification have been used to study the fate and transformations of GFNs (mostly graphene) in aqueous systems.[37, 42] Quantification of isotopically labeled GFNs allows for laboratory studies to be carried out at very low GFN concentrations (ng/L to µg/L range)—much lower than would be possible with most other analytical techniques.[37, 42] The radioactivity of isotopically labeled GFNs is quantified using liquid scintillation counting (LSC) with or without combustion of the graphene; combustion transforms the GFN to $^{14}CO_2$ prior to the LSC analysis. In one study, direct addition of a FLG suspension to scintillation cocktail followed by scintillation counting was hypothesized to underestimate FLG radioactivity due to interferences with beta emissions, likely from self-quenching of graphene agglomerates or within the layers of the FLG.[58] Higher radioactivity recovery rates have been achieved by combustion of FLG stock suspensions in a biological oxidizer with capture of the released $^{14}CO_2$ in scintillation cocktail followed by quantitation using LSC; biological oxidation eliminated the potential for self-quenching, but led to a lower precision.[58]

In biological systems, labeling GFNs with metals, fluorescent dyes, and ^{14}C isotopes may enhance the ability to detect and quantify intracellular GFNs *in situ*. Labeling of GFNs with materials such as fluorescene isothiocyanate (FITC) and ^{14}C-isotopes allows for their detection and quantification using techniques such as LSCM and radioactivity measurements, respectively.[37, 42, 159] Label-based GFN detection techniques are also capable of providing information on the bioaccumulation and translocation of GFNs within biological matrices.[58, 154,

[159, 178] Real time investigation of uptake and localization within small organisms (such as zebrafish embryos) is also possible with FITC-labeled GFNs.[159] In addition, GFNs can also be labeled with metals (preferably metals that are not inherently found in cells and tissues) which can be used as a tracer for the GFNs. One important consideration is that metallic and organic labels may interfere with normal cellular processes and/or may be toxic to organisms.[141, 179] The potential occurrence of these label-induced adverse effects to cells or organisms should be tested before using labeled GFNs in laboratory studies.

In soils and sediments, some techniques using labeled GFNs may be less prone to interferences compared to other types of techniques. Specifically, ^{14}C-labeled GFNs can typically be detected and quantified at low concentrations in soils and sediments.[57] In contrast, metal ions in these soils/sediments are often present at high concentrations and can interfere with metal ion labels. For fluorescence, the potential for fluorescence quenching by components in soils and sediments may limit the applicability of this type of labeling approach. Overall, labeling is very useful for laboratory studies that model outdoor conditions, but is unlikely to be used for detection and quantification of GFNs found in the environment.

DIFFERENTIATION OF OTHER CARBONACEOUS NANOMATERIALS FROM GFNS

All of the techniques applied to GFNs in Table 1 have been previously used for other graphenic nanomaterials such as CNTs and fullerenes; the differences in CNM structure are shown in Figure S3.[89] Therefore, the ability to differentiate GFNs from other carbonaceous nanomaterials must be considered as techniques are developed for the detection and quantification of GFNs present in environmental matrices. With the exception of microscopy, almost all of the techniques presented cannot completely differentiate GFNs from other graphenic nanomaterials.[16] For example, CNTs and fullerenes absorb/optically scatter light, have D and G bands at similar wavenumbers in Raman spectroscopy, and can have similar thermal profiles.[89] However, subtle differences between spectra and the use of nanomaterial controls can sometimes be used to differentiate CNMs. For example, the wavelength of absorption in UV-Vis spectroscopy is strongly dependent on the nanomaterial structure and dispersability.[92] In Raman spectroscopy, GFNs tend to have larger D bands relative to CNTs since there are more edge defects per total area in a flat structure than the number of defects present at the ends of a CNT cylinder (where the majority of defects reside). Thus, the D/G ratio can be much larger for two-dimensional versus three-dimensional graphitic carbon structures.[135, 180] Due to their strained curvature, CNTs and fullerenes tend to be less thermally stable than graphene. Thus, graphene may be differentiated from CNMs using thermal techniques with proper controls.[181] Microscopic analysis allows for differentiation of fullerenes, carbon nanotubes, and GFNs based on their unique physical structures and through the use of EFTEM utilizing differences in their electron energy loss spectra. However, GFNs can be difficult to detect with microscopy as a result of their

two dimensional structure.[16] Microwave-induced heating methods have been successfully used to quantify MWCNTs in biological samples by measuring the rapid thermal response of MWCNTs relative to the surrounding matrix, but a slower microwave-induced heating response was found for graphene powder. Thus, microwave-induced heating methods have not yet been shown to be useful for GFNs.[182] Other techniques such as metal ion labeling combined with inorganic elemental analysis and isotopic labeling can be used to differentiate GFNs from other types of graphitic nanomaterials but are limited to laboratory studies. Overall, the techniques previously developed for CNTs and fullerenes can be similarly applied to GFNs, with only some small differences that permit GFN differentiation in a CNM mixture.

EFFECT OF GFN INTERACTIONS AND TRANSFORMATION ON GFN QUANTIFICATION

In general, GFNs possess extremely large surface areas and highly negative charge densities when oxidized (i.e., GO),[3, 92] which enable them to adsorb various organic and inorganic compounds in the aqueous phase—including nutrients, NOM, and metal ions.[183-189] These interactions, mediated by π-bonding and hydrophobic interactions, electrostatic interactions, and hydrogen bonding, influence the surface charge of GFNs and thus their homo-agglomeration, and heteroagglomeration with other particles in aquatic systems such as clays, metallic colloids and organic particles.[30, 97, 188, 190, 191] Adsorption of inorganic ions (e.g., metal ions) neutralizes the surface charge of GFNs, which typically leads to decreased colloidal stability;[34, 37, 99, 114] while adsorption of organic materials (such as NOM) increases the colloidal stability of GFNs via increased electrostatic repulsion and/or steric hindrance.[37, 114] The interactions of organic and inorganic compounds (including other colloids) with GFNs also lead to formation of agglomerates with different morphological conformations,[34, 97, 192] which (like changes to colloidal stability) can interfere with techniques such as UV-Vis spectroscopy and fluorimetry. More readily water-dispersible GO will also decrease in colloidal stability if the salt concentration is sufficiently high due to suppression of GO's electric double-layer by the cations in salts.[30, 32-34, 99] Importantly, the adsorption of organic compounds (such as NOM) and nutrients onto GFNs may contribute to the signals obtained from non-specific analytical methods such as UV-Vis and TOC analysis.

Transformations of GFNs have been shown in natural conditions, and these transformations can potentially interfere with GFN detection and quantification. For instance, exposure of graphene to water changes its morphology, and results in greater disorder of the structure (increased D/G band ratio), and expansion of the d-spacing (the distance between adjacent planes in the crystal structure).[193] These physicochemical changes are further enhanced when graphene is exposed simultaneously to water and visible light.[193] In the case of GO, sunlight reduces the primary particle size and colloidal stability, which may interfere with techniques such as UV-Vis spectroscopy and fluorimetry.[40, 43, 72, 95] These transformations should

be considered when quantifying sunlight-exposed GO with spectroscopic and thermal techniques.[8, 40] GFNs in the natural environment are also subject to chemical transformation by strong, naturally occurring oxidizing agents such as hydrogen peroxide (H_2O_2), found in rain and natural waters. The degradation of graphene (and most likely, other GFNs) can occur at concentrations of H_2O_2 that naturally occur in surface waters (51 mg/L to 231 mg/L or 1 x 10^{-3} mol/L to 7 × 10^{-3} mol/L),[147, 194] leaving defects on the surface of the GFN. Similarly, iron/H_2O_2-driven Fenton chemistry (with or without UV irradiation), a treatment technique commonly applied in wastewater treatment plants, can generate reactive oxygen species (ROS; such as hydroxyl radical, •OH) which can cause defects in the GFN structure, and even lead to complete degradation to CO_2 at sufficiently high concentrations of the reactants.[42, 93, 195] Structural defects can make the detection and quantification of GFNs more complicated when using analytical techniques that rely on structural properties. Furthermore, when using non-degraded GFNs for calibration, the use of UV-Vis spectroscopy and fluorimetry to quantify degraded GFNs may lead to inaccurate estimation of GFN concentration.[8, 40, 93, 195]

Transformation of GFNs arising from interactions with cells and organisms has not been widely investigated but a few studies have shown that GO can be reduced by microorganisms via direct contact and electron shuttling.[196-199] Reduction of GO by bacterial genera (including *Shigella*, *Shewanella*, and *Escherichia*) occurs as the nanomaterial acts as an electron acceptor for the electrons generated during respiration. Similar reduction of GO has been shown by other biological molecules, including plant extracts.[200, 201] As mentioned earlier, reducing the oxygen-containing functional groups on GO will influence its spectroscopic and thermal response and thus may interfere with measurements. The size and thickness of graphene were shown to decrease in a chemical reaction catalyzed by horseradish peroxidase, showing that the enzyme can change the morphology of GFNs.[202] The effects of horseradish peroxidase (in the presence of H_2O_2) on the structure of GO was even stronger (than that of graphene)—resulting in the formation of holes (up to 27 nm wide after 10 days) in the graphitic lattice of the basal plane, and complete oxidation to CO_2 after 20 days.[203] The study however found no effects of horseradish peroxidase on rGO possibly due to tighter binding between the rGO and enzymes, which retarded the dynamic motion of the enzymes.[203] Overall, these enzyme-catalyzed changes should be considered when analyzing enzyme-exposed GFNs with spectroscopic and microscopic techniques.

Changes in the physicochemical state of GFNs in soils and sediments can further complicate their measurements in these matrices. For example *Shewanella*, a microorganism that has the ability to transfer electrons extracellularly (i.e., an exoelectrogen), which has been shown to reduce GO in laboratory studies, is present in freshwater and marine sediments and may also use GO as a terminal electron acceptor in these matrices.[204] In fact, in a study testing five strains of *Shewanella* obtained from different natural environments, the strain obtained from marine sediments (Pacific Ocean) achieved the highest GO reduction—more than 95% of the carbon left in the GO was in a reduced state after 24 h (compared to 83% obtained by using hydrazine, a

commonly used GO reducing agent).[196] Additionally, *E. coli*, a bacterium found in almost all environmental media, including soils and sediments, has also shown the ability to reduce GO.[10, 199] Chemical degradation of GFNs can occur via Fenton reactions in soils and sediments but has not been studied. In addition, all GFNs are likely to strongly bind to dissolved organic carbon and colloids in marine sediments due to the high ionic strength of marine waters. Further, GFNs with low surface charges (i.e., graphene and rGO) will adsorb to soil/clay particles due to weak repulsive forces.[98, 205, 206] It is also likely that GFNs will behave similarly to CNTs and strongly interact with organic matter in soils and sediments.[207-209] These interactions will affect the bioavailability of the nanomaterials as well as their extractability (and thus, measurements in soils and sediments).

EXTRACTION

Isolation of GFNs from other materials present in an environmental matrix is an important component of GFN detection since GFNs can have similarities to other matrix materials, which can also be carbonaceous and graphenic, and can hinder identification of GFN via microscopy. The process of isolating GFNs from an environmental matrix by transfer of the GFNs from the matrix phase to another phase is termed extraction.[89] Extraction methods typically involve transfer of the GFNs out of the initial matrix phase into a phase where the interfering compounds are less soluble. Conversely, removal of the interfering compounds to another phase can also be applied.

Methods to extract GFNs from environmental matrices can be considered in the context of CNT and fullerene extraction methods that have already been successfully employed. For examples, CNTs have been extracted from environmental and biological matrices with asymmetric field flow fractionation (AF4),[210] matrix digestion,[211] and sonication with surfactants.[82] Techniques used for CNT purification (i.e., separating a distribution of CNTs into homogeneous fractions) such as gel permeation chromatography, capillary electrophoresis, density ultracentrifugation, and two-phase polymer extraction may also be considered with respect to the extraction of GFNs from environmental matrices.[212-214] Fullerene extraction has been even more thoroughly studied than CNT extraction, most likely due to having a less heterogeneous distribution of particles, at least in terms of size, and their affinity for many organic solvents such as toluene. Fullerenes have been extracted from complex matrices using solid phase extraction techniques (i.e., chromatography) and liquid-liquid phase extraction, mostly with toluene as the non-polar phase, and sometimes, the addition of salt to destabilize the nC_{60} particles.[80, 91, 215] Importantly, extraction approaches have been successfully used to enable quantification of fullerene concentrations in complex matrices such as sediments,[216, 217] soils,[218, 219] and organisms.[220, 221] An approach for detection of oxidized fullerenes (i.e., fullerols) has been the addition of salt and toluene for liquid or solid phase extraction, and, occasionally, solid phase extraction of oxidized fullerenes in an aqueous phase after less oxidized fullerenes are separated out using toluene.[80, 215] Since GFNs have a different shape than CNTs and fullerenes, a distribution and range of physical dimensions, surface chemistries that can range from

hydrophobic to hydrophilic, and are affected by transformation processes in the environment, testing of CNT and fullerene extraction methods with GFNs needs to be attempted and modified as needed. Although it is unlikely that a "one-size-fits-all" approach will work considering the range of physicochemical properties that GFNs can have, development of efficient and simple extraction techniques for GO, rGO, FLG, and single-layer graphene along with extraction techniques for GFNs with various lateral sizes and thicknesses would be very useful. Furthermore, extraction of all types of carbonaceous nanomaterials requires development of more general combined strategy approaches of filtration, differential extraction and functionalization/defunctionalization.[91]

Only a few studies have used extraction methods to isolate GFNs from environmental matrices. In Doudrick et al,[96] both FLG and GO were extracted from biomass grown from return activated sludge. Solvable, a tissue solubilizer consisting of sodium hydroxide, C10-16-alkyldimethyl, N-oxide, and C11-15-secondary, ethoxylated alcohol, was used for matrix digestion (at 60 °C for 24 h). For GO, a sodium borohydride ($NaBH_4$) reduction step was required to remove surface-bound oxygen from GO and thus increase its hydrophobicity for pellet formation during centrifugation. The samples were centrifuged, washed twice with water, and the formed pellet was analyzed with PTA. Extraction of 20 µg GFNs from 200 mg dried biomass/L wastewater followed by PTA analysis yielded recoveries of 52 ± 8 % and 80 ± 6 % for FLG and GO, respectively.[96] The authors also tried phase-separation of reduced GO by heating for longer times in the $NaBH_4$ step and allowing the surfactant phase of Solvable, containing rGO, to separate from the aqueous phase; this increased the reduced GO recovery, but was not successful at phase-transferring the FLG.[96] Overall, this study demonstrates the challenge in recovering GFNs with a range of surface functionalities, especially without reduction of GO to a more thermally stable form. In another study, graphene was extracted from water using oil, toluene, and hexanes, but this study did not consider the effect of these solvents on environmental media where other hydrophobic components would transfer to the hydrophobic phase.[222] Finally, a separate study made use of GO and rGO as a microbarrier between water and dispersed organic droplets, where spontaneous assembly of GO and rGO sheets was observed at the droplet interfaces with 'tunability' of the process possible using multivalent cations.[223] Applications of approaches such as this to environmental matrices may be worthwhile to investigate further as there are currently no studies involving GFN extraction methods for cells, tissues, soils, sediments, and complex waters other than wastewater. The approaches may, in some cases, be matrix specific such as lysing cells first in biological systems or filtration techniques in the case of soils and sediments.

Ideally, extraction techniques should be able to completely separate interfering materials or compounds in the matrix from the GFN of interest or completely eliminate the interfering materials while preserving the GFN. However, methods that can sufficiently reduce the amount of interfering material such these substances no longer overwhelm the signal from the GFN of interest may also be successful. For instance, thermally stable or graphenic materials (other than

GFNs) from complex media such as soil and sediment must not phase-transfer when using techniques such as PTA and Raman spectroscopy, but if they do, microscopy (which is time-consuming) may become a more viable option since the matrix materials will be less abundant. For released GFNs from polymer nanocomposites, many GFNs will be encased partially or fully in polymer fragments, which will alter their ability to phase-transfer, and extraction techniques that phase-transfer particular polymer types might be more suitable or need to be used in addition to GFN extraction techniques.

Currently, the ability to distinguish other carbonaceous nanomaterials (of high thermal stability) from GFNs with the centrifugal separation method using Solvable or the other methods described for CNTs in this section have not been thoroughly explored, and further study would be useful. In one study, a quantitative method based on isolating CNTs with specific DNA oligomers successfully separated CNTs from GO.[168] However, it is unknown if the method will be applicable to isolating GFNs from other natural and engineered CNMs.[168] Future application of this approach to a range of GFNs is worthwhile since this method was useful for extraction from an environmental matrix.[168] In addition, the orientation of engineered CNMs (such as CNTs, graphene, and GO) in suspension can be ordered upon the application of external stimuli such as induced flow, or magnetic or electric field.[64, 224, 225] The differences in the degree and potential for ordering of different CNMs under different scenarios may be useful for separating GFNs from other types of engineered CNMs, and this possibility is worth exploring.

GFN DETECTION/QUANTIFICATION CASE STUDY

For GFN quantification, one of the first considerations is which instruments are available in the testing laboratory. In Figure 4, the availability of different techniques for GFN quantification is compared based on their availability for purchase and their availability in environmental testing laboratories. It is interesting to note that most of the techniques discussed in this paper are commercially available, but most of the techniques are not typically available in environmental testing laboratories. Another important consideration relates to the method used to stabilize the GFN in water. If a dispersant is needed, that could influence the technique selected, while the potential for the GFNs to agglomerate in the water could also impact the feasibility of using some techniques. One helpful approach to understand the appropriate choice and use of GFN quantification techniques is to consider two case studies where there are clear advantages to using different sets of techniques. Two realistic scenarios for graphene quantification involve 1) regulatory toxicity testing of a GFN using an acute immobilization method for *Daphnia magna* (OECD method 202) and 2) monitoring the concentration of a GFN released from a manufacturing plant in industrial effluent discharged into a receiving river to insure GFN concentrations in the river are below a specific hazard level. To describe how to quantify the GFNs in the case studies described above, we will assume that all commercially available techniques are available to discuss how quantification could be addressed in a best-case scenario without instrumental limitations. Compared to the effluent scenario, it will be more straightforward to analyze the suspended GFN in the toxicity testing stock suspensions for the *D.*

magna assay since there will be fewer interfering compounds present. In contrast, when the effluent is mixed with the natural water present in the river, there will likely be NOM and other suspended organic and inorganic particles. For the *D. magna* assay, the technique to use depends mainly on the detection limit needed, and if the stock suspension or the suspension in the test media before or after the test is being analyzed. As described in Table S1, a relatively low detection limit can be obtained with PTA analysis for the stock suspension and this technique should work in this stock suspension regardless of the GFN thermal properties because there are no other compounds present that would interfere with GFN detection (unless a dispersant is used to help suspend the GFN). Other techniques may also be applicable but would depend on their limits of detection, which would need to be tested given the lack of data on this topic in the published literature, and if interferences in the test media could impact the measurement. For example, one complication for UV/Vis spectroscopy analysis is that the GFNs may agglomerate during the exposure and this could impact the absorption coefficient which may in turn bias the concentrations measured.[226] The situation is more complex for the river water scenario as described above. A first step is to collect water prior to the point where the manufacturing plant contacts the river (i.e., the influent), and to assess to what degree the properties of the river water could impact the test results for various techniques (e.g., the impact of NOM on TOC or Raman measurements). If the GFN to be tested is available, it could then be dispersed and spiked into the river water to evaluate potential matrix effects and recovery for different techniques which include Raman spectroscopy, PTA, and extraction followed by UV-Vis. Based on these results and the detection limits needed, the best technique can be selected to evaluate the test sample. As a last resort due its time-consuming nature, SEM or TEM microscopy could be applied with dried-down river water samples for counting, assuming the matrix does not overwhelm identification of the GFNs.

OUTLOOK AND FUTURE RESEARCH TOPICS

The concentration of GFNs released into the environment needs to be better understood. The lack of data on environmental GFN concentrations is a result of scattered information on the number of products on the market that hinders predictive modeling, the difficulty of detecting the very low GFN concentrations (e.g., ng/L) expected in the environment, environmental transformations of GFNs or interactions with other particles or NOM in the environment, and the lack of techniques for GFN detection in complex environmental media and biological systems. The quantity of GFNs released from polymer nanocomposites and other consumer-relevant matrices is also largely unknown, especially relative to CNTs where the high aspect ratio, molecular structure, and entanglement have shown low CNT release (undetectable to µg level).[90, 111, 112, 149, 227]

Currently, the ability to quantify GFNs in purified, natural and synthetic aqueous media is in its infancy and requires further development. For example, it may be possible to further

improve PTA by combining that analysis with Raman given that the D to G band ratios may reveal insights into the thermal stability of the GFN, as has been previously demonstrated for CNTs,[170] and therefore inform the thermal program to use. Additional work on hybrid Raman-PTA instruments could be valuable as could investigations into different carrier gases or the addition of new detectors to PTA instruments. Detection and quantification are even more challenging in complex matrices where creative approaches would be most valuable for environmentally relevant studies. Complex matrices often possess unique interferences that affect GFN detection and quantification. The potential biases and limitations of each method can potentially be overcome by using multiple analytical techniques for a given GFN/matrix. Some methods such as thermal analysis and UV-Vis spectroscopy were found to provide similar detection events for GFNs and other CNMs, such as CNTs, while other methods such as Raman spectroscopy and microscopy, have subtle or substantial differences allowing for a more unique level of detection of GFNs in the presence of other CNMs. Techniques such as Raman spectroscopy require further development to permit quantification in addition to detection, while labeling (e.g., with ^{14}C or FITC) has been successfully used in multiple laboratory studies. Other techniques that can distinguish between GFNs and other carbonaceous (nano)materials, such as microscopy, may be combined with thermal techniques for a more reliable quantification of GFNs in complex aqueous matrices.

Extraction is often necessary for reliable quantification of GFNs, especially when GFNs are present in natural aquatic and terrestrial media, and biological matrices. However, methods for extracting GFNs from natural and synthetic matrices are currently scarce.[96] Furthermore, the mass concentration of GFNs in the environment is expected to be in the ng/L range, and without isolation and concentration of GFNs, the use of the techniques described in this text are limited for GFN detection in environmental samples. Developing robust extraction methods can also enable more cost-effective options for environmental testing laboratories to detect and quantify GFNs present in environmental matrices. Extraction techniques must also be designed to consider the range of physicochemical properties that GFNs can have in order to specifically target GO, rGO, FLG, or single-layer graphene along with GFNs having a range of lateral sizes and thicknesses. Currently, it is not clear if the extraction methods previously developed for carbon nanotubes (CNTs) will be as effective for GFNs due to differences in physicochemical properties.[82, 96, 168, 228, 229] Also, existing extraction methods are not likely to effectively distinguish between the different types of CNMs (i.e., CNTs, fullerenes, GFNs, etc.), should they co-exist in a matrix. In addition to selectivity between the different engineered CNMs, such techniques should also be able to separate GFNs from incidental and naturally-occurring carbonaceous particles such as soot and black carbon. Once methods for extracting and analyzing GFNs in natural and synthetic matrices are readily available, the ability to comprehensively quantify GFN exposure will be achieved. Consequently, research geared towards the development of extraction techniques that are specific to GFNs and work across relevant matrices is needed.

As reported in this review, several studies have shown that transformations of GFNs (including graphene and GO) can occur in aquatic ecosystems via irradiation, and chemical reactions involving enzymes, ROS, and reducing agents.[40, 42, 202, 203] These transformations are important because they influence not only the fate and ecotoxicological effects of the nanomaterials,[43, 50] but also how their extraction, detection and quantification is approached (especially when using spectroscopic, thermal, and microscopic techniques).[203] While several studies have investigated the transformation of GFNs in aqueous media, information on the transformations of the nanomaterials in other relevant natural and anthropogenic matrices is rare. Therefore, studies investigating the transformation (both physical and chemical) of GFNs in cells and tissues, nanocomposites, soils and sediments are urgently needed to reduce biases that can result from not accounting for different GFN forms. Developing methods to extract, detect and quantify GFNs, including transformed GFNs, in complex natural systems is critical to understanding the effects of these nanoparticles on human health and ecological systems.

Increased implementation of GFNs in consumer products requires a reduction in the uncertainty surrounding their environmental impact. This reduction in uncertainty can be accomplished by the continued progress of analytical method development to detect and quantify GFNs in environmentally relevant matrices. The capacity to detect GFNs in these matrices at increasingly lower concentrations with greater precision and selectivity is expected to yield new insights into the toxicity mechanisms of GFNs in cells and organisms. It will also help accurately model the environmental fate and transformations of these materials in the natural environment. This information will ultimately enable the optimal design of GFN-enabled products that utilize their superior properties while minimizing potential adverse effects.

Supporting Information

Tables describing lowest reported detection limits and potential matrix interferences for GFN quantification, and figures showing the structures of different carbonaceous nanomaterials, thermogravimetric profiles of GFNs and matrices, and examples of biases in different matrices. This information is available free of charge via the Internet at http://pubs.acs.org.

Disclaimers

Certain commercial products or equipment are described in this paper in order to specify adequately the experimental procedure. In no case does such identification imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that it is necessarily the best available for the purpose. This is NHEERL Contribution ORD-022716. The views expressed in this article are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency. Any mention of trade names, products, or services does not imply an endorsement by the U.S.

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Tables

Table 1 Selected techniques for GFN characterization and quantification

Method	Overview	Strengths	Limitations
		Spectroscopic	
Absorbance [8, 32, 40, 44, 92, 96, 113, 114, 116, 230]	Measures absorbance of aqueous sample; can include ultraviolet, visible, or near infrared wavelengths; absorbance can be related to mass concentration using the Beer-Lambert law; with analytical ultracentrifugation (AUC), different fragment sizes of material can be measured with absorbance	Except for AUC, absorbance spectrophotometers are readily available in many environmental laboratories	Interference from other sample components; relatively high detection limit; only applicable for aqueous samples; controlled GFN dispersion quality required
Raman [113, 136, 137, 145, 159, 160, 231-233]	Measures G, D and G' vibrational bands in dry powder, polymer nanocomposites, and tissues	Minimal sample preparation; enables GFN characterization; compatible with *in vitro* and *in vivo* samples; can be used with a microscope; low detection limits achieved using resonance Raman conditions	Some matrices may produce interferences; sensitive to laser power; requires calibration or a reference peak for quantitative analysis; background fluorescence can interfere; samples dispersed in an organic solvent are less common but this is possible
Fluorescence [103]	Measures fluorescence emission intensity after excitation of GFN at a known adsorption band	Available in many environmental laboratories; fluorimetry is highly sensitive; rapid technique	Interference of other fluorescent materials (e.g. polymer or environmental matrix); non-specificity of GFN signal; only applicable for aqueous samples; controlled GFN dispersion quality required; may work better for graphene oxide (GO) versus graphene because GO is more highly fluorescent
X-ray Photoelectron Spectroscopy (XPS) [234-236]	Measures the atomic surface concentration of carbon (top 10 nm) and can provide some information on oxidation state; relative concentration of GFN can be determined in a dry matrix if matrix has a very different conductivity relative to the GFN	Provides atomic information and oxidation state of GFN	Requires dry down and a high vacuum environment; doesn't distinguish nanomaterial carbon from background carbon unless charging occurs and background material identity is known
		Spectrometric	
Inorganic Elemental Analysis of Metal Coordination to GFN Functional Groups [141, 155]	Measures divalent metal cations coordinated to GO functional groups	Multi-elemental capability and extreme sensitivity of ICP-MS allow for an accurate and selective determination of metal content coordinated to GFN in a wide range of matrices at ngL^{-1} or sub ngL^{-1} levels, the rapid sample throughput of this method is attractive for routine screening; potential for covalent attachment of metals rather than coordination to minimize	Carbon is generally not detectable with standard ICP-MS methods; metal release from carboxyl groups using strong acid is required prior to analysis; other carboxyl groups in environmental samples can interfere; carboxyl group content can vary between different GFNs; divalent metal cations can dissociate from carboxyl groups since they are not covalently attached; divalent metal cations can increase agglomeration state in water samples; will not work for pristine

		desorption of the metal tags during measurements	graphene since it does not contain functional groups
Microscopic			
Atomic Force Microscopy (AFM) [32, 113, 115, 237-241]	Measures the surface features of a sample by dragging or tapping a cantilever over the sample; the dimensions of identifiable GFN particles can be determined by the movements of the cantilever	A reliable technique for determining sheet thickness and lateral dimensions	Deposition bias, measurement bias, and detection errors are all possible in most samples
Hyperspectral Imaging [156, 242-247]	Measures reflectance (or absorbance and transmittance) spectra of GFNs in a darkfield (visual near infrared /short-wave infrared spectral range) mode using a high-power halogen light source, resulting in 2D-optical images with full spectral information (400 nm to 1000 nm or 900 nm to 1700 nm, respectively) in each pixel (a pixel can be as small as 128 nm) [248]	Easy sample preparation, provides optical and spectral information, allows spatial localization of particles without the need for labelling, can provide semi-quantitative information	Spectral mixing in complex samples/composites, long analysis times, spatial resolution may not be sufficient to differentiate individual small-sized GFNs from their aggregates (especially when stacked), which might impact quantification. Relatively expensive instrumentation.
Scanning Electron Microscopy (SEM) [137, 158, 159, 237, 247, 249]	Measures the interaction of a finely collimated electron beam with the GFNs; secondary electrons emitted by atoms excited by the electron beam can be used for image formation	Provides 3-D morphological properties of GFNs; GFNs may be identifiable in complex matrices based on morphological criteria	Labor-intensive, often only qualitative information
Transmission Electron Microscopy (TEM) and Scanning Transmission Electron Microscopy (STEM) [112, 113, 157, 159, 160, 178, 237, 244, 250]	A TEM passes a parallel beam of electrons through a selected sample area and detects the transmitted electrons that pass through the samples. The main difference with the STEM mode is that it scans very finely focused beam of electrons over the sample selected area in a raster pattern.	Provides morphological properties of GFNs; GFNs can be identified in energy filtered TEM images	Challenging sample preparation for tissues; it may be very hard to detect GFNs in complex samples at low concentrations
Laser Scanning Confocal Microscopy [110, 159, 178, 179]	Uses a laser to excite fluorophores from a fluorescent marker tagged to GFNs or optically detects reflected light. The technique generates a series of focused image planes in the z direction by scanning with point illumination suppressing out-of-focus signal using a pinhole in front of the detector; three dimensional images are generated by combining the series of focused image planes.	Relatively easy technique for tracking translocation of GFNs in biological tissues	Only qualitative, or at best, semi-quantitative. Fluorescence probes may photo-bleach, and may be cytotoxic or interfere with normal biological processes. Reflection mode may be unable to distinguish GFN from other materials in the matrix that scatter light similarly.

Transient Absorption Microscopy [133, 251-254]	A typical pump–probe technique whereby a modulated pump field (typically a pulsed laser) excites the electrons in the sample. A probe (another light source) then interacts with the photoexcited sample to obtain an absorption spectrum	Relatively fast, highly sensitive, and label-free technique that can be used to visualize GFNs in living cells and live animals. May provide quantitative data in well-dispersed GFNs	Light-absorbing matrices may introduce strong background signals. GFNs may have to be functionalized to improve their dispersability for quantitative analyses
Thermal			
Thermal Gravimetric Analysis (TGA) [173-176]	Quantification of mass percentage of phases with distinct thermal stabilities under a variety of reactive gases (usually inert or air) and relatively rapid temperature programs (e.g., heating rates of 5 °C/min to 20 °C/min; room temperature to ca. 950 °C); each sample takes 1 h to 2 h total; a systematic shift in the TGA profile as a function of GFN loading can potentially be measured since GFNs can enhance the thermal stability of materials	A rapid technique that allows for the quantification of multiple phases in a single sample; good for complex matrices; no special sample preparation needed	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 since only small masses can be analyzed, potential for interferences between sample matrix (e.g., polymer, other carbon nanomaterials, soot, or black carbon) and GFN decomposition temperatures; good GFN dispersion quality required for systematic TGA profile shift; drying required
Differential Scanning Calorimetry (DSC) [173]	Measures the thermal transitions of materials relative to a reference pan. The relative energy required or released is measured as a material is heated or cooled through a thermal transition; this technique has been used to measure the shift in the glass transition temperature (T_g) as a function of GO loading	A rapid technique and good for complex matrices; no special sample preparation needed	Thermal ramp rate can affect the transition temperatures; detection limits are relatively high for solid matrices; good GFN dispersion quality required for systematic DSC profile shift; dry-down required; might only be useful for samples containing polymer
Total Organic Carbon (TOC) Analysis [255-257]	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 types of detectors	TOC analysis has been used successfully with CNTs and fullerenes and once with few layer graphene (FLG) to investigate binding of NOM to FLG	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 will 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; with the more common instrument setups (680 °C maximum temperature), the temperature used is not sufficiently high to combust the FLG but would most likely be high enough for GO to combust
Programmed Thermal Analysis (PTA) [96, 170, 211, 258]	While the temperature is ramped, there are two phases: inert followed by oxidizing for measuring organic and elemental carbon, respectively. Detects carbon by having evolved organic carbon be converted to CO_2, then converted back to methane, and	Very reliable technique for detecting elemental carbon in environmental matrices, this technique could differentiate between types of GFNs based on their thermal stability; there is an ability to quantify mass	Too much organic carbon in a sample causes peak overlapping between elemental and organic carbon which affects the accuracy; similar carbonaceous materials such as CNTs and fullerenes will be counted in the GFN peak if they exist in the sample; unless the peak from GFN is far enough from the peaks for other carbonaceous material, it is difficult to

	analyzed using a flame ionization detector. If organic carbon is converted to elemental carbon during the inert phase, there is a correction that can be performed.		exclude the other carbonaceous materials, however, adjusting the temperature program might help to some extent; GO does not separate from matrix unless a strong reducing agent is used followed by extraction prior to sample analysis
Isotopic Labeling			
Carbon-14 Labeling [37, 42, 57, 106, 154]	Can be used to quantify carbon-14 labeled GFNs following combustion in a biological oxidizer or direct addition to a scintillation cocktail; measures beta emissions using liquid scintillation counting (LSC); autoradiography can provide spatial distribution of radioactivity	Provides definitive quantification of GFNs in complex matrices; can be used as an orthogonal technique to develop other analytical techniques; can be used to identify degradation products and GFN quantities in tissues or released from polymer nanocomposites	High cost to synthesize radioactively labeled GFN; safety concerns; limited availability of radioactively labeled GFN; C-14 not inherently part of GFN that would be released into the environment
Additional Techniques			
Gravimetric [259, 260]	GFN mass concentration in air is estimated by determining total particle number (e.g. during GFN production) while accounting for background particle concentration. In suspensions, GFN concentration is estimated by drying a fraction of the suspension and weighing it, or by determining the fraction of GFNs not suspended by weighing the mass of GFN particles settled at the bottom of the container	Uses readily available equipment except in airborne measurements which require special instrumentation	Limited to high GFN concentrations, except in airborne measurements where the sensitivity of equipment may be reasonably high. The technique is nonspecific, and thus only applicable in relatively simple systems/matrices

1730

Figures

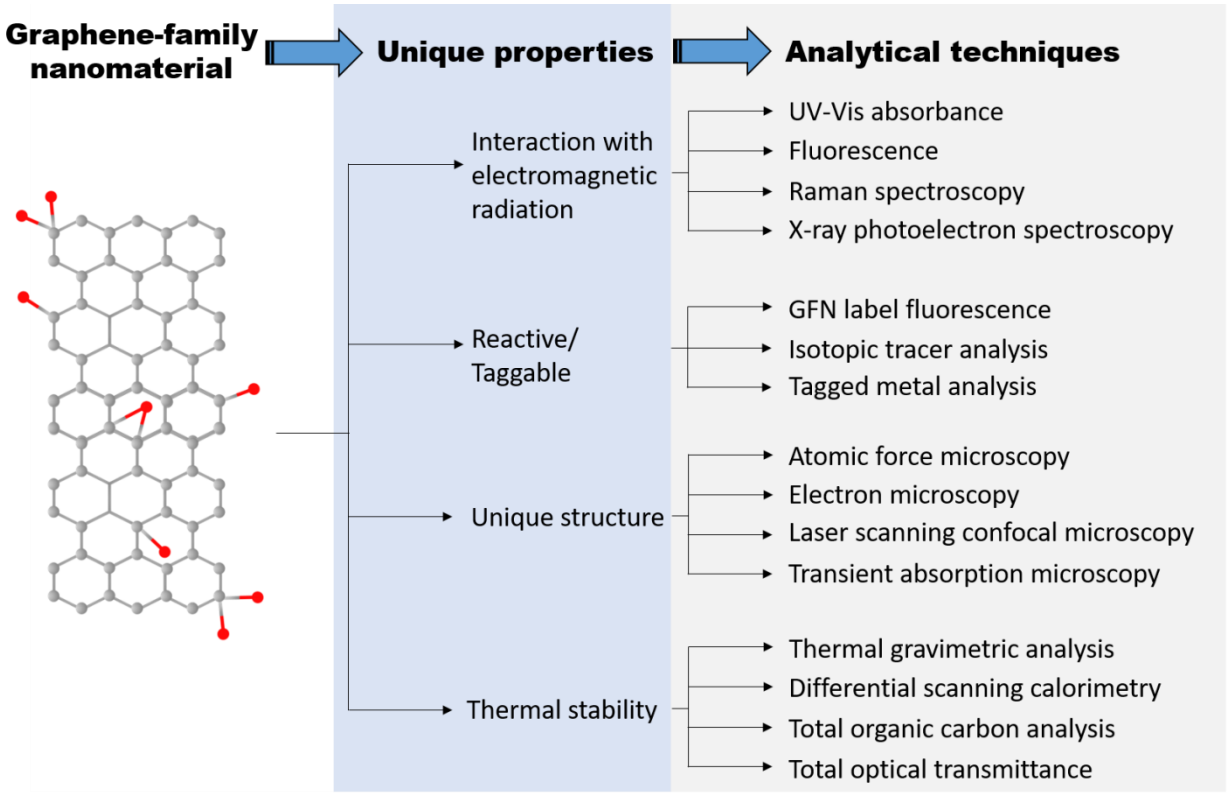

Figure 1 A selection of unique graphene-family nanomaterial (GFN) properties and the analytical techniques that can be used to measure these properties.

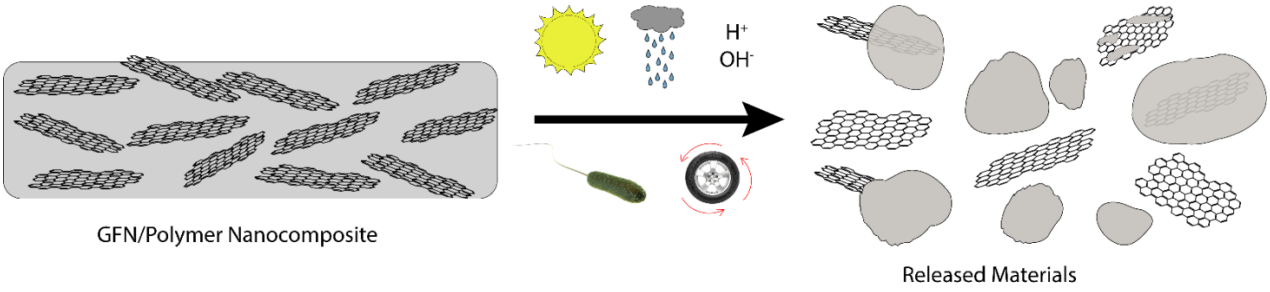

Figure 2 Degradation of GFN/polymer nanocomposites by environmental processes such as UV-weathering, rain, acid rain, alkaline conditions, microbial activity, and mechanical wear can lead to the release of a heterogeneous mixture of polymer fragments, polymer fragments containing GFNs, GFNs coated in polymer, and free GFNs.

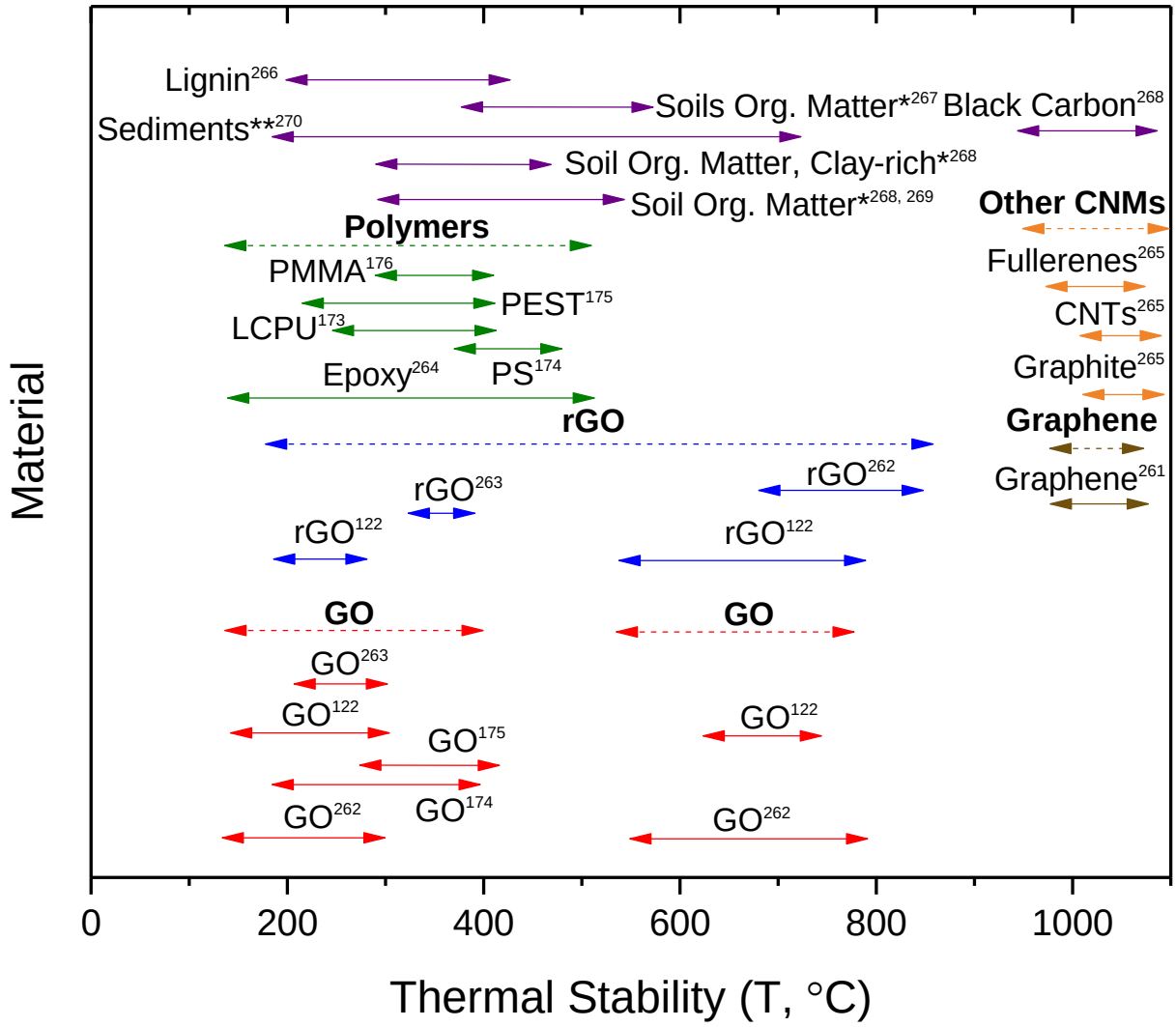

Figure 3 The thermal stability as a function of mass loss for graphene,[261] graphene oxide[122, 174, 175, 262, 263] and reduced graphene oxide[122, 262, 263] relative to polymer matrices (LCPU = liquid crystalline polyurethane, PS = polystyrene, PMMA = poly(methyl methacrylate), PEST = polyester, and epoxy),[173-176, 264] other carbonaceous nanomaterials,[265] and plant material (lignin),[266] soils or soil materials,[267-269] and sediments.[270] An asterisk (*) indicates that clay was not included as part of the thermal gravimetric analysis (TGA) profile while a double asterisk (**) indicates that clay was included as part of the TGA profile. The plot shows where overlap can occur between the thermal profile of the CNM and the thermal profile of the matrix. Ranges provided are the most dramatic change(s) observed with TGA under inert conditions (N_2 or Ar) with ramp rates ranging from 5 °C/min to 20 °C/min.

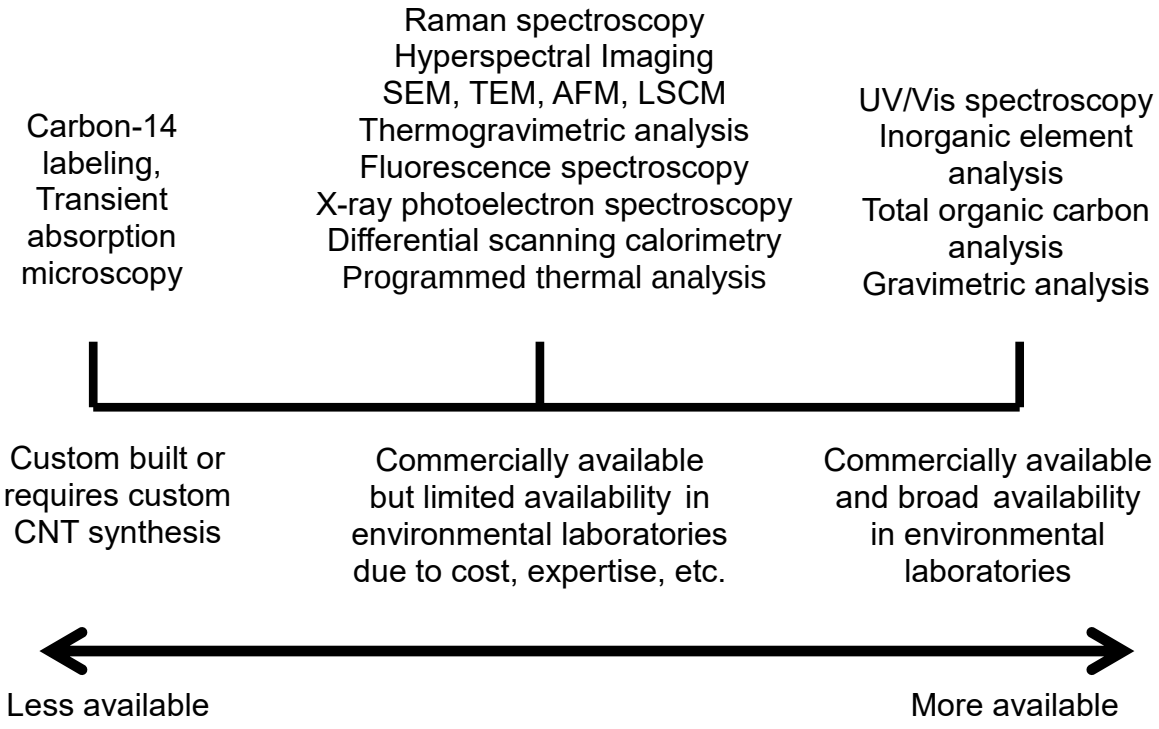

Figure 4 The availability of different techniques for GFN quantification. Techniques are compared based on their availability for purchase and their availability in environmental testing laboratories