



Published in final edited form as:

Sci Total Environ. 2019 June 20; 670: 1140–1145. doi:10.1016/j.scitotenv.2019.03.311.

Interaction between functionalized multiwalled carbon nanotubes and MS2 bacteriophages in water

Anna E. Merryman^{a,1}, Indu Venu Sabaraya^{a,1}, Lewis Stetson Rowles III^a, Aleesha Toteja^a, Sofia I. Carrillo^a, Tara Sabo-Attwood^b, Navid B. Saleh^{a,*}

^a Department of Civil, Architectural and Environmental Engineering, The University of Texas at Austin, Austin, TX 78712, United States of America

^b Department of Environment and Global Health, University of Florida, Gainesville, FL 32610, United States of America

Abstract

Fate and transport of carbon nanomaterials can be strongly dependent on the interaction with secondary particulates in the aquatic systems. Bio-particulates in water, e.g., viruses with charged and hydrophobic surface moieties, may profoundly influence the interfacial behavior and hence the environmental fate of nanomaterials (and vice versa). This study systematically evaluates the interfacial interaction of acid-functionalized multiwalled carbon nanotubes (MWNTs) with MS2 bacteriophages, or heteroaggregation behavior of these particulates, under mono- and di-valent cations and with Suwannee River humic acid (SRHA). Results indicate that the highest concentration of MS2 (i.e., MWNT:MS2 of 100:1) renders exceptional stability of MWNTs, even in high salinity conditions. However, at lower MS2 concentrations (i.e., MWNT:MS2 of 1000:1 and 10,000:1), the suppression of MWNT heteroaggregation rate is not as significant. The observed enhanced stability is likely caused by the preferential attachment of the MS2 capsids onto MWNT surfaces, which is mediated by electrostatic attraction (between negatively charged oxygen-containing moieties on MWNTs and positively charged amino acid residues on MS2 surfaces) and/or by MS2 capsids with positive hydrophobicity index (indicating strong hydrophobicity). Presence of SRHA also shows stability enhancement; however, at lower MS2 concentrations, SRHA dominated the heteroaggregation behavior. These results implicate that preferential interaction between virus capsids (i.e., MS2 and may be other waterborne viruses) and carbonaceous nanomaterials may influence environmental transport of both in aquatic environments.

Graphical Abstract

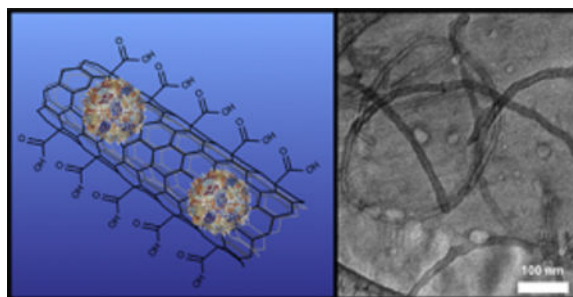
Multiwalled carbon nanotubes preferentially interact with MS2 capsids and can result in long-range transport of both.

* Corresponding author. navid.saleh@utexas.edu (N.B. Saleh).

¹ These authors contributed equally.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2019.03.311>.



Keywords

Heteroaggregation; Virus; Cryo-TEM; Fate and transport

1. Introduction

The likelihood of release of tubular carbon allotropes or carbon nanotubes (CNTs) during manufacture, use, or from CNT-enabled products at the end-of-life disposal has been shown to increase (Wiesner et al., 2006; Baughman et al., 2002; Grassian et al., 2016; Wohlleben et al., 2016). Recent studies estimate that CNTs can be released in ng/L to µg/L concentrations to surface waters and wastewater effluents, respectively, while near mg/kg quantities of CNTs may be found in the sediment environment (Gottschalk et al., 2013). Release of CNTs in the natural aquatic environments will result in interaction with naturally occurring particulates, including bio-particulates, such as viruses. Understanding the interfacial interaction between carbonaceous nanomaterials and bio-particulates is thus imperative to assess the fate and transport of both entities in natural waters.

Viruses, one of the most important bio-particles, are known to bear a large burden of the global waterborne diseases (Gorchev and Ozolins, 1984). Norwalk virus, now called norovirus, is known to cause 18% of all the diarrheal disease incidences worldwide (Ahmed et al., 2014). Bacteriophage MS2 is a positive-sense single stranded RNA virus, that infect several types of enterobacteriophages. These have similar surface composition and average virion diameter (27 nm), similar to those of several other viruses, e.g., those of norovirus. MS2 thus can be easily used as a model surrogate for norovirus in systematic environmental studies (Hoelzer et al., 2013). Due to their size and surface chemistry, these viruses have elongated residence time in the water column or in the subsurface pore-water. Though a wealth of literature is available on fate and transport of MS2 capsids (Mylon et al., 2010), as well as on inactivation of such phages with nanomaterials (Gutierrez et al., 2009; You et al., 2011; Huo et al., 2017), none of the studies consider biophysical interfacial interaction of these bio-particles with engineered carbon-nanoparticulates in the natural environment. Similarly, the studies focusing on viral removal and inactivation with different nanoparticles and with immobilized carbon nano-filters (Brady-Estévez et al., 2010; Rahaman et al., 2012) identify pore-size (i.e., not interfacial physicochemical factors) to be the key factor responsible for the demonstrated performances. These observations highlight the need for studies that evaluate the interfacial interaction of nano and bioparticles in natural aqueous systems.

Homoaggregation of single-walled (Wang and Hobbie, 2003) and multiwalled CNTs (Saleh et al., 2008) has been extensively studied under a wide range of aquatic conditions (pH, ionic strength and composition (Saleh et al., 2008), NOM (Saleh et al., 2010)) and as a function of surface functionality (Khan et al., 2013a; Smith et al., 2009) and atomic structure (e.g., chirality (Khan et al., 2013b)). Results conclusively demonstrate that these carbon allotropes aggregate in water, but can be electrosterically stabilized with geo- and bio-macromolecules (Saleh et al., 2010; Hyung and Kim, 2008; Hyung et al., 2006; Chen et al., 2017). Aggregation of CNTs in the presence of a secondary particulate is known to be facilitated or suppressed, depending on the ionic condition and concentration of the secondary particulates (Khan et al., 2013b; Bedran-Russo et al., 2013; Schierz et al., 2014). MS2 capsids on the other hand, consist of small icosahedral shells ($T=3$), composed of 90 dimers of the coat protein, which protects the genomic RNA inside (Valegård et al., 1990). The virus coat proteins include various amino acid residues, both on the interior (20–90 residues) and the exterior (1–20 and 90–129 residues) of the capsids (Penrod et al., 1996). These amino acids present positively and negatively charged moieties that include protonated amine and diaminoiminium groups and carboxylates, respectively, which display a pH dependent (Penrod et al., 1995) net surface charge on the MS2 (Penrod et al., 1996). Furthermore, a number of the residues on the capsid exterior exhibit positive hydrophathy index values, indicating strong hydrophobicity (Penrod et al., 1996; Kyte and Doolittle, 1982). Aggregation studies of these MS2 bacteriophages show that these bio-particles are highly stable and can be destabilized by elevated ionic strength and by a change in pH (Mylon et al., 2010; Langlet et al., 2007). Strong hydrophobicity and the presence of surface moieties on these colloiddally stable MS2 capsids have been shown to facilitate attachment to CNTs; however, no systematic heteroaggregation studies have been performed to assess the fate and transport of both.

This study presents a systematic assessment of acid functionalized multiwalled carbon nanotubes (MWNTs as primary particles) and MS2 (as secondary particle) interaction in aqueous environments. Different MWNT:MS2 ratios are used to evaluate the role of particle concentration on heteroaggregation kinetics under a range of monovalent (NaCl) and divalent (CaCl_2) salt concentrations and in the presence of Suwannee River humic acid (SRHA). A subset of these heterogeneous interactions is then visualized with cryogenic TEM (cryo-TEM) to decipher mechanisms of heteroaggregation.

2. Materials and methods

2.1. Preparation and characterization of MWNTs and MS2 samples

Multiwalled carbon nanotubes (MWNTs) with 8–15 nm outside diameter were procured from Cheap Tubes Inc., Brattleboro, VT. These pristine MWNTs were acid functionalized using a previously established protocol (details in SI) (Smith et al., 2009). Bacteriophage MS2 15597-B1 was obtained from American Type Culture Collection (Manassas, VA). Particle number of MWNT and MS2 suspensions were estimated using Nanoparticle Tracking Analysis (NTA) (LM10, NanoSight Ltd., Amesbury, U.K.), equipped with a 405 nm violet laser. Transmission electron microscopy (TEM; FEI Tecnai F20 200 kV S/TEM, FEI Company, Hillsboro, OR) was used to assess physical morphology, while Zetasizer-ZS

(Malvern Instruments Ltd., Worcestershire, U.K.) was used to measure the surface potential of the particles (details in SI).

2.2. Solution chemistry

Reagent grade NaCl and CaCl₂ stock solutions (Sigma-Aldrich, St. Louis, MO) were used to simulate a wide range of aquatic conditions. Suwannee River Humic Acid (SRHA) stock solution (Standard II, International Humic Substances Society) was prepared and the details are described in earlier studies (Saleh et al., 2010; Khan et al., 2013a) as well as in the SI.

2.3. Aggregation kinetics

Time-resolved dynamic light scattering (TR-DLS) was employed using a goniometer system to determine hydrodynamic radii of MWNT and MS2 aggregates during both homoaggregation and heteroaggregation. Details are described in earlier studies (Saleh et al., 2008; Saleh et al., 2010; Khan et al., 2013a) and in the SI.

2.4. Cryogenic transmission electron microscopy

The samples for cryogenic TEM were vitrified in liquid ethane (at or below −182 °C) and analyzed with a FEI Tecnai F20 200 kV S/TEM (FEI Company, Hillsboro, OR). This study closely followed a previously published protocol (Bedran-Russo et al., 2013) (details in SI).

3. Results and discussion

3.1. Physicochemical properties of MWNTs and MS2

The electron micrographs show debundling of MWNTs when acid etched, with approximately 15–20 nm outer-diameter and little presence of catalyst particles. The MS2 capsids are 35.6 ± 11.3 nm in diameter (Fig. S1), and this observed size is in agreement with previous literature reports (Redman et al., 1997). The bacteriophages tend to form small clusters in the stock solution, often appearing in doublets and triplets. Hydrodynamic radii obtained from dynamic light scattering (DLS) measurements for MWNTs and MS2 are 74.0 ± 1.6 nm and 44.6 ± 2.0 nm, respectively (Fig. S2).

3.2. Electrokinetic properties of MWNTs and MS2

The electrophoretic mobility (EPM) values of MWNTs and MS2 are shown in Fig. S3(a) and (b), respectively. Carboxyl groups (pK_a = 4–5) on functionalized MWNTs are expected to dissociate at stock pH conditions, resulting in negative surface charge (Fig. S4) throughout the pH range of interest (i.e., 2–10). MS2 capsids also display negative surface charge in most pH conditions and the point of zero charge is near pH 3.0 (Fig. S4); this value is consistent with previous literature reports (Penrod et al., 1996; Redman et al., 1997; Zerda and Gerba, 1984; Langlet et al., 2008a). The driver for such surface charge trend can be identified as the dissociable functional groups (Fig. S4). EPM values of both MWNTs and MS2 are observed to decrease with the increase in salt concentration, which is caused by either double-layer compression or counter ion adsorption indicating a classical electrokinetic behavior.

3.3. Homoaggregation behavior

Homoaggregation of MWNTs and MS2 follow classical Derjaguin-Landau-Verwey-Overbeek (DLVO) type behavior with defined reaction- and diffusion-limited regimes (Fig. S5). Under mono-valent NaCl, the MS2 capsids do not show any aggregation tendency and the attachment efficiency values thus cannot be estimated with reasonable confidence. Estimated critical coagulation concentration (CCC) values are 139 mM NaCl for MWNTs and 1.34 mM and 147 mM CaCl_2 for MWNTs and MS2, respectively. The MWNT aggregation is induced by double layer compression and cation adsorption, while the MS2 aggregation is likely caused by surface adsorption of divalent Ca^{2+} and subsequent bridging with amino acid residues on the MS2 capsids (Mylon et al., 2010). These results strongly agree with the literature-reported homoaggregation behavior of MWNTs (Saleh et al., 2008; Smith et al., 2009) (reported CCC of 25–93 mM NaCl and 1.2–2.6 mM CaCl_2) and MS2 (Mylon et al., 2010) (CCC is 160 mM CaCl_2).

3.4. Heteroaggregation behavior: MS2 stabilizes MWNTs profoundly

Aggregation of MWNTs (10^{10} particles/mL) in the presence of secondary MS2 capsids (between 10^6 and 10^8 particles/mL) under both monovalent NaCl and divalent CaCl_2 has been suppressed substantially (Fig. 1). MS2, when present at the highest concentration (MWNT:MS2 of 100:1), reduce the rate of aggregation of MWNTs (i.e., homoaggregation) by 2 to 3 orders of magnitude (from 2×10^{-1} to 3×10^{-4} nm/s) in mono-valent electrolytes and by more than 4 orders of magnitude (from more than 1.0 to 5×10^{-4} nm/s or lower) in di-valent electrolytes (Fig. 1). The presence of lower MS2 concentrations (i.e., MWNT:MS2 of 1000:1 and 10,000:1) also cause a reduction in MWNT aggregation rate, but by no more than an order of magnitude (when compared to the homoaggregation rates).

Such suppression of MWNT aggregation propensity is likely due to preferential attachment of MS2 capsids onto the tubular surfaces. Though the overall surface potential of MS2 is slightly negative at the pH of the heteroaggregation studies (Fig. S4), the attachment may have been facilitated by electrostatic attraction between the positively charged amine or diaminoiminium groups in MS2 amino acid residues and negatively charged carboxylate moieties on the MWNTs (Penrod et al., 1995). MS2 may also preferentially attach to the MWNT surfaces due to the positive hydrophathy index (Penrod et al., 1996; Kyte and Doolittle, 1982; Wang et al., 2003) of the capsids, indicating strongly hydrophobic exterior of these virus surrogates (Penrod et al., 1996; Lytle and Routson, 1995; Shields, 1986). Penrod et al. used the hydrophobicity or hydrophathy scale developed previously (Penrod et al., 1996; Kyte and Doolittle, 1982). The Kyte-Doolittle scale is a commonly cited scale based on the physicochemical properties of the amino acid side-chains (Kyte and Doolittle, 1982). An algorithm was developed by Kyte and Doolittle to calculate the hydrophathy values of a protein along its sequence, where the weighted sum of the assigned hydrophathy values within a segment was sequentially plotted. The assigned hydrophathy value was computed by utilizing previously published (Chothia, 1976) data on water-vapor transfer free energies and the interior-exterior distribution of amino acid side-chains. A ‘moving window’ or a ‘moving segment’ approach assigned an average hydrophathy score for a predetermined segment length of residues and plotted the average hydrophathy of the segment against the positions of the amino acid residues starting from the amino- until the carboxyterminus. The increased

stability of the MWNTs under di-valent cations likely has resulted from a higher number of MS2 capsid attachments through cation bridging between MS2 particles and MWNTs and subsequent soft-repulsion (Langlet et al., 2008b) between the MS2-attached MWNTs. Long-range electrostatic and short-range hydrophobic interactions likely mediate preferential attachment, which has likely resulted in such colloidal stability of this heterogeneous particulate system.

3.5. Cryogenic TEM to decipher attachment mechanism

Since conventional TEM cannot visualize MS2 without addition of a stain and also suffers from the limitation of agglomeration caused by sample drying, cryo-TEM is identified as an appropriate method for obtaining visual analysis of heteroaggregation systems (Huynh et al., 2014). Representative cryo-TEM images of heteroaggregate structures formed in MWNT:MS2 suspensions of 100:1 (frozen at 45 min) and of 10,000:1 (frozen at 25 min) ratios after the addition of 100 mM NaCl are presented in Fig. 2. The MWNTs interacting in such a binary particle system exhibit a preferential association with MS2 capsids (Figs. 2 and S6). The MS2 bio-particles are found to be attached to the surface of the MWNTs in both low and high MS2 concentration cases (Fig. 2). Though cryo-TEM images present a temporal snapshot of the dynamic heteroaggregation process, these results provide convincing visual evidences of preferential interaction between MWNTs and MS2.

3.6. influence of humic acid on heteroaggregation

Heteroaggregation studies are also repeated with the addition of SHRA (as 2.5 mg/L TOC) at all solution chemistries tested previously (Fig. 3). Typical heteroaggregation histories up to 1500 s in the presence of SRHA and salts are shown in Fig. S7. Results indicate that SRHA has reduced the homoaggregation rate of the MWNTs by 0.5–1 order of magnitude compared to the rates with no SRHA. Heteroaggregation rates are also suppressed by 2–3 orders of magnitude, but only for the highest MS2 concentrations in monovalent (high salinity) and di-valent salt conditions (Fig. 3). Little change in MWNT heteroaggregation rate with lower concentrations of MS2 indicates that SRHA is controlling the aggregation process, likely by adsorption onto MWNT surfaces and by providing steric hindrances to attachment. It is likely that SRHA at lower MS2 concentrations is outcompeting MS2 for attachment onto MWNT surfaces and thus dominating the aggregation behavior under these conditions.

4. Conclusions

Interfacial interaction of MWNTs and MS2 is an important process that drives fate and transport of both particles in natural systems. The results presented indicate that MWNTs and MS2 have preferential interaction in water, mediated by long-range electrostatic and/or short-range hydrophobic forces, which are induced by the surface functional moieties in MS2 capsids and functionalized MWNTs. Such interaction stabilizes MWNTs in water, even at high salinity conditions, and thereby will likely facilitate long-range transport of both carbon nanotube and bio-particulates in surface and groundwater systems. In the presence of SRHA and high MS2 concentrations, the stability is further enhanced, which may extend the residence time of MWNTs and MS2 in natural waters. However, the

presence of MS2 in low concentrations in natural waters with SRHA may not result in an increase in stability. It is also notable that stability of particulates in heterogeneous systems will likely be altered as the naturally occurring biomacromolecule composition changes. The implication of this work may extend beyond MS2 capsids and into other similar waterborne viruses (e.g., rotavirus, adenovirus, etc.), since many such virus capsids are decorated with similar amino acid residues and functional moieties (Gutierrez et al., 2009; Dawson et al., 2005; Armanious et al., 2016). However, further studies are necessitated that involve MWNTs (functionalized with other functional groups) and other relevant waterborne viruses to assess the influence of virus capsids on facilitated transport of carbonaceous nanomaterials in the aquatic environment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The authors thank Dr. Dwight Romanovicz at the Center for Biomedical Research Support for his guidance on cryo-TEM sample preparation and imaging.

Funding

This work was supported by the National Institutes of Health (R01HL114907 to TSA and NBS) and United States Department of Agriculture (USDA NIFA 00092821 to TSA).

Abbreviations

CCC	critical coagulation concentration
CNT	carbon nanotubes
DLS	dynamic light scattering
DLVO	Derjaguin-Landau-Verwey-Overbeek
EPM	electrophoretic mobility
MWNT	multiwalled carbon nanotubes
RNA	ribonucleic acid
SRHA	Suwannee River humic acid
TEM	transmission electron microscopy
TR-DLS	time-resolved dynamic light scattering

References

Ahmed SM, Hall AJ, Robinson AE, Verhoef L, Premkumar P, Parashar UD, Koopmans M, Lopman BA, 2014 Global prevalence of norovirus in cases of gastroenteritis: a systematic review and meta-analysis. *Lancet Infect. Dis* 14 (8), 725–730. 10.1016/S1473-3099(14)70767-4. [PubMed: 24981041]

- Armanious A, Aeppli M, Jacak R, Refardt D, Sigstam T, Kohn T, Sander M, 2016 Viruses at solid-water interfaces: a systematic assessment of interactions driving adsorption. *Environ. Sci. Technol* 50 (2), 732–743. 10.1021/acs.est.5b04644. [PubMed: 26636722]
- Baughman RH, Zakhidov AA, De Heer WA, 2002 Carbon nanotubes — the route toward applications. *Science*, 787–792 10.1126/science.1060928.
- Bedran-Russo AK, Karol S, Pashley DH, Viana G, 2013 Site specific properties of carious dentin matrices biomodified with collagen cross-linkers. *Am. J. Dent* 26 (5), 244–248. 10.1007/s11103-011-9767-z. [PubMed: 24479274]
- Brady-Estévez AS, Nguyen TH, Gutierrez L, Elimelech M, 2010 Impact of solution chemistry on viral removal by a single-walled carbon nanotube filter. *Water Res.* 44 (13), 3773–3780. 10.1016/j.watres.2010.04.023. [PubMed: 20569966]
- Chen M, Zeng G, Xu P, Yan M, Xiong W, Zhou S, 2017 Interaction of carbon nanotubes with microbial enzymes: conformational transitions and potential toxicity. *Environ. Sci.: Nano* 4 (10), 1954–1960. 10.1039/C7EN00512A.
- Chothia C, 1976 The nature of the accessible and buried surfaces in proteins. *J. Mol. Biol* 105 (1), 1–12. 10.1016/0022-2836(76)90191-1. [PubMed: 994183]
- Dawson DJ, Paish A, Staffell LM, Seymour IJ, Appleton H, 2005 Survival of viruses on fresh produce, using MS2 as a surrogate for norovirus. *J. Appl. Microbiol* 98 (1), 203–209. 10.1111/j.1365-2672.2004.02439.x. [PubMed: 15610433]
- Gorchev HG, Ozolins G, 1984 WHO Guidelines for Drinking-Water Quality.
- Gottschalk F, Sun T, Nowack B, 2013 Environmental concentrations of engineered nanomaterials: review of modeling and analytical studies. *Environ. Pollut* 181, 287–300. 10.1016/j.envpol.2013.06.003. [PubMed: 23856352]
- Grassian VH, Haes AJ, Mudunkotuwa IA, Demokritou P, Kane AB, Murphy CJ, Hutchison JE, Isaacs JA, Jun Y-S, Karn B, et al., 2016 NanoEHS — defining fundamental science needs: no easy feat when the simple itself is complex. *Environ. Sci.: Nano* 3 (1), 15–27. 10.1039/C5EN00112A.
- Gutierrez L, Li X, Wang J, Nangmenyi G, Economy J, Kuhlenschmidt TB, Kuhlenschmidt MS, Nguyen TH, 2009 Adsorption of rotavirus and bacteriophage MS2 using glass fiber coated with hematite nanoparticles. *Water Res.* 43 (20), 5198–5208. 10.1016/j.watres.2009.08.031. [PubMed: 19766286]
- Hoelzer K, Fanaselle W, Pouillot R, Van Doren JM, Dennis S, 2013 Virus inactivation on hard surfaces or in suspension by chemical disinfectants: systematic review and meta-analysis of norovirus surrogates. *J. Food Prot* 76 (6), 1006–1016. 10.4315/0362-028X.JFP-12-438. [PubMed: 23726196]
- Huo Z-Y, Luo Y, Xie X, Feng C, Jiang K, Wang J, Hu H-Y, 2017 Carbon-nanotube sponges enabling highly efficient and reliable cell inactivation by low-voltage electroporation. *Environ. Sci.: Nano* 4 (10), 2010–2017. 10.1039/C7EN00558J.
- Huynh KA, McCaffery JM, Chen KL, 2014 Heteroaggregation reduces antimicrobial activity of silver nanoparticles: evidence for nanoparticle-cell proximity effects. *Environ. Sci. Technol. Lett* 1 (9), 361–366. 10.1021/ez5002177.
- Hyung H, Kim JH, 2008 Natural organic matter (NOM) adsorption to multi-walled carbon nanotubes: effect of NOM characteristics and water quality parameters. *Environ. Sci. Technol* 42 (12), 4416–4421. 10.1021/es702916h. [PubMed: 18605564]
- Hyung H, Fortner JD, Hughes JB, Kim J-H, 2006 Natural organic matter stabilizes carbon nanotubes in the aqueous phase. *Environ. Sci. Technol*, 0–5 10.1021/es061817g.
- Khan IA, Aich N, Afrooz ARMN, Flora JRV, Schierz PA, Ferguson PL, Sabo-Attwood T, Saleh NB, 2013a Fractal structures of single-walled carbon nanotubes in biologically relevant conditions: role of chirality vs. media conditions. *Chemosphere* 93 (9), 1997–2003. 10.1016/j.chemosphere.2013.07.019. [PubMed: 23920360]
- Khan IA, Afrooz ARMN, Flora JRV, Schierz PA, Ferguson PL, Sabo-Attwood T, Saleh NB, 2013b Chirality affects aggregation kinetics of single-walled carbon nanotubes. *Environ. Sci. Technol* 47 (4), 1844–1852. 10.1021/es3030337. [PubMed: 23343128]
- Kyte J, Doolittle RF, 1982 A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol* 157 (1), 105–132. 10.1016/0022-2836(82)90515-0. [PubMed: 7108955]

- Langlet J, Gaboriaud F, Gantzer C, 2007 Effects of PH on plaque forming unit counts and aggregation of MS2 bacteriophage. *J. Appl. Microbiol.* 103 (5), 1632–1638. 10.1111/j.1365-2672.2007.03396.x. [PubMed: 17953574]
- Langlet J, Gaboriaud F, Duval JFL, Gantzer C, 2008a Aggregation and surface properties of F-specific RNA phages: implication for membrane filtration processes. *Water Res.* 42 (10–11), 2769–2777. 10.1016/j.watres.2008.02.007. [PubMed: 18329685]
- Langlet J, Gaboriaud F, Gantzer C, Duval JFL, 2008b Impact of chemical and structural anisotropy on the electrophoretic mobility of spherical soft multilayer particles: the case of bacteriophage MS2. *Biophys. J* 94 (8), 3293–3312. 10.1529/biophysj.107.115477. [PubMed: 18192368]
- Lytle CD, Routson LB, 1995 Minimized virus binding for tests of barrier materials. *Appl. Environ. Microbiol* 61 (2), 643–649. [PubMed: 7574603]
- Mylon SE, Rinciog CI, Schmidt N, Gutierrez L, Wong GCL, Nguyen TH, 2010 Influence of salts and natural organic matter on the stability of bacteriophage MS2. *Langmuir* 26 (2), 1035–1042. 10.1021/la902290t. [PubMed: 19775143]
- Penrod SL, Olson TM, Grant SB, 1995 Whole particle microelectrophoresis for small viruses. *J. Colloid Interface Sci.* 521–523 10.1006/jcis.1995.1354.
- Penrod SL, Olson TM, Grant SB, 1996 Deposition kinetics of two viruses in packed beds of quartz granular media. *Langmuir* 12 (23), 5576–5587. 10.1021/la950884d.
- Rahaman MS, Vecitis CD, Elimelech M, 2012 Electrochemical carbon-nanotube filter performance toward virus removal and inactivation in the presence of natural organic matter. *Environ. Sci. Technol* 46 (3), 1556–1564. 10.1021/es203607d. [PubMed: 22196381]
- Redman J, Grant S, Olson T, Hardy M, 1997 Filtration of recombinant Norwalk virus particles and bacteriophage MS2 in quartz sand: importance of electrostatic interactions. *Environ. Sci. Technol* 31 (12), 3378–3383.
- Saleh NB, Pfefferle LD, Elimelech M, 2008 Aggregation kinetics of multiwalled carbon nanotubes in aquatic systems: measurements and environmental implications. *Environ. Sci. Technol* 42 (21), 7963–7969 Aggregation. 10.1021/es801251c. [PubMed: 19031888]
- Saleh NB, Pfefferle LD, Elimelech M, 2010 Influence of biomacromolecules and humic acid on the aggregation kinetics of single-walled carbon nanotubes. *Environ. Sci. Technol* 44 (7), 2412–2418. 10.1021/es903059t. [PubMed: 20184360]
- Schierz A, Espinasse B, Wiesner MR, Bisesi JH, Sabo-Attwood T, Ferguson PL, 2014 Fate of single walled carbon nanotubes in wetland ecosystems. *Environ. Sci.: Nano* 1 (6), 574–583. 10.1039/C4EN00063C.
- Shields PA, 1986 Factors Influencing Virus Adsorption to Solids. Ph.D. Dissertation. University of Florida, Gainesville Vol. PhD.
- Smith B, Wepasnick K, Schrote KE, Bertele AR, Ball WP, O'Melia C, Fairbrother DH, 2009 Colloidal properties of aqueous suspensions of acid-treated, multi-walled carbon nanotubes. *Environ. Sci. Technol.* 43 (3), 819–825. 10.1021/es802011e. [PubMed: 19245021]
- Valegård K, Liljas L, Fridborg K, Unge T, 1990 The three-dimensional structure of the bacterial virus MS2. *Nature* 345 (6270), 36–41. 10.1038/345036a0. [PubMed: 2330049]
- Wang H, Hobbie EK, 2003 Amphiphobic carbon nanotubes as macroemulsion surfactants. *Langmuir* 19 (8), 3091–3093. 10.1021/la026883k.
- Wang S, Humphreys ES, Chung S-Y, Delduco DF, Lustig SR, Wang H, Parker KN, Rizzo NW, Subramoney S, Chiang Y-M, et al., 2003 Peptides with selective affinity for carbon nanotubes. *Nat. Mater.* 2 (3), 196–200. 10.1038/nmat833. [PubMed: 12612679]
- Wiesner MR, Lowry GV, Alvarez P, Dionysiou D, Biswas P, 2006 Assessing the risks of manufactured nanomaterials. *Environ. Sci. Technol.* 40 (14), 4336–4345. 10.1021/es062726m. [PubMed: 16903268]
- Wohlleben W, Meyer J, Muller J, Müller P, Vilsmeier K, Stahlmecke B, Kuhlbusch TAJ, 2016 Release from nanomaterials during their use phase: combined mechanical and chemical stresses applied to simple and multi-filler nanocomposites mimicking Wear of nano-reinforced tires. *Environ. Sci.: Nano* 3 (5), 1036–1051. 10.1039/C6EN00094K.

- You J, Zhang Y, Hu Z, 2011 Bacteria and bacteriophage inactivation by silver and zinc oxide nanoparticles. *Colloids Surf. B: Biointerfaces* 85 (2), 161–167. 10.1016/j.colsurfb.2011.02.023. [PubMed: 21398101]
- Zerda KS, Gerba CP, 1984 Agarose isoelectrofocusing of intact virions. *J. Virol. Methods* 9 (1), 1–6. 10.1016/0166-0934(84)90077-6. [PubMed: 6094606]

HIGHLIGHTS

- Heteroaggregation between multiwalled carbon nanotubes and MS2 phages is studied.
- MS2 phages stabilize the carbon nanotubes even in high salinity conditions.
- The stability is a likely result of MS2 preferential-attachment on nanotube surface.
- Electrostatic attraction between MS2 and nanotubes is a driver for attachment.
- Positive hydropathy index (strong hydrophobicity) of MS2 can be another driver.

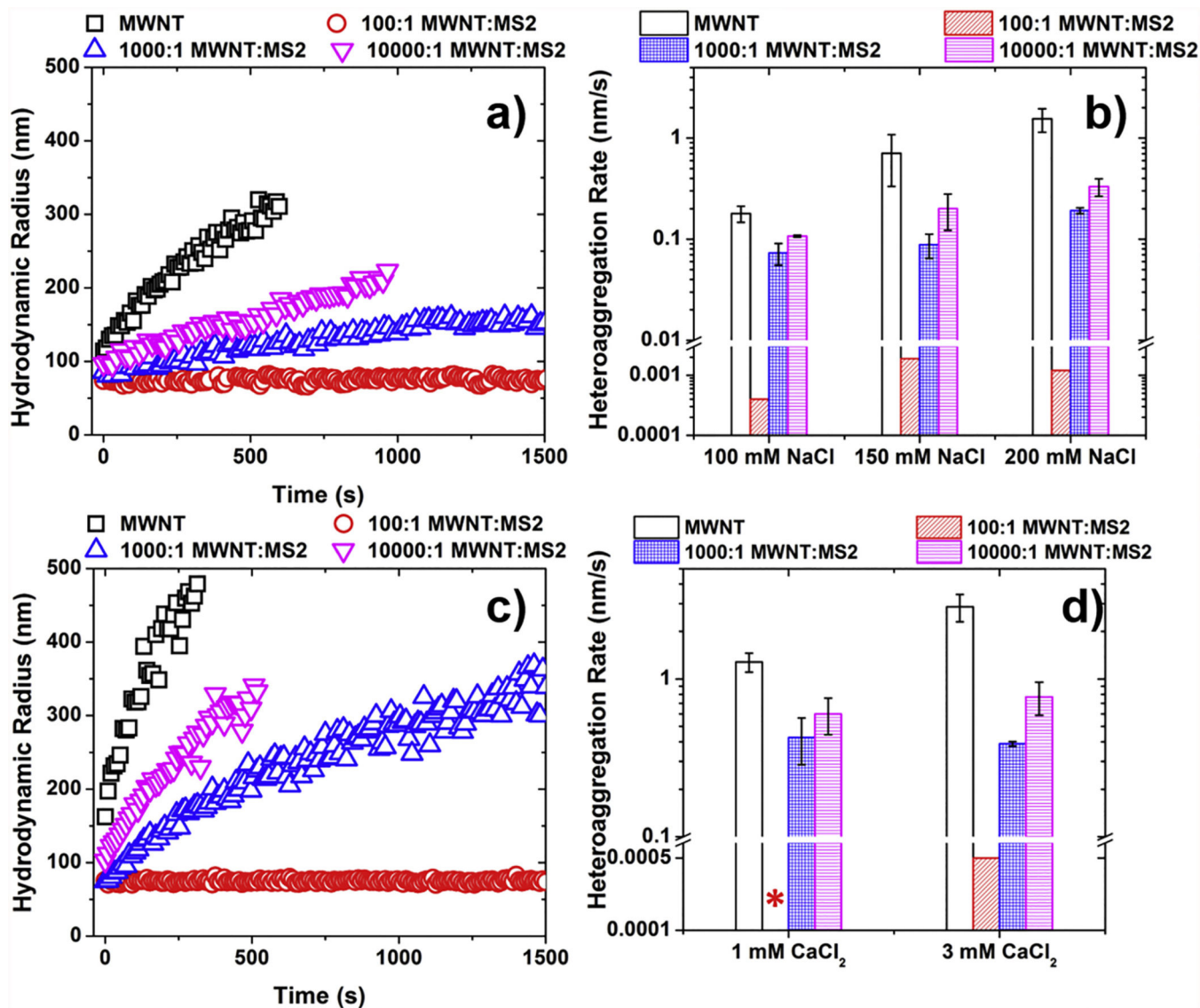


Fig. 1. Aggregation history and aggregation rates with (a–b) monovalent NaCl and (c–d) di-valent CaCl₂. Error bars in (b) and (d) represent standard deviations from at least three replicates. The asterisk represents near-zero aggregation rate, which could not be estimated with a reasonable accuracy. All measurements were conducted at pH 5.6.

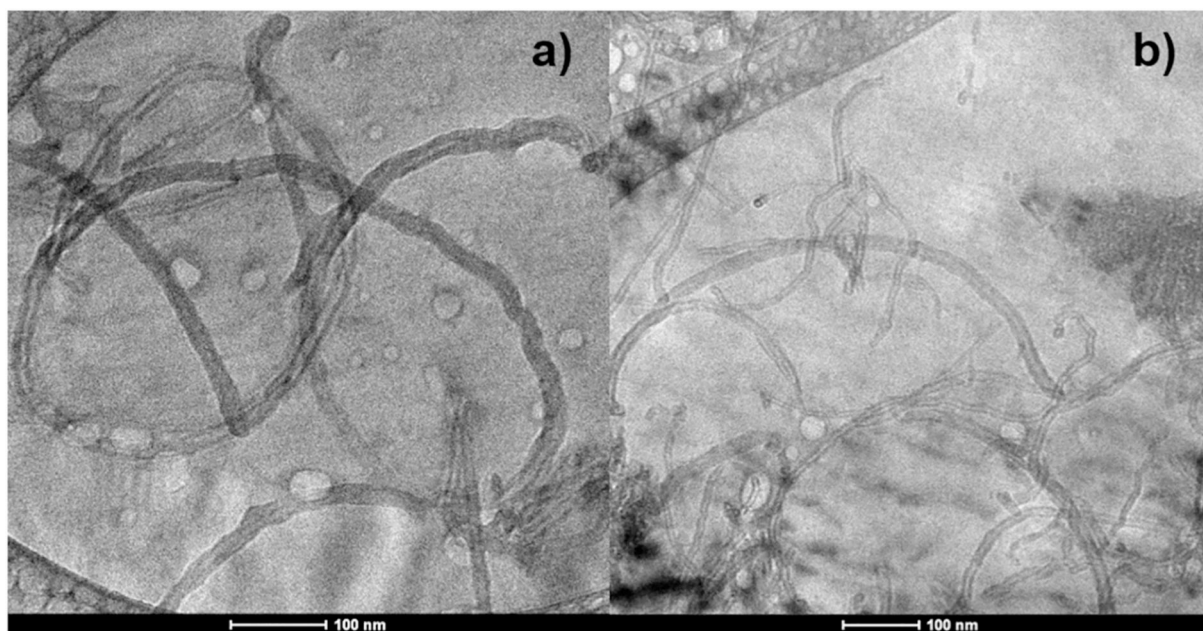


Fig. 2. Representative cryogenic TEM images of heteroaggregates at MWNT:MS2 of (a) 100:1 and (b) 10,000:1. Samples were frozen after 45 and 25 min for 'a' and 'b', respectively. The background electrolyte concentration was 100 mM NaCl.

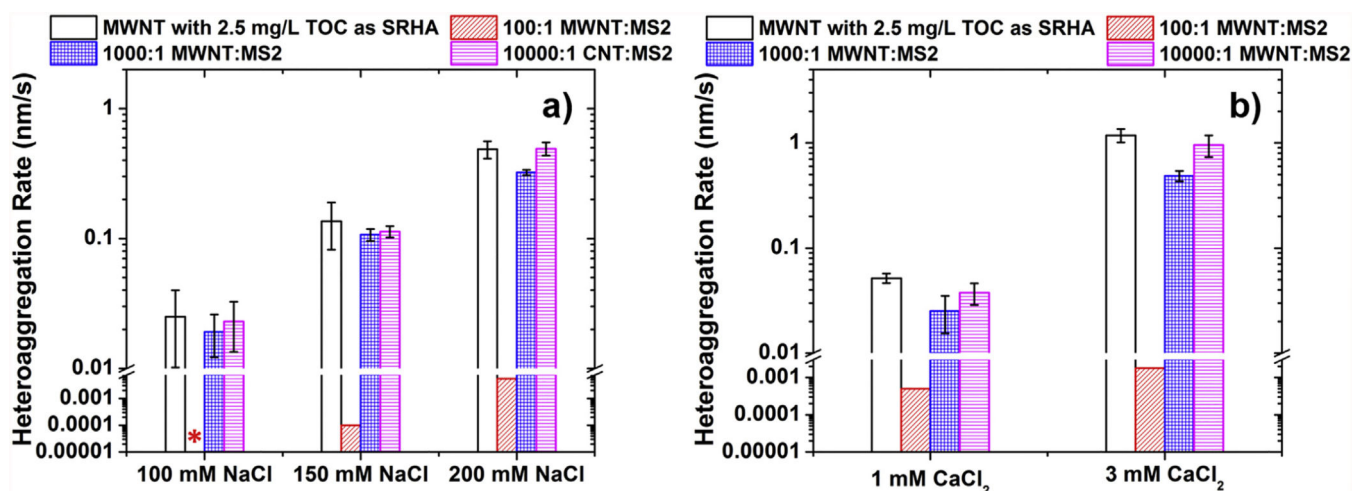


Fig. 3.

Effect of Suwannee River humic acid (SRHA) on heteroaggregation under (a) monovalent salt (NaCl) and (b) di-valent CaCl₂. SRHA was used at 2.5 mg/L TOC concentration for all the above measurements. Error bars in (b) and (d) represent standard deviations from at least three replicates. The asterisk represents near-zero aggregation rate, which could not be estimated with a reasonable accuracy. All measurements were conducted at pH 5.6.