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## Influence of the initial state of carbon nanotubes on their colloidal stability under natural conditions

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The colloidal stability of CNTs varies a lot depending on the initial state of the CNTs (dry vs. pre-dispersed), the applied dispersant for pre-suspension, and the composition of the medium.

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### ABSTRACT

The colloidal stability of dry and suspended carbon nanotubes (CNTs) in the presence of amphiphilic compounds (i.e. natural organic matter or surfactants) at environmentally realistic concentrations was investigated over several days. The suspensions were analyzed for CNT concentration (UV–vis spectroscopy), particle size (nanoparticle tracking analysis), and CNT length and dispersion quality (TEM). When added in dry form, around 1% of the added CNTs remained suspended. Pre-dispersion in organic solvent or anionic detergent stabilized up to 65% of the added CNTs after 20 days of mild shaking and 5 days of settling. The initial state of the CNTs (dry vs. suspended) and the medium composition hence are critical determinants for the partitioning of CNTs between sediment and the water column. TEM analysis revealed that single suspended CNTs were present in all suspensions and that shaking and settling resulted in a fractionation of the CNTs with shorter CNTs remaining predominantly in suspension.

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### 1. Introduction

Along with the forecasted increase in manufacturing and use of carbon nanotubes (CNTs), unintended and accidental releases of CNTs to the environment are becoming likely (Nowack and Bucheli, 2007). Exposure might occur during the production, use and disposal of CNTs and CNT-containing products and is largely determined by the matrices in which these materials are found (Köhler et al., 2008; Wiesner et al., 2009). CNTs may be released into the aquatic environment from point sources, such as factories, landfills and wastewater effluents or from nonpoint sources, such as storm-water runoff, attrition from composites and from wet deposition from the atmosphere (Wiesner et al., 2006). According to first modeling approaches the concentrations of CNTs in the aquatic environment are expected to be in the ng/L range (Mueller and Nowack, 2008) and sediments are predicted to be the major environmental sink (Gottschalk et al., 2009; Koelmans et al., 2009). From an ecological point of view, the partitioning between sediment and the water column is a critical determinant of suspendability with regard to potential impacts of CNTs on the ecosystem. The formation

of agglomerates and their subsequent settling initiates partitioning. It is assumed that the better de-bundled and individually suspended the CNTs are, the longer they remain in the water column.

Upon release of CNTs into the aquatic environment, water composition and properties (e.g. ionic strength, pH) affect the behavior of CNTs. Pristine CNTs are extremely hydrophobic and cannot easily be dispersed in distilled water (Girifalco et al., 2000). However, it has been shown that natural organic matter (NOM), such as humic and fulvic acid, present in natural systems (Hyung et al., 2007; Kennedy et al., 2009) enhances the solubility of CNTs at environmentally relevant concentrations. The dissolved organic carbon (DOC) concentrations in natural waters range from 0.5 to 30 mg/L (Thurman, 1985). On average, humic acid and fulvic acid accounts for 10% and 40% of the DOC, respectively (Thurman, 1985). It has been proposed that the hydrophobic moieties of NOM adsorb to the hydrophobic surfaces of the CNTs whilst hydrophilic moieties stretch towards the solution and thereby enhance the dispersability of CNTs (Hyung et al., 2007; Kennedy et al., 2009). Another possible interaction is hydrogen bonding between oxygen-containing functionalities on the NOM and polar functionalities (e.g. –COOH, –OH) on the graphene surface of CNTs (Wang et al., 2009). Even pristine CNTs often contain oxygen-containing functional groups, formed during purification or by incidental exposure to oxidizing agents after arrival in the environment (Cho et al., 2008). NOM

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adsorption leads to CNT stabilization, as it causes charge repulsion due to negative surface charge and steric hindrance due to its large molecular size (Hyung and Kim, 2008).

Surfactants have the same amphiphilic molecular structure as NOM and therefore have the same dispersing effect on CNTs (Chappell et al., 2009). The non-polar tail interacts with the tube wall whilst their polar head is orientated towards the solution. The steric stabilization due to the size of the surfactant molecule seems to be more important than the charge (Moore et al., 2003). Different arrangements of the non-polar hydrocarbon tail on the CNT surfaces have been proposed (Richard et al., 2003; Tummala and Striolo, 2009). Discharged CNTs may be stabilized by the interaction with detergents from raw wastewater or surface waters. Surfactant concentrations in raw waste waters are in the low mg/L range (Matthijs et al., 1999). In surface waters, concentrations in the  $\mu\text{g/L}$  range have been monitored (Ying, 2006). CNTs are often processed in suspended form for industrial applications. Organic solvents or surfactants at relatively high concentrations (g/L range) (Moore et al., 2003) are commonly used to obtain stable suspensions.

Dry CNT powder and pre-dispersed CNTs are possible forms in which CNTs potentially reach the aquatic environment (Han et al., 2008; Wang et al., 2008a; Holbrook et al., 2010; Lin et al., 2010). However, the long-term partitioning of dry and pre-dispersed CNTs in aqueous solutions under environmentally realistic conditions is poorly known.

The majority of studies working with dry CNTs focused on the adsorption of different compounds onto CNTs (Lu and Su, 2007; Han et al., 2008; Hyung and Kim, 2008; Lin and Xing, 2008a,b; Wang et al., 2008b, 2009; Yang and Xing, 2009; Zhang et al., 2009). Only few studies investigated the stabilizing effect of various compounds on CNTs (Hyung et al., 2007; Kennedy et al., 2008, 2009; Chappell et al., 2009; Lin et al., 2009, 2010) and quantified the resulting amount of suspended CNTs (Hyung et al., 2007; Kennedy et al., 2008, 2009; Lin et al., 2009, 2010). Sonication, magnetic stirring and shaking were used to mechanically disperse dry CNTs in fate-studies. Although shaking is the most environmentally realistic scenario, it was applied only once and over a relatively short period of one day (Chappell et al., 2009). Suspended CNTs are commonly used to study their colloidal stability (Han et al., 2008; Lin et al., 2009, 2010), their aggregation kinetics (Saleh et al., 2008, 2010; Li and Huang, 2010), their mobility in porous media (Wang et al., 2008a) and their sorption to soils and sediments (Zhang et al., 2011). These studies described changes in dispersion quality with regard to colloidal properties over time (up to 5 days) and only rarely quantified the remaining suspended CNT concentration (Lin et al., 2010; Zhang et al., 2011).

The objective of this study was to investigate how the initial state of the CNTs (dry vs. pre-dispersed) affects their colloidal stability in the presence of environmentally realistic concentrations of humic and fulvic acids and various detergent concentrations as a comparison. In different media, the dispersion of dry CNTs and the stabilization of pre-dispersed CNTs was continuously measured over 20 days of mild shaking, simulating turbulences in the natural water body. Subsequently the samples were allowed to settle (5 days) whilst the colloidal stability of CNT suspensions was investigated. The supernatants were analyzed with respect to CNT concentration (UV–vis), particle sizes (NTA) and the quality of CNT dispersion and CNT length (TEM).

## 2. Materials and methods

### 2.1. Materials

All experiments were conducted with Baytubes C150 P, the commercially available multi-walled CNTs (MWCNTs) produced by Bayer Technologies Service. The characterization of the CNTs is described in the Supporting information. Humic

and fulvic acids from Suwannee River (International Humic Substances Society IHSS), SRHA (Standard II) and SRFA (Standard I), respectively, were chosen as model natural organic matter (NOM). The following detergents were used: SDS (sodium dodecyl sulphate; Fluka); anionic surfactant; LAS (alkylbenzene sulphonic acid; Mifa); anionic surfactant; LUT (LUTENSOL; Mifa) alcohol ethoxylate: non-ionic surfactant and REW (REWOQUAT; Mifa) esterquat: cationic surfactant. SDS (sodium dodecyl sulphate; Fluka) and NMP (1-methyl-2-pyrrolidinone; Sigma) were applied as dispersants in stock suspensions (Giordani et al., 2006; Bergin et al., 2008).

All samples were buffered to pH 7 using MOPS (3-morpholinopropanesulfonic acid, 10 mM; Fluka). Sodium azide ( $\text{NaN}_3$ , 0.5 mM; Aldrich) was added to suppress bacterial growth. Preliminary tests proved that 0.5 mM  $\text{NaN}_3$  effectively suppressed bacterial growth and that neither  $\text{NaN}_3$  nor MOPS affected the dispersability of the CNTs.

### 2.2. Experimental setup

Each sample comprised 50 mL test solution prepared in 50 mL glass bottles with screw cap (Schott Duran; Huber). All treatments were replicated three times ( $n = 3$ ). All samples contained the same background medium prepared in Millipore water: 10 mM MOPS, 3.8 mM NaOH and 0.5 mM  $\text{NaN}_3$  (pH 7; ionic strength  $I = 4.3$  mM).

#### 2.2.1. "Dry CNTs"

Dry CNT powder (50 mg/L) was added into background medium only and into background medium containing either 20 mg/L NOM (20 mg/L SRHA or 20 mg/L SRFA) or varying concentrations of detergents (5 g/L LAS, 5 g/L LUT, 2.5 g/L SDS, 20 mg/L SDS, LAS, LUT, REW). The samples were shaken on a horizontal shaker (150 rpm, bottles horizontally placed) for 1, 6, 8, 12 and 20 days. Subsequently the samples were removed from the shaker and the supernatants (approx. 0–2.5 cm depth) were analyzed after 0 h, 2 h, 1 day, 3 days and 5 days settling time.

#### 2.2.2. "Pre-dispersed CNTs"

A stock suspension of CNTs was produced in the presence of a dispersant (250 mg/L CNTs in SDS, 250 mg/L CNTs in LAS and 1 g/L CNTs in NMP) by a sonication tip (Bandelin Sonopuls HD 2200, tip MS 73, 60 W, 7 min continuously). Dispersant concentrations were 12.5 g/L SDS, 2.5 g/L SDS, 0.1 g/L SDS, 25 g/L LAS or 100% NMP (i.e. pure solvent with no aqueous medium), respectively. From the stock suspensions small volumes were added into the test solutions to achieve CNT concentrations of 50 mg/L CNTs. The pre-dispersed CNTs were added, to background medium only and to background medium containing either NOM (CNTs pre-dispersed in NMP added in 20 mg/L SRHA; CNTs pre-dispersed in NMP or SDS added in 20 mg/L SRFA) or varying concentrations of detergent (CNTs pre-dispersed in NMP added in 5 g/L and 250 mg/L LAS). The samples were horizontally shaken (150 rpm, bottles horizontally placed) for 5 h, 1 day, 8 days, 12 days and 20 days. Subsequently the samples were removed from the shaker and the supernatants (approx. 0–2.5 cm depth) were analyzed after 0 h, 2 h, 1 day, 3 days and 5 days.

### 2.3. Quantification of CNTs

The concentration of CNTs in the supernatant was quantified by UV–vis spectroscopy (Uvikon 930) at 800 nm (Hyung et al., 2007). A separate calibration was made for each experimental setup because slight differences were observed in the UV-absorption of different compositions of the medium (standard deviation 8%). CNT-stock suspensions (4 mg/L CNTs in SDS, 4 mg/L in LAS and 16 mg/L in NMP) were prepared in the presence of the different dispersants (12.5 g/L SDS, 2.5 g/L SDS, 25 g/L LAS and 100% NMP) by sonication tip. After sonication no agglomerates were visible in suspension, at the suspension-surface, at the walls or at the bottom of the vessel. Small volumes of the stock suspensions were added into experimental medium (20 mg/L SRHA, 20 mg/L SRFA, 5 g/L and 250 mg/L LAS) followed by brief magnetic stirring and measured immediately by UV–vis. For each experimental setup the corresponding calibration was used. For the quantification of dry CNTs present in the supernatant after shaking and settling, a mean value of all calibrations curves was applied.

Each sample replicate was measured 3 times by UV–vis at 800 nm. The detection limit (DL) was defined as three times the standard deviation of the measurement replicates of the background medium and was 0.001 a.u., which is equivalent to 0.1% of the added CNTs (50  $\mu\text{g/L}$ ). The samples were background corrected with the absorption signal of the compounds present besides the CNTs (SRHA, SRFA, cationic, non-ionic, anionic detergents, buffer MOPS, NaOH,  $\text{NaN}_3$ ). The samples were measured in plastic cuvettes (Fisherbrand, FB 55143, 1 cm, 4 mL). For the stability analysis of the sample over 5 days settling time, the filled cuvettes were stored in a closed box with 100% humidity to prevent evaporation of the sample. Samples were carefully taken out to avoid re-suspension and placed in the spectrophotometer.

### 2.4. Particle size

Nanoparticle tracking analysis (NTA 1.5.1, LM20, NanoSight Ltd.) was used to measure the particle sizes (Montes-Burgos et al., 2010). The results correspond to an equivalent hydrodynamic diameter of a sphere that has the same diffusion properties

as the CNT. The number of particles present in the media containing no CNTs but humic and fulvic acids was checked and found to be negligible.

The quality of CNT dispersion and the length of the CNTs in the supernatant were investigated by transmission electron microscopy (TEM, Morgagni, FEI). The TEM was operated at an acceleration voltage of 100 kV and the images were recorded on a CCD camera. CNTs suspended in the supernatants were deposited directly on TEM grids (Cu grids:  $\varnothing$  3.05 mm, carbon formvar coated, SPI) by ultracentrifugation (28,000 rpm  $\approx$  125,000  $\times$  g, 5 h). These conditions result in a total deposition of particles  $> 5$  nm (equivalent spherical diameter, density 1.3 g/cm<sup>3</sup>) suspended in the water column above the grid (volume = 73.1  $\mu$ L). The formation of CNT-agglomerate-artefacts during centrifugation due to deposition of CNTs on each other could be excluded, as TEM pictures with CNT-agglomerates in the supernatant clearly differ from pictures resulting from individually suspended CNTs. TEM images therefore provide an insight as to whether the CNTs are predominantly present in the supernatant as individually dispersed tubes or as small agglomerates.

The length distributions of the CNTs were derived by applying images analysis (IA) tools (Russ, 2002) on several images. The weak contrast between the CNTs and the background precluded the direct application of the IA on the original TEM images. Thus, the CNTs were manually traced along their curved contours (avoiding

crossings) on hardcopies to enhance the contrast. These images were then scanned and used for the IA procedure. Several hundred CNTs were evaluated (on average about  $\approx$  540 CNTs) for each sample to derive a length distribution.

### 3. Results

#### 3.1. Dispersability of CNTs over time

Fig. 1a depicts the amount of “dry CNTs” that remained suspended in different media. The CNT amounts measured in the background medium remained below the detection limit over the entire shaking period. In suspensions containing 20 mg/L SRHA and SRFA, the amount of CNTs found in the supernatant increased with shaking time. However, after 8 days shaking the amount of CNTs in suspension was around the detection limit of 0.1% of the added CNTs, and the amount of CNTs suspended after 20 days reached

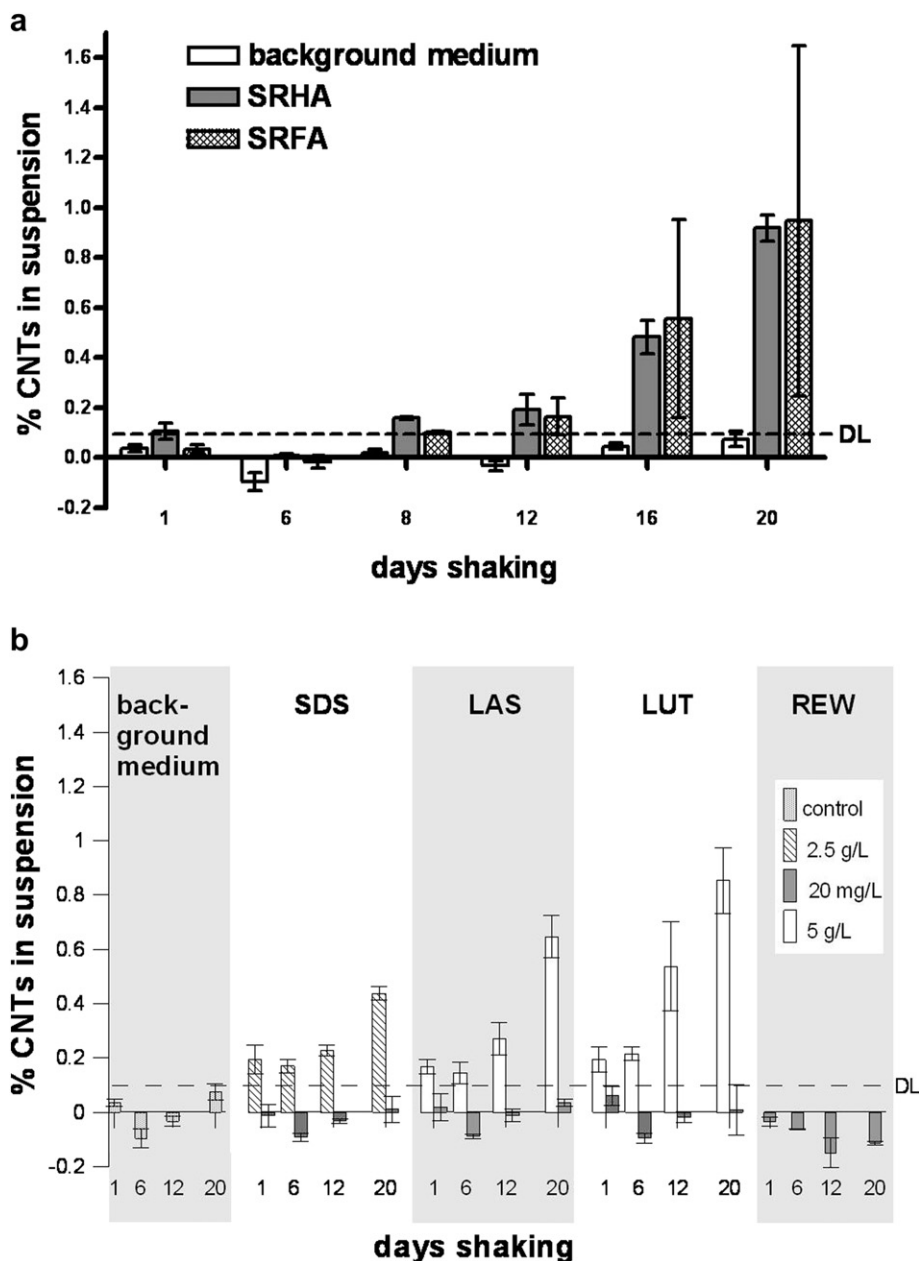


Fig. 1. Dispersion of dry CNTs (50 mg/L) in a) 20 mg/L SRHA and SRFA and b) in varying surfactant concentrations over time. Measured after 5 days of settling. Error bars  $\sigma = 1$ ,  $n = 3$ . Measured absorption values below the detection limit of 0.1% of the added CNTs are reported for illustrative purposes but cannot be quantified.

only 1% for SRHA and SRFA. There was no clear difference between the two NOMs with regard to their dispersion potential.

The ability of different detergents to suspend “dry CNTs” is shown in Fig. 1b. At higher concentrations of SDS (2.5 g/L), LAS (5 g/L) and LUT (5 g/L) the dispersed fraction increased over time. However, the amount of CNTs found in the supernatant did not exceed 1% of the added CNTs. The REW at higher concentrations was not measured because it also absorbed at 800 nm. The dispersing effect of detergents at 20 mg/L, a surfactant concentration occurring in raw waste waters (Matthijs et al., 1999), was similar to that of the controls and remained below the detection limit of 0.1% of the added CNTs for all three detergent types (anionic, cationic and non-ionic detergent).

Table 1 gives an overview of the CNT dispersability of “pre-dispersed CNTs” as a function of shaking time. The subsequent settling period of all these samples lasted 5 days. For CNT pre-dispersion in different stock suspension followed by addition in background medium only, the CNT-stability depended on the chosen dispersant and its resultant concentration in the sample. CNTs in NMP were only marginally stable (<1% of the added CNTs) when mixed with background medium. In the case of CNT pre-dispersion in a 12.5 g/L SDS stock suspension, there were 18% CNTs left in the supernatant after 20 days of shaking and 5 days of settling. A CNT pre-dispersion in LAS (25 g/L) resulted in similar percentages of CNTs in the supernatant (20%) of background medium at the end of the experiments.

The ability of SRHA and SRFA to suspend pre-dispersed CNTs differed. SRHA was quite effective in keeping CNTs pre-dispersed in NMP in suspension. After 5 h of shaking, 36% of the CNTs remained in suspension. During the subsequent days of shaking a re-suspension of CNTs occurred. Humic acid was able to prevent further sedimentation of CNTs and even increased the CNTs present in the supernatant over time. Within the first 5 h of shaking SRFA stabilized only 18% CNTs from the same NMP-stock suspension. Over the next 8 days of shaking the amount decreased to 3%. SRFA was more efficient in dispersing CNTs derived from SDS stock suspensions. CNT pre-dispersed in three different SDS concentrations (12.5 g/L, 2.5 g/L and 0.1 g/L) were added to SRFA. For all setups there was a decrease with shaking time. However, at the highest SDS concentrations there was hardly any precipitation within the first 5 h of shaking followed by 5 days of settling. After 20 days of shaking there was still 65% of the CNTs stable in suspension.

The influence of varying detergent concentrations in the medium on CNT dispersability was tested as well. CNTs were pre-dispersed in NMP and added into background medium containing 5 g/L and 250 mg/L LAS. In the presence of 5 g/L LAS, a considerable amount of CNTs were stabilized (around 48%) and the variation in

CNT concentrations over 20 days of shaking was below 10%. The presence of 250 mg/L LAS had a similar effect. The suspensions were rather stable, containing around 40% CNTs. The influence of sample preparation is revealed when comparing the results of the samples using LAS (5 g/L) once as a dispersant and once as a compound. Pre-dispersion followed by addition into LAS-medium stabilizes around 20% CNTs more in the supernatant than pre-dispersion in LAS and addition into background medium only (Table 1).

### 3.2. Settling of CNT suspensions

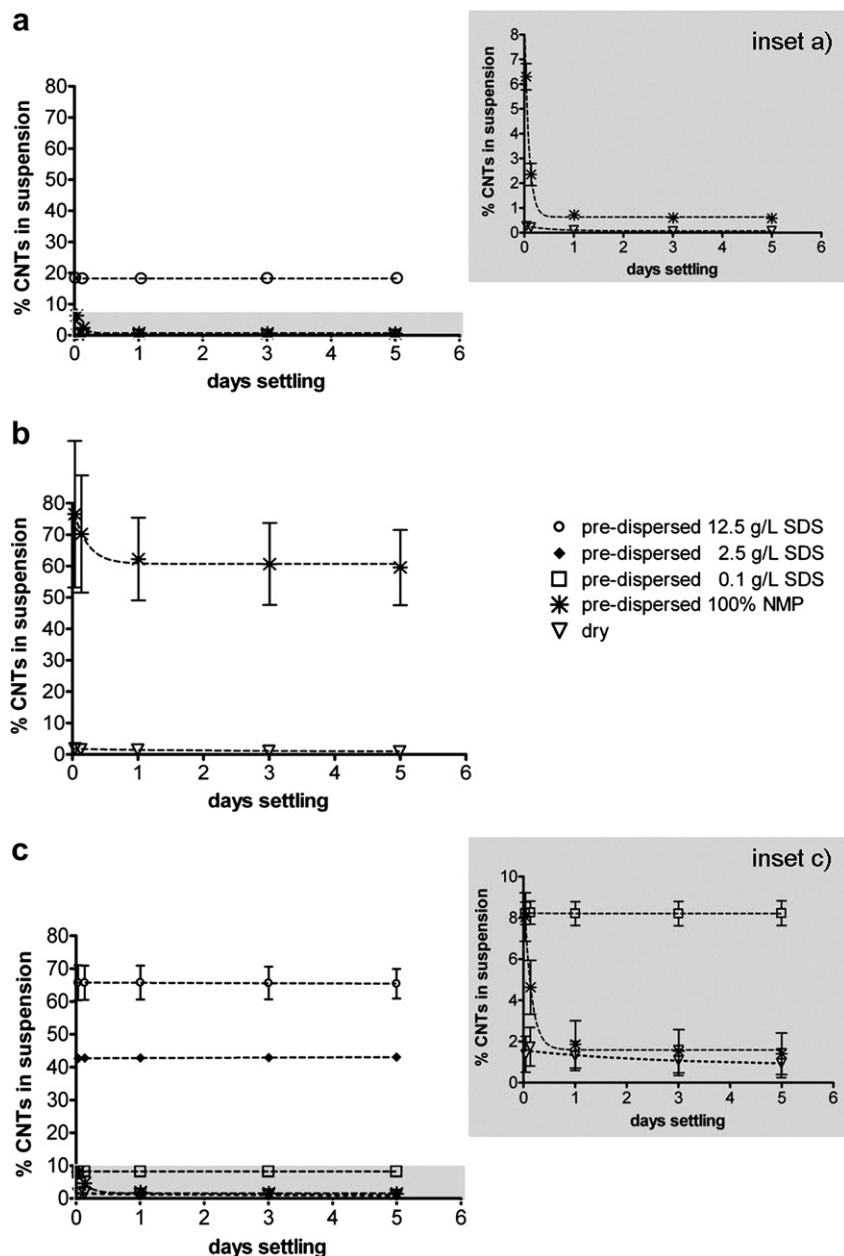
The colloidal stability of suspensions of dry and pre-dispersed CNTs for up to 5 days is presented in Fig. 2. All initial suspensions exhibited characteristic sedimentation kinetics. The marginal CNT amount found in the supernatant after shaking of “dry CNTs” settled steadily over 5 days ( $\approx 1.9$ – $0.1\%$  CNTs). The colloidal stability of CNTs pre-dispersion in NMP was characterized by a distinct sedimentation within the first 24 h, until reaching a plateau. Shaking of CNTs pre-dispersed in SDS resulted in remarkably stable suspensions. For all the samples it was found that once having reached a plateau, the concentration hardly changed for up to 10 days of settling (results not shown).

### 3.3. Particle characterization by NTA and TEM

The particle sizes determined by NTA (Table 2) indicate differences according to the experimental setup. The particle sizes of “dry CNTs” decreased in all media (SRFA, SRHA, SDS) with shaking time. SRHA was able to stabilize the largest particles. After 20 days of shaking, the particles detected in SRHA samples were around 150 nm and the ones in SRFA and SDS had mean size values of around 100 nm (particle size distribution see Supporting information). Shaking of CNTs pre-dispersed in NMP resulted in the smallest particle sizes, when added to a stabilizing medium (SRFA, SRHA or LAS). The same trend was observed in the case of CNT pre-dispersion in SDS. CNT–SDS–stock suspensions added to background medium resulted in larger particle sizes than when added into SRFA. NTA yields hydrodynamic diameters for 3D structures present in the measured samples. The experimental suspensions contained either single suspended flexible rod-like structures with adsorbed compounds or agglomerates thereof. CNTs pre-dispersed and added to background medium only had larger diameters due to faster agglomeration compared to when stabilizing agents were present. The smaller the particle sizes based on NTA measurements, the more likely the particles are present as single suspended CNTs.

**Table 1**  
Dispersion of CNTs (50 mg/L) in different media: % CNTs in the supernatant; shaken for 5 h, 1 day, 8 days, 12 days and 20 days followed by 5 days settling. The standard deviations of the 3 replicates are in parentheses.

Stock suspension			Background medium plus		Shaking				
Dispersant	Conc. stock		Compound	Conc. compound [mg/L]	5 h [wt% CNTs]	1 day [wt% CNTs]	8 days [wt% CNTs]	12 days [wt% CNTs]	20 days [wt% CNTs]
	In stock	In sample							
NMP	100%	5%	–	–	0.5 ( $\pm 0.3$ )	0.4 ( $\pm 0.2$ )	0.5 ( $\pm 0.1$ )	0.4 ( $\pm 0.2$ )	0.6 ( $\pm 0.1$ )
SDS	12.5 g/L	2.5 g/L	–	–	27 ( $\pm 0.3$ )	24 ( $\pm 0.2$ )	20 ( $\pm 0.3$ )	19 ( $\pm 0.8$ )	18 ( $\pm 0.4$ )
LAS	25 g/L	5 g/L	–	–	26 ( $\pm 0.2$ )	24 ( $\pm 0.1$ )	23 ( $\pm 0.2$ )	22 ( $\pm 0.2$ )	20 ( $\pm 0.5$ )
NMP	100%	5%	SRHA	20	36 ( $\pm 0.6$ )	39 ( $\pm 3.7$ )	51 ( $\pm 8.4$ )	55 ( $\pm 10.0$ )	60 ( $\pm 12.0$ )
NMP	100%	5%	SRFA	20	18 ( $\pm 3.5$ )	11 ( $\pm 2.4$ )	3 ( $\pm 2.8$ )	2 ( $\pm 1.6$ )	1 ( $\pm 1.0$ )
SDS	12.5 g/L	2.5 g/L	SRFA	20	93 ( $\pm 0.4$ )	84 ( $\pm 0.8$ )	76 ( $\pm 1.9$ )	73 ( $\pm 2.8$ )	65 ( $\pm 4.5$ )
SDS	2.5 g/L	0.5 g/L	SRFA	20	69 ( $\pm 0.8$ )	58 ( $\pm 0.9$ )	49 ( $\pm 0.5$ )	47 ( $\pm 0.2$ )	43 ( $\pm 1.0$ )
SDS	0.1 g/L	0.02 g/L	SRFA	20	17 ( $\pm 1.0$ )	13 ( $\pm 0.8$ )	12 ( $\pm 1.2$ )	10 ( $\pm 0.4$ )	8 ( $\pm 0.6$ )
NMP	100%	5%	LAS	5000	51 ( $\pm 0.5$ )	53 ( $\pm 1.5$ )	48 ( $\pm 1.4$ )	48 ( $\pm 1.8$ )	44 ( $\pm 1.3$ )
NMP	100%	5%	LAS	250	43 ( $\pm 0.6$ )	41 ( $\pm 1.0$ )	40 ( $\pm 5.7$ )	41 ( $\pm 7.8$ )	38 ( $\pm 6.5$ )



**Fig. 2.** Dispersion of CNTs (50 mg/L) in different media: a) background medium, b) 20 mg/L SRHA and c) 20 mg/L SRFA as a function of settling time after 20 days of shaking. The different symbols represent the different setups. Grey highlighted are enlargements of a) and c). DL = 0.1%, Error bars  $\sigma = 1$ ,  $n = 3$ .

TEM images of dry shaken CNTs showed few tubes, of which the majority occurred as individual CNTs (Fig. 3a). TEM grids of samples from pre-dispersed CNTs, were heavily loaded with CNTs. Pre-dispersion in NMP resulted in many homogeneously dispersed single CNTs (Fig. 3b), whilst pre-dispersion in SDS produced flocks of CNTs alternated with single dispersed CNTs (Fig. 3c).

TEM images qualitatively agreed with NTA results. TEM images of samples, where NTA analysis identified the smallest particle sizes, showed individual well dispersed CNTs (e.g.: Fig. 3b, Table 2: CNTs pre-dispersed in NMP given in SRFA, particle size distribution see Supporting information). On the other hand, in samples with larger NTA-particle sizes, CNT-flocks were observed on TEM images (e.g.: Fig. 3c, Table 2: CNTs pre-dispersed in SDS given in SRFA, particle size distribution see Supporting information). We assume

that the largest flocks were not captured by the NTA evaluation. According to the NTA-particle size distributions the majority of the particles were 10–300 nm. Some particles between 300 nm and 400 nm occurred. Few particles were detected above 400 nm. A CNT-agglomerate of 400 nm diameter needs approximately 10 days (density  $1.3 \text{ g/cm}^3$ ) to travel 2.5 cm based on Stoke's Law. The sample for NTA measurement was taken from the upper 2.5 cm of the supernatant after 7 days of settling. From a physical point of view flocks up to 475 nm can still be present in these supernatants. This is supported by our observation of the TEM images, for which sample preparation took place after 6 days of settling (Fig. 3c). The size distribution from NTA was thus derived from the single dispersed tubes and from small CNT-agglomerates present in these samples. Small CNT-agglomerates were most likely identified as single larger particles.

**Table 2**

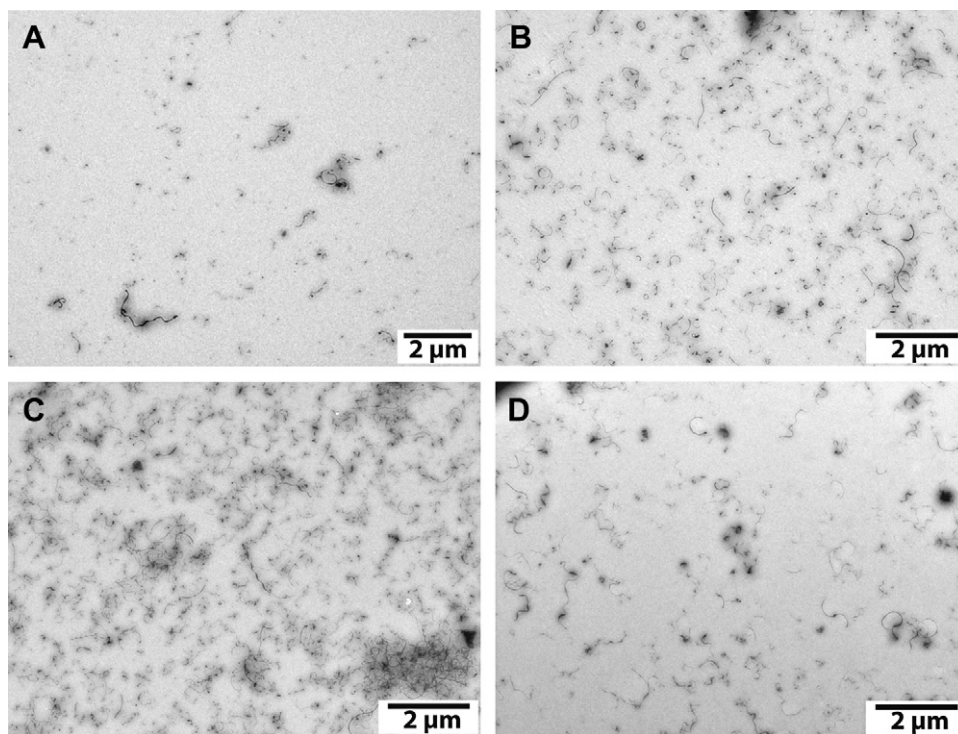
Particle size analysis by NTA in supernatant after 6 and 20 days shaking followed by 7 days of settling, n = 1.

Treatment	Dispersant conc. in sample	Medium		6 Days shaken		20 Days shaken	
		Substance	Conc. medium	Mode diameter [nm]	Mean diameter [nm]	Mode diameter [nm]	Mean diameter [nm]
Dry CNTs, shaken	–	SRFA	20 mg/L	111	106	85	99
	–	SRHA	20 mg/L	204	207	194	154
	–	SDS	2500 mg/L	204	131	64	91
In NMP pre-dispersed CNTs, shaken	5%	Background medium	–	157	135	–	–
	5%	NMP	100%	28	98	–	–
	5%	SRFA	20 mg/L	25	53	–	–
	5%	SRHA	20 mg/L	33	97	–	–
	5%	LAS	250 mg/L	38	118	–	–
In SDS pre-dispersed CNTs, shaken	2.5 g/L	Background medium	–	164	146	–	–
	0.02 g/L	SRFA	20 mg/L	112	131	–	–

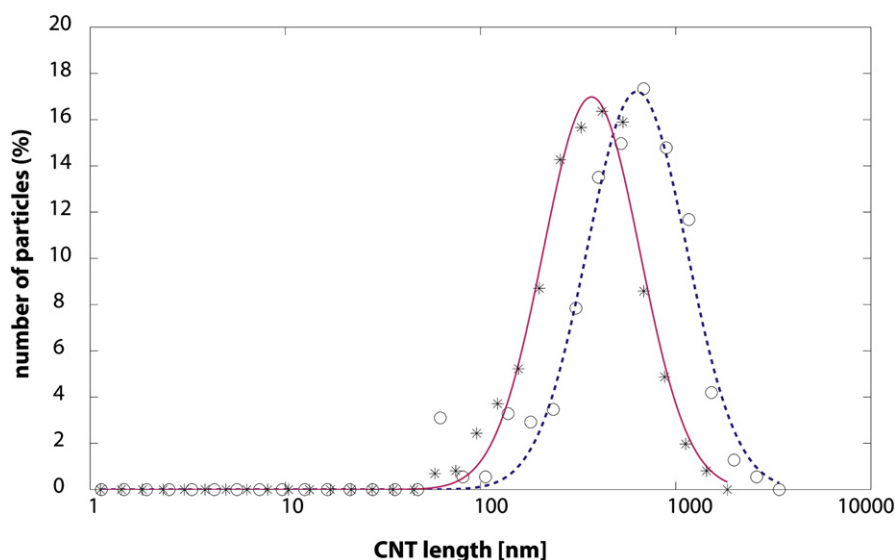
The CNT lengths in the stock suspension and in the shaken and settled supernatants were compared. In the NMP-stock suspension the CNTs had mean lengths of 633 nm (95% confidence bounds: 603–665 nm) (Fig. 3d, Fig. 4, dashed line). Similar values were detected in the case of the SDS–CNT-stock suspension (667 nm; 95% confidence bounds: 634–701 nm). This values are comparable to published data on length of Baytubes in suspension after sonification (Pauluhn, 2010). Another shift towards shorter tube lengths was detected after 20 days of shaking and 6 days of settling. The distribution of samples starting with CNTs pre-dispersed in NMP had mean lengths of 370–450 nm (Fig. 4). This fractionation of the tube lengths during the experiment occurred as well in the sample starting with CNT pre-dispersion in SDS (365 nm; 95% confidence bounds: 352–380 nm). This decrease in tube length suggests that shorter tubes remained for longer time in suspension or were better stabilized by amphiphilic compounds compared to longer ones.

#### 4. Discussion

This study provided experimental data about the partitioning of CNTs in the aqueous environment from a long-term (days–weeks) perspective. The experiments showed that the state of the CNTs at the moment of release is crucial for their environmental fate. Dry CNTs hardly get suspended irrespective of the medium composition. Only after several days of shaking a slight suspending effect of the media was detected. Mild shaking (20 days) simulating natural conditions was not sufficient to separate and suspend agglomerated dry CNTs effectively. According to the results, longer shaking times might further increase the suspended amount of CNTs. Nevertheless, for “dry CNTs” the sediments are likely to be the major sink and only minor amounts will be present as stable suspended particles. When starting with CNT-stock suspensions, shaking in the presence of an amphiphilic compound stabilized



**Fig. 3.** TEM pictures: a) supernatant of “dry CNTs” added to 20 mg/L SRFA shaken for 20 days and settled for 6 days, b) supernatant of in NMP pre-dispersed CNTs added to 20 mg/L SRFA shaken for 20 days and settled for 6 days, c) supernatant of in 0.1 g/L SDS pre-dispersed CNTs added to 20 mg/L SRFA shaken for 20 days and settled for 6 days, d) in NMP pre-dispersed CNTs immediately after sonication (1/5000 diluted).



**Fig. 4.** CNT length distribution in NMP-stock suspension (dashed line) and after 20 days of shaking in 20 mg/L SRFA followed by 5 days of settling (solid line). Number of evaluated particles was 548 and 862, respectively.

between 1 and 65% of the CNT material over longer time, depending on the composition of the stock suspension and the medium. This wide range of CNTs concentrations shows that the final deposition of pre-dispersed CNTs cannot be clearly ascribed to one compartment. The water column and the sediments can both be affected.

The previous findings that amphiphilic compounds enhance the solubilization of dry CNTs were confirmed. However, the amount of dry CNTs suspended was smaller than in most published studies, which were carried out with various CNT types. In this study around 0.5 mg/L dry CNTs were suspended in the presence of SRHA and SRFA (20 mg/L) after 20 days shaking followed by 5 days of settling. Hyung et al. (2007) detected 1.15 mg/L out of 50 mg/L CNTs dispersed in Suwannee River NOM (25 mg/L SR-NOM) after one hour of vigorous agitation followed by 4 days of settling. Kennedy et al. (2009) added 100 mg/L CNT powder and found less than 5 mg/L CNTs and around 15 mg/L CNTs in the presence of fulvic acids (16 mg/L) and humic acids (16 mg/L), respectively, after 7 days of magnetic stirring and 1 day settling. Lin et al. (2010) detected stabilization of particulate CNTs (8 mg/L) after 7 days shaking and 2 days settling in a surface water having a high TOC (16.15 mg/L) and a moderate ion concentration (4 mmol/L). In the referenced study the stabilization of pre-dispersed CNTs was investigated as well. It was found that in anionic and non-ionic surfactants pre-dispersed CNTs remained stable after mixing into water samples (36 h shaking, 2 days settling) with less than 50% of the added CNTs (13 mg/L and 17 mg/L CNTs, respectively) deposited. The comparison of these studies indicates agreement with the general findings here; however, the CNT characteristics, the applied time frame for the experiment, the dispersion method (sonication, stirring, shaking) as well as the chosen arrangement for sedimentation are critical determinants of suspension concentration. We have focused on an environmentally realistic dispersion method, a long-term approach and industrially relevant CNTs.

Hyung and Kim (2008) and Kennedy et al. (2009) found dispersion of CNT powder in humic acid to be more effective than in fulvic acid. It was assumed that fulvic acid has less capability to suspend CNTs due to its lower aromaticity and molecular mass (SRHA: aromatic C 31%, molecular weight 1066 Da (Aiken et al., 1989); SRFA: aromatic C 24%, molecular weight 800 Da (Aiken

et al., 1989)). In the current study a significant difference in effectiveness of SRHA and SRFA occurred only when starting with pre-dispersed tubes. We assume that in the case of shaking, dry CNTs were bundled too strongly for the SRHA to exhibit its higher potency.

Far higher surfactant concentrations were needed to suspend the same amount of CNTs-powder compared to NOM (Hyung et al., 2007; Wang et al., 2008a). For “dry CNT” we can conclude that the tested surfactants have no mobilizing potential at concentrations relevant to wastewater and surface waters. For “pre-dispersed CNTs” a slight effect of surfactants when present in the medium at environmentally realistic concentrations is possible.

According to TEM images, single suspended tubes were present in the supernatant of all samples. However, the number varied depending on the composition of the suspension. According to the CNT length evaluation, the shorter CNTs remained in suspension over a longer period of time. This might be due to the fact that the number of CNT-compound interactions needed to sustainably stabilize a short CNT is smaller compared to that of a longer one. As the shorter ones are more mobile in the suspension, their likelihood for interaction is greater.

In an environmental system the initial CNT concentration is expected to be lower than the concentration added in this study (50 mg/L) and the retention time in the water column might be longer due to larger dimensions of the water body. As a consequence the probability for inter-tube-collision (homoagglomeration) decreases whilst the one for heteroagglomeration increases. The proportion of single suspended CNTs present in the water column depends on their interaction with environmental colloids. In natural systems the ratio of NOM concentration to CNTs would be higher and therewith CNT suspendability is expected to be favored. However NOM is only on possible environmental colloid type. The interaction of CNTs with other natural colloids (Buffle, 2006) may promote sedimentation of CNTs due to their role as adsorption surfaces. From an environmental point of view the presence of single suspended CNTs is more problematic than large agglomerates because they are more mobile. This study identified that the release of pre-dispersed CNTs into the aquatic environment poses a greater chance for formation of large numbers of single suspended CNTs than dry CNTs.

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## Appendix A. Supplementary data

Supplementary data associated with this article, can be found in the online, at doi:10.1016/j.envpol.2011.02.044.

## References

- Aiken, G.R., Brown, P.A., Noyes, T.I., Pinckney, D.J., 1989. Molecular Size and Weight of Fulvic and Humic Acids from the Suwanee River, Humic Substances in the Suwanee River, Georgia: Interactions, Properties, and Proposed Structures. U.S. Geological Survey, Denver, Colorado.
- Bergin, S.D., Nicolosi, V., Streich, P.V., Giordani, S., Sun, Z., Windle, A.H., Ryan, P., Niraj, N.P.P., Wang, Z.-T.T., Carpenter, L., Blau, W.J., Boland, J.J., Hamilton, J.P., Coleman, J.N., 2008. Towards solutions of single-walled carbon nanotubes in common solvents. *Advanced Materials* 20, 1876–1881.
- Buffle, J., 2006. The key role of environmental colloids/nanoparticles for the sustainability of life. *Environmental Chemistry* 3, 155–158.
- Chappell, M.A., George, A.J., Dontsova, K.M., Porter, B.E., Price, C.L., Zhou, P.H., Morikawa, E., Kennedy, A.J., Steevens, J.A., 2009. Surfactive stabilization of multi-walled carbon nanotube dispersions with dissolved humic substances. *Environmental Pollution* 157, 1081–1087.
- Cho, H.-H., Smith, B.A., Wnuk, J.D., Fairbrother, D.H., Ball, W.P., 2008. Influence of surface oxides on the adsorption of naphthalene onto multiwalled carbon nanotubes. *Environmental Science & Technology* 42, 2899–2905.
- Giordani, S., Bergin, S.D., Nicolosi, V., Lebedkin, S., Kappes, M.M., Blau, W.J., Coleman, J.N., 2006. Debundling of single-walled nanotubes by dilution: observation of large populations of individual nanotubes in amide solvent dispersions. *Journal of Physical Chemistry B* 110, 15708–15718.
- Girifalco, L.A., Hodak, M., Lee, R.S., 2000. Carbon nanotubes, buckyballs, ropes, and a universal graphitic potential. *Physical Review B* 62, 13104.
- Gottschalk, F., Sonderer, T., Scholz, R.W., Nowack, B., 2009. Modeled environmental concentrations of engineered nanomaterials (TiO<sub>2</sub>, ZnO, Ag, CNT, fullerenes) for different regions. *Environmental Science & Technology* 43, 9216–9222.
- Han, Z., Zhang, F., Lin, D., Xing, B., 2008. Clay minerals affect the stability of surfactant-facilitated carbon nanotube suspensions. *Environmental Science & Technology* 42, 6869–6875.
- Holbrook, R.D., Kline, C.N., Filliben, J.J., 2010. Impact of source water quality on multiwall carbon nanotube coagulation. *Environmental Science & Technology* 44, 1386–1391.
- Hyung, H., Fortner, J.D., Hughes, J.B., Kim, J.H., 2007. Natural organic matter stabilizes carbon nanotubes in the aqueous phase. *Environmental Science & Technology* 41, 179–184.
- Hyung, H., Kim, J.-H., 2008. Natural organic matter (NOM) adsorption to multi-walled carbon nanotubes: effect of NOM characteristics and water quality parameters. *Environmental Science & Technology* 42, 4416–4421.
- Kennedy, A.J., Gunter, J.C., Chappell, M.A., Goss, J.D., Hull, M.S., Kirgan, R.A., Steevens, J.A., 2009. Influence of nanotube preparation in aquatic bioassays. *Environmental Toxicology and Chemistry* 28, 1930–1938.
- Kennedy, A.J., Hull, M.S., Steevens, J.A., Dontsova, K.M., Chappell, M.A., Gunter, J.C., Weiss, C.A., 2008. Factors influencing the partitioning and toxicity of nanotubes in the aquatic environment. *Environmental Toxicology and Chemistry* 27, 1932.
- Koelmans, A.A., Nowack, B., Wiesner, M.R., 2009. Comparison of manufactured and black carbon nanoparticle concentrations in aquatic sediments. *Environmental Pollution* 157, 1110–1116.
- Köhler, A.R., Som, C., Helland, A., Gottschalk, F., 2008. Studying the potential release of carbon nanotubes throughout the application life cycle. *Journal of Cleaner Production* 16, 927–937.
- Li, M.H., Huang, C.P., 2010. Stability of oxidized single-walled carbon nanotubes in the presence of simple electrolytes and humic acid. *Carbon* 48, 4527–4534.
- Lin, D., Liu, N., Yang, K., Xing, B., Wu, F., 2010. Different stabilities of multiwalled carbon nanotubes in fresh surface water samples. *Environmental Pollution* 158, 1270–1274.
- Lin, D., Liu, N., Yang, K., Zhu, L., Xu, Y., Xing, B., 2009. The effect of ionic strength and pH on the stability of tannic acid-facilitated carbon nanotube suspensions. *Carbon* 47, 2875–2882.
- Lin, D.H., Xing, B.S., 2008a. Adsorption of phenolic compounds by carbon nanotubes: role of aromaticity and substitution of hydroxyl groups. *Environmental Science & Technology* 42, 7254–7259.
- Lin, D.H., Xing, B.S., 2008b. Tannic acid adsorption and its role for stabilizing carbon nanotube suspensions. *Environmental Science & Technology* 42, 5917–5923.
- Lu, C.Y., Su, F.S., 2007. Adsorption of natural organic matter by carbon nanotubes. *Separation and Purification Technology* 58, 113–121.
- Matthijs, E., Holt, M.S., Kiewiet, A., Rijs, G.B.J., 1999. *Environmental Monitoring for Linear Alkylbenzene Sulfonate, Alcohol Ethoxylate, Alcohol Ethoxy Sulfate, Alcohol Sulfate, and Soap*. Wiley Periodicals, Inc., pp. 2634–2644.
- Montes-Burgos, I., Walczyk, D., Hole, P., Smith, J., Lynch, I., Dawson, K., 2010. Characterisation of nanoparticle size and state prior to nanotoxicological studies. *Journal of Nanoparticle Research* 12, 47–53.
- Moore, V.C., Strano, M.S., Haroz, E.H., Hauge, R.H., Smalley, R.E., Schmidt, J., Talmon, Y., 2003. Individually suspended single-walled carbon nanotubes in various surfactants. *Nano Letters* 3, 1379–1382.
- Mueller, N.C., Nowack, B., 2008. Exposure modeling of engineered nanoparticles in the environment. *Environmental Science & Technology* 42, 4447–4453.
- Nowack, B., Bucheli, T.D., 2007. Occurrence, behavior and effects of nanoparticles in the environment. *Environmental Pollution* 150, 5–22.
- Pauluhn, J., 2010. Subchronic 13-week inhalation exposure of rats to multiwalled carbon nanotubes: toxic effects are determined by density of agglomerate structures, not fibrillar structures. *Toxicological Sciences* 113, 226–242.
- Richard, C., Balavoine, F., Schultz, P., Ebbesen, T.W., Mioskowski, C., 2003. Supramolecular self-assembly of lipid derivatives on carbon nanotubes. *Science* 300, 775–778.
- Russ, J.C., 2002. *The Image Processing Handbook*, fourth ed. CRC Press, Boca Raton.
- Saleh, N.B., Pfefferle, L.D., Elimelech, M., 2008. Aggregation kinetics of multiwalled carbon nanotubes in aquatic systems: measurements and environmental implications. *Environmental Science & Technology* 42, 7963–7969.
- Saleh, N.B., Pfefferle, L.D., Elimelech, M., 2010. Influence of biomacromolecules and humic acid on the aggregation kinetics of single-walled carbon nanotubes. *Environmental Science & Technology* 44, 2412–2418.
- Thurman, E.M., 1985. *Organic Geochemistry of Natural Waters*. United States Geological Survey, Denver, Colorado, USA.
- Tummala, N.R., Striolo, A., 2009. SDS surfactants on carbon nanotubes: aggregate morphology. *ACS Nano* 3, 595–602.
- Wang, P., Shi, Q.H., Liang, H.J., Steuerman, D.W., Stucky, G.D., Keller, A.A., 2008a. Enhanced environmental mobility of carbon nanotubes in the presence of humic acid and their removal from aqueous solution. *Small* 4, 2166–2170.
- Wang, X.L., Lu, J.L., Xing, B.S., 2008b. Sorption of organic contaminants by carbon nanotubes: influence of adsorbed organic matter. *Environmental Science & Technology* 42, 3207–3212.
- Wang, X.L., Tao, S., Xing, B.S., 2009. Sorption and competition of aromatic compounds and humic acid on multiwalled carbon nanotubes. *Environmental Science & Technology* 43, 6214–6219.
- Wiesner, M.R., Lowry, G.V., Alvarez, P., Dionysiou, D., Biswas, P., 2006. Assessing the risks of manufactured nanomaterials. *Environmental Science & Technology* 40, 4336–4345.
- Wiesner, M.R., Lowry, G.V., Jones, K.L., Hochella, J.M.F., Di Giulio, R.T., Casman, E., Bernhardt, E.S., 2009. Decreasing uncertainties in assessing environmental exposure, risk, and ecological implications of nanomaterials. *Environmental Science & Technology* 43, 6458–6462.
- Yang, K., Xing, B., 2009. Adsorption of fulvic acid by carbon nanotubes from water. *Environmental Pollution* 157, 1095–1100.
- Ying, G.G., 2006. Fate, behavior and effects of surfactants and their degradation products in the environment. *Environment International* 32, 417–431.
- Zhang, L., Petersen, E.J., Huang, Q., 2011. Phase distribution of 14C-labeled multi-walled carbon nanotubes in aqueous systems containing model solids: peat. *Environmental Science & Technology* 45, 1356–1362.
- Zhang, S., Shao, T., Bekaroglu, S.S.K., Karanfil, T., 2009. The impacts of aggregation and surface chemistry of carbon nanotubes on the adsorption of synthetic organic compounds. *Environmental Science & Technology* 43, 5719–5725.