Effect of primary particle size on colloidal stability of multiwall carbon nanotubes
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ABSTRACT
Multiwall carbon nanotubes (MWCNTs) are one type of nanoparticles that have various special properties and potential applications. Due to their increasing production and potential toxicological effects, the environmental behavior and transport of MWCNTs have become important research topics. Particle size is one of the important properties of nanoparticles, yet its effects on MWCNT environmental behaviors have not been fully investigated. In this study, we tested how the length of MWCNTs influenced their settling in the presence of Na\(^+\) and/or natural organic matter (NOM), and postulated the governing mechanisms. The results showed that when adding Na\(^+\) the shorter MWCNTs exhibited preferential aggregation and settling. One possible reason could be that shorter MWCNTs possess larger specific surface area and consequently stronger attraction forces. However, NOM strongly mitigated such aggregation, and helps to disperse MWCNTs regardless of their length and aqueous conditions.

Key words | aggregation, colloidal stabilization, NOM, settling, transport

INTRODUCTION
The development of nanotechnology has resulted in successful applications of various nanomaterials and will encourage further expansion (Piccinno et al. 2012). Carbon nanotubes (CNTs) are among the nanomaterials that have been studied extensively due to their prominent electronic, physicochemical, optical, and mechanical properties (Baughman et al. 2002). CNTs are allotropes of carbon that can be illustrated as graphene sheet(s) rolled into a cylindrical shape, while multiwall carbon nanotubes (MWCNTs) are composed of a series of coaxially arranged graphene sheets. In some of the applications, such as drug delivery vessel and sorbents (Baughman et al. 2002; Mauter & Elimelech 2008), CNTs are required to be dispersed stably in aqueous phase. Upon release to environmental aquatic bodies, whether CNTs can stably suspend in water will influence their mobility, environmental distribution and bioavailability (Gottschalk & Nowack 2011; Petersen et al. 2011; Piccinno et al. 2012).

The size of MWCNTs falls into the colloidal range. Studies showed that MWCNTs with hydrophilic surface functional groups, i.e., carboxyl, hydroxyl, carbonyl, etc., can stably suspend in water to form a colloidal dispersion. However, common cations such as Na\(^+\), K\(^+\), Ca\(^{2+}\), and Mg\(^{2+}\) can induce their aggregation by suppressing electrostatic repulsion between the CNTs, and this process can be reasonably modeled using the Derjaguin–Landau–Verwey–Overbeek theory (Kennedy et al. 2008; Saleh et al. 2008; Zhang et al. 2012). MWCNT aggregates, when they reach certain sizes, can readily settle from water, and consequently their mobility is reduced. Therefore aggregation is an important process governing the environmental fate of MWCNTs in aqueous systems.

In addition to aqueous conditions, e.g., cation valence, cation concentration, and pH, MWCNT properties, e.g., surface charge and functional groups, also affect their colloidal stability (Smith et al. 2009a, b). However, the impact of other properties such as size and morphology has not been fully investigated, yet may be important factors controlling CNT aggregation and stability according to the traditional colloid science (Tsuruta et al. 1995; Chin et al. 2000; Kallay & Zalac 2002; He et al. 2008). Particle size is one important physical property of CNTs influencing many important phenomena, e.g., cellular interactions and uptake, percolation thresholds, strength of interactions with external fields, self-assembly, and optical properties (Dubnikova et al. 2010; Fagan et al. 2011); thus particle size also likely has influence on the
colloidal stability of MWCNTs. The research on this topic usually studied particles of a spherical shape or that can be approximated or assumed as a spherical shape. CNTs were, however, rod-shaped (Park et al. 2008). The effect of size on the colloidal stability of tubular particles is largely unknown. Moreover, in literature, the size of CNTs was often described by equivalent spherical size, e.g., hydrodynamic diameter, a way that does not differentiate individual particles and their aggregates. To precisely describe a CNT size requires two parameters, i.e., diameter and length, both of which may pose impact on the CNT behavior in aqueous phase. For example, O’Driscoll et al. (2010) found that decreasing diameter enhanced the colloidal stability of MWCNTs dispersed by natural organic matter (NOM), whereas the effect of MWCNT length is still unknown.

In order to investigate the size effect to the colloidal stability of a polydispersed MWCNT suspension, we used scanning electron microscopy (SEM) to characterize the length distribution of primary MWCNTs, as well as dynamic light scattering (DLS) to measure the hydrodynamic diameter. The effect of size on MWCNT settling was examined under two aqueous conditions: varying sodium ion concentrations (0, 4 and 40 mmol L\(^{-1}\)) and the absence or presence of NOM. Possible mechanisms of the phenomenon were discussed.

**METHODS**

**\(^{14}\)C-labeled MWCNT**

Carbon-14 labeling was used to unambiguously quantify MWCNTs in aqueous phase. \(^{14}\)C-labeled MWCNTs were synthesized using a modified chemical vapor deposition technique as described in our previous study (Petersen et al. 2008; Zhang et al. 2011). The MWCNTs were treated with hydrochloric acid followed by a mixture of concentrated sulfuric and nitric acid (3:1 v/v) to remove impurities and add hydrophilic functional groups to the nanotube surfaces. The MWCNTs were then ultrasonicated (34.7 ± 0.8 W; uncertainty indicates the standard error) in de-ionized water (>18 M\(\Omega\)) for 6 h, centrifuged at 3,500 g for 5 min to produce a stable dispersion of 67 mg L\(^{-1}\) determined by the radioactivity. This MWCNT dispersion was fully characterized in our previous study (Zhang et al. 2011). Briefly, the surface oxygen contents were determined to be ~8.6\% by X-ray photoelectron spectroscopy (Kratos Analytical Axis Ultra X-ray photoelectron spectrometer).

The point of zero charge is around pH 1.5 in a monocation solution at 0.1 mol L\(^{-1}\).

The MWCNT concentration was diluted to ~10 mg L\(^{-1}\) before initiating the experiments. MWCNT concentrations were quantified by radioactivity using a Beckman LS 5801 liquid scintillation counter (Brea, CA) after mixing a 3 mL sample with 3 mL of scintillation cocktail (Insta-Gel Plus, PerkinElmer, MA, USA) (Zhang et al. 2011). These initial dispersions were sampled and subjected to the SEM measurement as described in ‘Particle size characterization: length’.

**Settling experiment**

The effect of particle length on the colloidal stability of MWCNTs was investigated by comparing the length distribution of MWCNTs before and after settling. If MWCNTs in certain size range are more stable than those in other ranges, the relative abundance of them will increase after the settling and be reflected in the length distribution histogram. Aside from length, we also examined the effect of two aqueous conditions using a factorial design, i.e., Na\(^+\) concentrations and with or without NOM (in Figures 1 and 2 noted as ‘Blank’ or ‘NOM’, respectively). The experiment was conducted in 150 mL flasks; each contained 10 mL of a MWCNT suspension. The tested Na\(^+\) concentrations were 0, 4 and 40 mmol L\(^{-1}\) adjusted by adding NaCl.

![Figure 1](https://iwaponline.com/wst/article-pdf/68/10/2249/471570/2249.pdf)

**Figure 1** | Mass percentage of MWCNTs removed from the aqueous phase after 7 days of settling experiment with different aqueous conditions, i.e., NaCl concentration and NOM addition. The error bars represent the standard deviations of the triplicates. The different letters (i.e., a and b) above the bars indicate statistically significant difference with each other, at the confidence level of 0.1 according to the pair-wised comparisons among conditions across the same length range using Tukey’s test.
The NOM is the water-soluble portion of a natural soil organic matter, Canadian peat (Quebec, Canada). Peat is one typical soil organic matter, usually composed of recently deposited plant organic materials, and is thus a diagenetically young organic matter (Huang & Weber 1997). Thus it contains relatively higher oxygen to carbon content and less compact structure compared to the aged organic matter. The total organic carbon content of the final solution containing this NOM was 7.67 mg C L\(^{-1}\). Each condition was prepared in triplicate. The samples were hand-shaken initially and then set still in room temperature for 7 days to allow settling. At the end of the 7-day period, a 5-mL portion was taken from each sample and centrifuged at 3,500 g for 5 min in a glass centrifuge tube of 1.2 cm diameter to obtain a stable suspension. The supernatant of the top 3-cm layer was sampled from the centrifuge tube for further measurements: 1) 0.2 mL for length distribution of the MWCNTs by SEM; 2) 3 mL for quantification of MWCNTs by \(^{14}\)C radioactivity.

**Particle size characterization: length**

SEM was employed to measure sizes of individual MWCNTs. The MWCNTs examined by SEM were from the initial dispersion without adding NaCl or NOM or from the stable dispersions after settling experiments. An aliquot of 0.2 mL of each sample was mixed with 1.8 mL methanol; 10 \(\mu\)L of the mixture was applied on a silicon substrate and vacuum dried. The substrate was then examined using an FEI Inspect F50 FEG SEM at an accelerating voltage of 10.00 or 15.00 kV according to image clarity. Images at 20 kx magnification were taken for each sample at random positions on the silicon substrate. The lengths of individual MWCNTs on the microscopic images were quantified using SigmaScan Pro. 5.0. The curved
MWCNTs were measured using a customized ruler. The length distribution histogram was number-averaged by at least 150 MWCNTs for each sample. The statistical analysis was performed using SAS ver. 9.2.

**Particle size characterization: hydrodynamic diameter**

The average sizes of MWCNTs and their aggregates were described using the hydrodynamic diameter as well as polydispersity index (PDI) measured by DLS on a Malvern Zetasizer Nano ZS instrument (Malvern Instruments, UK) operating at 25°C. MWCNT dispersions were measured immediately after the addition of NaCl or after the settling experiment for each condition. Samples were transferred to a macro-cuvette (pass length: 10 mm) and the scattering angle was 173°. The instrument automatically chose the number of runs performed on each sample depending on the quality of the autocorrelation function; typically 10 to 30 runs were performed.

**RESULTS AND DISCUSSION**

**Effect of individual length**

The mass of 14C-MWCNT remaining in the dispersion after settling was measured using the liquid scintillation counter and the percentage was calculated according to the radioactivity (Figure 1). In the absence of Na⁺ and NOM, 13.3 ± 8.2% (n = 3, uncertainties represent standard deviations) of MWCNTs were removed during the settling. The addition of Na⁺ significantly increased the removal of MWCNTs to 61.2 ± 18.9% and 89.3 ± 2.5% for 4 and 40 mmol L⁻¹ Na⁺, respectively.

Scanning electron micrographs of the initial dispersed MWCNTs are shown in Figure 2(a). According to the graph, most of the MWCNTs were individually dispersed. By visual comparison between the SEM images before and after settling (Figure 2(a) and (c)), the amount of individually dispersed MWCNTs after the addition of NaCl is less than the initial sample; instead, most of them exist in bundles. This showed that the cation induced MWCNT aggregation, and the aggregation is likely to cause the removal of MWCNTs from aqueous phase by settling.

The initial length distribution (Figure 1 and Figure S1) indicates a broad range of MWCNT length ranging from less than 100 nm to more than 2,000 nm, a result similar to that previously determined for a different set of MWCNTs prepared using the same synthesis method (Petersen et al. 2009). Most (73.2%) of the MWCNTs were shorter than 400 nm, and the average length (by number) was approximately 353 nm. Diameters ranged mainly from 16 to 92 nm with a mean at 34.8 ± 13.3 nm (n = 50; uncertainties indicate standard deviations). The polydispersed nature of the MWCNT suspension makes it difficult to separately track the behaviors of MWCNTs within different length range. Instead, the relative abundance of MWCNTs in each length range is quantifiable. Its change can reflect the preferential removal of MWCNTs with regard to length. Therefore the length distributions after settling under different condition were analyzed and are shown in Figure 5. Given the resolution of the SEM images and challenges associated with distinguishing between MWCNTs and dust or debris on the wafer, MWCNTs less than 100-nm long were not considered.

As shown in Figure 3(a), after settling the percentage of the shorter fraction of MWCNTs significantly decreased, and the decrement was correlated with Na⁺ concentration. This suggests the preferential removal of the MWCNTs with shorter length. Generally speaking, the MWCNTs falling in the 100 to 400 nm length range were relatively less stable than those in other length ranges in this experiment, showing higher tendency to be removed from aqueous phase and consequently lower mobility in the environment. Aggregation is the prerequisite of the MWCNT settling, and the factors influencing the aggregation can also influence the settling. Therefore the preferred settling of shorter MWCNTs over the longer ones likely resulted from the preferential aggregation of shorter MWCNTs in the lower length range. This result is consistent with the colloidal stability studies of small particles in nanoscale (Tsuruta et al. 1995; Kallay & Zalac 2002; He et al. 2008). A study using standard material, polystyrene microspheres (mean diameter ranged from 76 to 1,010 nm), stabilized by negative sulfate charges revealed that at a given salt concentration, stability of this colloid increases, reaches a maximum, and then decreases as a function of size increase (Tsuruta et al. 1995). Kallay & Zalac (2002) concluded that for the very small colloid particles in nanometer range, smaller particles are less stable by theoretical calculation using the Bronsted concept based on the transition state theory. This theorem has been experimentally verified by hematite particles (radius of 12, 32 and 65 nm) (He et al. 2008). However, within a larger diameter range such as 890 ± 0.2 nm, this trend was reversed in that the secondary-minimum aggregation of hematite particles was enhanced by increasing particle size (Chin et al. 2000). Thus in the lower size range, the spherical particles with smaller diameter are easier to
aggregate. Our result showed that this phenomenon may also be applied to non-spherical particles such as MWCNTs.

In addition, MWCNTs of different length may form aggregates with different density, which partially explains the preferential removal of the shorter MWCNTs. A previous study reported that the acid-treated single-walled CNTs (SWCNTs) yielded looser aggregate structures at large length scale and more compact structures at smaller length scale (Chen et al. 2004). This conclusion could also be applied to MWCNTs. In this case, with increased density the aggregates of shorter MWCNTs tend to have greater settling velocity according to the Stokes' law as described in Supplementary Material (available online at http://www.iwaponline.com/wst/068/489.pdf), and consequently become less stable than the longer MWCNTs.

As shown in Figure 1, MWCNTs dispersed in the NOM solution showed 28.8 ± 8.8% settling, larger than the amount of the control without NOM addition (13.3 ± 8.2%). This may have resulted from the mineral salts associated with the NOM. The enhanced settling compared to control has also caused the percentage of the shorter MWCNTs to slightly decrease, however not significantly (Figure 3(b)). In contrast to the treatments without NOM present, increasing the cation concentration did not significantly increase the amount of MWCNT settling, 33.0 ± 7.0% and 38.1 ± 6.5% for 4 and 40 mmol L⁻¹ Na⁺, respectively.

With the presence of NOM, even if the concentration of Na⁺ reached 40 mmol L⁻¹, the length distribution did not change significantly after 7 days with one exception, i.e., length range between 400 and 700 nm when NaCl concentration was 40 mmol L⁻¹ (Figure 1(b)). This phenomenon suggested the prominent ability of NOM to disperse MWCNTs in water regardless of not only the sodium concentration but also the length. Previous studies have shown that NOM can be adsorbed on MWCNT surfaces and stabilize MWCNTs by steric hindrance and/or electrostatic repulsion (Hyung et al. 2007; Hyung & Kim 2008; Zhang et al. 2011). As a result the MWCNTs were mostly individually dispersed and the aggregation was significantly reduced as shown in the SEM images (Figure 2). The steric stabilization results from displacement of water molecules into the bulk phase, which is caused by the interpenetration of the hydrophilic portion of the NOM that extends into the solution phase when two CNT particles approach each other (Chen et al. 2004). This stabilization is relatively inert to the change of ionic strength in solution phase. Therefore the NOM can stabilize MWCNTs even under relatively high Na⁺ concentration of 40 mmol L⁻¹.

The preferential aggregation of the shorter MWCNTs could be explained by the fact that longer length may result in lower specific surface area. Lower specific surface area will in turn lead to weaker van der Waals attractions and hydrophobic interactions between MWCNTs, which result in less aggregation and settling. However, higher specific surface area will also lead to stronger interactions.

Figure 3 | Length distributions of MWCNTs in dispersions as measured by SEM. Each bar in this graph shows the number-averaged percentage of MWCNTs from certain length ranges (x-axis) before or after settling for 7 days with different aqueous conditions. ‘NOM’ in (b) denotes treatments with peat NOM in solution and ‘Blank’ in (a) and (b) means without NOM in the solution. The concentrations indicated, i.e., 0, 4 or 40 mM (mmol L⁻¹), are for the NaCl added. The error bars represent the standard deviations of the triplicates. The different letters (a, b, or c) above the bars indicate statistically significant difference between each other at the confidence level of 0.05 according to pair-wised comparisons among conditions across the same length range using Tukey’s test.

between NOM and MWCNTs, and consequently the smaller MWCNTs will adsorb more NOM per unit mass. This may be the reason why MWCNTs with smaller diameter are preferentially suspended in NOM solutions, according to O’Driscoll et al. (2010). In our study, the adsorption of NOM compensated for the extra instability of shorter MWCNTs and made them equally stable even under the ‘stress’ of relatively high \( \text{Na}^+ \) concentration.

Hydrodynamic diameter and polydispersity of MWCNT dispersions

The average hydrodynamic diameters and polydispersity factors of MWCNT dispersions at the initial state of settling and stable state after settling are shown in Table 1. The hydrodynamic diameter measures an effective size of a particle in liquid environment and includes the thickness of the thin, electric dipole layer adhering to the particle surface. Thus it is usually larger than the actual size of particle measured by microscopic techniques. In addition, as mentioned before, it does not differentiate primary particles and their aggregates. However, it is an important parameter when considering a particle’s movement in aqueous phase. We can see in Table 1 that the average hydrodynamic diameter at the initial state of settling showed a significant increase corresponding to increasing \( \text{Na}^+ \) concentration, which means the addition of \( \text{Na}^+ \) immediately initiated the aggregation process and increased the apparent size of MWCNTs, especially when \( \text{Na}^+ \) concentration was high, e.g. 40 mmol L\(^{-1}\). However the MWCNT aggregates with larger size were unstable and settled out during the 7-d period, leaving the smaller ones suspended in the aqueous phase. Thus the average hydrodynamic diameters decreased, and the difference between various \( \text{Na}^+ \) concentrations was far less pronounced as when the \( \text{Na}^+ \) was first added.

PDI value indicates the extent that the MWCNT suspension diverged from a monodispersed solution which has very narrow size distribution. Given the wide length range of the MWCNTs revealed by SEM, the polydispersity in the suspensions of MWCNT is likely greater than the monodispersed spherical nanoparticles. In addition, the hydrodynamic diameter determined by the DLS method for high aspect ratio nanoparticles, such as MWCNT, will be neither the length nor the width of that particle, and is orientation-dependent, because it involves a calculation based on the assumption that the particles are spherical. The tube-like shapes orientate themselves in different ways in aqueous phase and thus appear to have wider size distribution. After 7-day settling, the polydispersity indices decreased, suggesting that the hydrodynamic sizes of the dispersed particles became more consistent. It is likely that the smaller MWCNTs tended to aggregate, consequently approaching the stable size range, while the aggregates larger than the stable size range tended to settle.

### CONCLUSIONS

The effect of MWCNT sizes on their environmental behaviors and impact is of great significance. Our research showed that the smaller fraction of MWCNTs below 400 nm long is less stable than the longer fraction, and consequently easier to be removed from the aqueous phase in wastewater treatment. This may partially compensate for the higher environmental risk of the shorter CNTs. Research showed that only SWCNTs shorter than 200 nm can negatively affect the metabolism of several mammalian cell lines (Fagan et al. 2011). Similarly, the smaller fraction of SWCNTs can reduce the life cycle of the estuarine copepod \textit{Amphiascus tenuiremis} and increase the mortality (Templeton et al. 2006).

However, with the presence of NOM, this preferential removal is mitigated. Besides, CNTs with smaller diameter were shown to be preferentially suspended in NOM.

### Table 1

<table>
<thead>
<tr>
<th>Na\textsuperscript{+} conc. (mmol L\textsuperscript{-1})</th>
<th>Initial state Z-average\textsuperscript{a} ( d ) (nm)</th>
<th>Polydispersion index</th>
<th>Stable state Z-average\textsuperscript{a} ( d ) (nm)</th>
<th>Polydispersion index</th>
<th>Stable state with NOM\textsuperscript{b} Z-average\textsuperscript{a} ( d ) (nm)</th>
<th>Polydispersion index</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>162.3 (1.8)</td>
<td>0.302</td>
<td>190.9 (1.9)</td>
<td>0.272</td>
<td>188.6 (13.6)</td>
<td>0.280</td>
</tr>
<tr>
<td>4</td>
<td>202.8 (9.5)</td>
<td>0.305</td>
<td>200.4 (1.3)</td>
<td>0.278</td>
<td>184.5 (19.2)</td>
<td>0.338</td>
</tr>
<tr>
<td>40</td>
<td>818.9 (20.7)</td>
<td>0.375</td>
<td>250.5 (2.9)</td>
<td>0.288</td>
<td>202.5 (37.1)</td>
<td>0.387</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Values in parentheses are standard error. The Z-average diameter defines as the ‘harmonic intensity averaged particle diameter’. It is an intensity-based calculated value also known as the cumulants mean. The calculation is defined in ISO 13321 and ISO 22412.

\textsuperscript{b}The Z-averaged diameters of MWCNT-NOM composites with sodium ions. However, because some portion of the NOM may also be considered particles, these Z-averaged diameters displayed here include MWCNT, NOM, and MWCNT-NOM composites.
solutions (O’Driscoll et al. 2010), likely due to the larger specific area associated with smaller diameter. In addition, the settling of MWCNTs was significantly reduced by NOM with the presence of Na+. Therefore the smaller CNTs are likely to be more available for transport and dispersion in aquatic ecosystems with NOM.

Our work studied relatively small MWCNTs with the majority lengths less than 1,000 nm, the lower end of colloidal particle sizes, and our results showed that the smaller size MWCNTs tend to preferentially aggregate. According to the traditional colloidal study with regard to spherical particles, the behavior of preferential aggregation differs for particles with sizes smaller and larger than 890 ± 0.2 nm. For tubular shape nanoparticles like MWCNTs, whether there is such a transition in preferential aggregation and what is the threshold size is an interesting topic for further investigation.

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