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Effect of Kaolinite on the Fate and Transport of Carbon Nanotubes in Environmentally Relevant Conditions

By

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Abstract

Effect of Kaolinite on the Fate and Transport of Carbon Nanotubes in Environmentally Relevant Conditions

Fate and transport of the engineered nanomaterials (ENMs) in aquatic systems has been increasingly concerned due to their potential human exposure and reported toxicity to the living organisms. The ubiquitous naturally occurring colloids (NOC) is an important impacting factor controlling the aqueous stability and subsurface transport of ENMs in porous media. This study investigated the effect of kaolinite and dissolved natural organic matter on the aggregation, stability and subsurface mobility of carboxyl-functionalized multi-walled carbon nanotubes (COOH-MWCNT) under a range of environmentally relevant solution conditions.

The increase of ionic strength and decrease of pH enhanced the heteroaggregation, CNT and kaolinite can form both primary and secondary heteroaggregates under different solution conditions. The effect of heteroaggregation, strongly depends on the CNT-to-kaolinite ratio; it can either increase or decrease the stability of the suspension depending on the structure of the heteroaggregates formed. Ca$^{2+}$ and dissolved NOM played opposite roles on the stability of CNT. The addition of Ca$^{2+}$ induced the heteroaggregation through bridging effect and charge screening while NOM hindered the aggregation via steric effect in low Ca$^{2+}$ conditions. When Ca$^{2+}$ reached a high concentration range, the effect of Ca$^{2+}$ dominated and induced large heteroaggregates, destabilizing the CNT-kaolinite system. In natural surface waters, CNT exhibits decreased stability, a notable portion of which is attributed to the naturally occurring colloids.
The effect of other water quality parameters (e.g., ionic composition) also contributes to the decreased stability.

Flow cytometry, which is a common technique for cell analysis in biological field, was applied in analyzing aggregations in Alexa Fluor 633 dyed carbon nanotubes (AFCNT) and kaolinite mixture under various pH and ionic strength conditions. By testing the extremely low concentrations of AFCNT and kaolinite mixture samples, the flow cytometer rapidly provided data needed in quantitatively determining the degree of homo- and heteroaggregations. It can be applied on future aggregation studies of ENM-NOC systems and provide meaningful information for the risk management of ENMs in aquatic environments.

The mobility of CNT in porous media is strongly dependent on the degree of CNT-kaolinite heteroaggregation and the formation of the aggregates. Results demonstrated that significant CNT-kaolinite heteroaggregations occurred under extremely low pH (pH=3 in 1 mM NaCl) or extremely high ionic strength (pH=9, 100 mM NaCl) conditions. Under the conditions when large secondary aggregation formed at low pH, kaolinite hindered CNT mobility through straining effect. However, CNT transport was facilitated by kaolinite when primary heteroaggregates are the main formation in high ionic strength and high pH. When there were no CNT-kaolinite interactions, CNT mobility was enhanced probably because kaolinite competed with CNT for the adsorption sites on porous media.

Findings in the study highlighted the important role of naturally occurring colloids, dissolved natural organic matter and solution chemistry on environmental fate and transport of CNT. It provides fundamental information for the prediction of CNT and its risk assessment in natural water systems.
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Chapter 1

Introduction

1.1 Introduction

Due to their unique properties, carbon nanotubes (CNT) have been one of the most widely used engineered nanomaterials (ENMs) with significantly increasing world-wide production. The number of consumer products made from carbonaceous materials more than doubled between 2006 and 2001, with over 40% of the 91 products being made from CNT\(^1\). Thus, the release of CNT into natural water systems is inevitable. As a matter of fact, modelling studies predicted that the CNT concentration in surface water was 0.001 ng/L in the U.S. in 2008 with an annual increase in the sediment of 46 ng/(kg yr)\(^2\). Moreover, in recent years evidences of CNT toxicity have been reported, such as inflammation in rat lungs\(^3\), DNA damages\(^4\), bacterial cell membrane damage\(^5\) and pathological effects in mice\(^6\), etc. As a result, learning about CNT’s human exposure pathways in the aquatic systems has become an increasing concern. It is crucial to investigate fate and transport of CNT in surface water and sediments to better assess their potential human health and ecological impacts through these routes.

In most cases, ENMs do not necessarily transport in surface water and groundwater in isolation. On the contrary, a majority of disposed ENMs form aggregates either with themselves (homoaggregation) or with other particles (heteroaggregation) in the underground and surface water. In river systems, naturally occurring colloids (NOCs) such as inorganic minerals and natural organic matter (NOM) have concentrations much greater than those of ENMs. Thus,
heteroaggregation between ENMs and NOCs is highly likely to occur. This can lead to important changes in particle size distribution and surface physicochemical properties, which consequently affect the transport behaviors of the ENMs in the subsurface environments. Nevertheless, most studies on ENM transport have focused on ENMs alone. Only few recent studies investigated ENMs transport in the presence of NOCs. How various NOCs affect the aqueous stability of ENNs and their transport through subsurface porous media remains a largely unexplored area. This study investigates the stability of CNTs (i.e. the extent of CNTs remaining suspended in solution at equilibrium) aqueous phase and their transport in saturated porous media in the presence of kaolinite, one of the most common clay minerals. Because solution conditions such as ionic strength and pH can greatly alter ENM particle surface charge, they are expected to have significant impact on CNT-kaolinite interactions and co-transport processes. In natural systems, other environmental components such as calcium ions and NOM, which are factors known to affect colloidal behavior, may also play a key role in the fate, transport, and toxicity of CNT. However, there has been no systematic investigation on the effects of these environmental factors on CNT fate and transport in the presence of natural colloids.

This work seeks to address the following research gaps:

a. The majority of the CNT transport studies were carried out in CNT alone conditions. Research is needed to address the heteroaggregation and co-transport behaviors of CNT under the presence of natural colloids such as kaolinite. In addition, the role of CNT’s special long-aspect ratio and kaolinite surface charge heterogeneity on the heteroaggregation needs to be examined.

b. There is very limited information regarding the effect of coexisting environmental components on the stability and mobility of CNT-kaolinite binary system. Although the presence of NOM
and calcium ions is expected to affect particle interactions as well as transport in various solution pHs and salinity ranges, little is known about how they affect the above behaviors of CNT in the presence of kaolinite.

1.2 Research objectives and hypothesis

The main aim of this research is to examine the roles of kaolinite, solution chemistry and natural organic matter in the stability of CNTs in aqueous phase and their transport in saturated porous media, which will provide necessary information for the prediction of CNT fate and transport in natural aquatic environments.

Specific objectives and underlying hypotheses are as follows:

Objective 1. To explore the impact of kaolinite and various environmental compositions (solution pH, ionic strength, Ca$^{2+}$ and NOM) on the stability of CNTs in aqueous phase.

Hypothesis:

a. Stability of the CNT-kaolinite binary system could be decreased and heteroaggregation between CNT and kaolinite could occur as the solution pH decreases and ionic strength increases. For only negatively charged CNT (e.g., COOH-CNTs) and kaolinite-only suspensions, a decrease in pH and an increase in ionic strength each result in less negative surface charge and a thinner electrical double layer for the CNT and kaolinite particles. Thus, the repulsive electrostatic force among particles will decrease and the CNT-only or kaolinite mono-systems will be destabilized$^{7,8}$. For CNT-kaolinite binary systems, it has been reported that hydrophobicity and Louis acid-base interaction can lead to heteroaggregation$^{9}$. Thus, it is expected that electrostatic repulsion between CNT and kaolinite can be overcome, especially
when solution pH is lower than point of zero charge (PZC) of kaolinite and/or CNT and when the solution has a relatively high ionic strength (equivalent of ~100 mM NaCl).

b. Compared to NaCl at the same ionic strength, CaCl$_2$ destabilizes the CNT-kaolinite system and enhances heteroaggregation due to charge screening and bridging effects. NOM greatly stabilizes the system via the steric hindrance effect except under a certain Ca$^{2+}$/NOM range due to the adsorption of Ca$^{2+}$ on NOM, which then creates bridges among individual particles.

**Objective 2.** To investigate the effect of kaolinite and various solution conditions (ionic strength, pH, Ca$^{2+}$ and natural organic matter (NOM) on the subsurface transport of CNT in saturated porous media.

**Hypothesis:**

a. Kaolinite will either facilitate or hinder CNT transport depending on solution pH and ionic strength. Solution pH and ionic strength affect deposition behaviors of CNT-only and kaolinite-only systems by controlling their stability as well as through interaction with the porous media collectors$^{10-12}$. These solution chemistry factors may also change CNT mobility in co-transport scenarios. Specifically, under high pH and low ionic strength conditions, kaolinite may increase CNT mobility in porous media via competing with CNT adsorption sites on the porous media. Under intermediate solution conditions when heteroaggregation occurs but the attachment efficiencies of particles and porous media are low, kaolinite may facilitate CNT transport as CNT carriers. Under low pH and high ionic strength conditions, kaolinite could decrease the mobility of CNT by forming large homo- or heteroaggregates which block the pore channels in porous media (straining effect).
b. The presence of Ca\(^{2+}\) is expected to enhance the attachment of individual or aggregated particles (including homo- and heteroaggregates) onto porous media via charge screening or charge reversal effects on the surfaces of particles and porous media collectors. In the absence of Ca\(^{2+}\), an increase in NOM concentration is expected to increase the mobility of various compositions (free particles, homoaggregates and heteroaggregates) in the CNT-kaolinite binary system, because increase in NOM concentration has been reported to greatly increase the transport of CNT-only and kaolinite-only particle systems (mainly via the steric effect)\(^{13}\).

**Objective 3.** To study the structure and morphology of various compositions in CNT-kaolinite binary systems under various CNT-kaolinite concentration ratios and solution conditions. This structural and morphological information will help explain the mechanisms that cause the differences in the stability and mobility between CNT-only and CNT-kaolinite systems.

**Hypothesis:**

Under varying CNT/kaolinite concentration ratios and varying solution chemistry conditions, the percentage of heteroaggregates and the percentages of different structures of the heteroaggregates in the binary system will change. The expected heteroaggregate structures include but are not limited to: 1) particle coating of kaolinite with CNT, 2) secondary aggregates due to the attachment of CNTs to the kaolinite, 3) secondary aggregates due to side and edge contact of kaolinite platelets, and 4) stable primary heteroaggregates due to total coverage of CNT, which prevents further aggregation. NOM is expected to hinder aggregation via steric repulsion under high concentrations, and enhance aggregation via a bridging effect under low concentrations.
1.3 Research roadmap

This work focuses on the aqueous stability and subsurface transport of the CNT-kaolinite binary system. The impact of kaolinite, pH, ionic strength, Ca\(^{2+}\), and NOM on the stability of CNT in the aqueous phase will be studied through aggregation and sedimentation experiments. Aggregation behavior is studied by measuring changes in particle size via dynamic light scattering (DLS). Sedimentation behavior is characterized by measuring changes in light absorbance of the settling samples through a UV-vis spectrophotometer. To determine the degree of heteroaggregation, various compositions (free CNT, total CNT and kaolinite) are separated from the mixture by a series of centrifugation and ultra-sonication processes. In terms of the subsurface co-transport of CNT and kaolinite in saturated porous media, the effect of kaolinite, pH and ionic strength is investigated by detecting changes in the mobility of free CNT and total CNT through column experiments. To verify the speculations on the structure of the heteroaggregates and the applicability of flow cytometry on characterizing heteroaggregation, TEM imaging is performed to provide information on the morphology of various types of heteroaggregates. Flow cytometry is applied to quantitatively determine the extents of homo- and heteroaggregation in the system. The results of the above research will be interpreted in order to contribute to the prediction of the formation of CNT-NOC heteroaggregates and the transport of CNT, which will be helpful for assessing the environmental risk of CNT. A schematic of the research roadmap appears in Figure 1-1.
Study the impact of:
1. CNT/kaolinite ratio, solution pH and ionic strength
2. Ca^{2+} and NOM

Degree of heteroaggregation
- Separate the attached and free CNT from the mixture via centrifugation and ultrasonication
- Measure concentration of each component via UV-vis spectrophotometer
- Flow cytometry

Sedimentation experiment
- UV-vis spectrophotometer

Aggregation experiment
- Particle size measurement by Dynamic Light Scattering (DLS)

Subsurface Transport

Column experiment

Structure of heteroaggregates

TEM imaging

Figure 1-1 Schematic of the research roadmap
Chapter 2

Literature Review

2.1 Carbon nanotubes (CNT)

Carbon nanotubes are hollow tube-shaped materials with walls composed of one or more layers of graphite. They can be as small as 3 Å in diameter but up to 132,000,000,000 times that long. Since exhibiting new characteristics in mechanics, optics and electrical science, CNT have experienced an increasing trend in production and commercial application in various fields, such as composite materials, microelectronics, energy storage and biotechnology.

At the same time, given the increasing CNT production, researchers have been focusing on and reporting some important discoveries related to the toxicity of CNT. It has been shown that CNT can cause inflammatory effects in rat lungs, DNA damages, bacterial cell membrane damage and pathological effects in mice. Also, the CNT toxicity was found to be dependent on CNT properties such as size, composition and shape.

Based on the facts of increased CNT production coupled with their toxicity, human and biota exposure to CNT through environmental media (including water, soil and air) has become a concern. The fate and transport processes of CNT can not only influence the exposure level, but also change CNT properties such as size and zeta potential (e.g. through interactions with NOCs). Both of these properties can greatly alter the potential impact on humans and ecosystems. However, studies in this area fall behind the current intensive research on CNT synthesis and application.
In terms of the synthesis, surface functionalization techniques have been extensively used to improve CNT solubility\textsuperscript{23,24}. Among these studies, carboxyl functionalized CNT have drawn great attention\textsuperscript{25}. Carboxylic acid functional groups on CNT can act as ‘reactive sites’ for further surface modification\textsuperscript{25}, leading to their relatively high production. Moreover, carboxylated CNT are easily dispersed and highly stable in the aqueous phase because of their highly negative surface charges and low hydrophobicity. Therefore, these will probably transport further than non-functionalized CNT in the aqueous environment. The enhanced mobility will then largely affect its human exposure. Thus, in this research ENM was modeled with COOH-MWCNT.

2.2 Kaolinite

As is stated in the introduction, in natural water systems, the concentration of natural occurred colloids (NOCs) is typically much larger than that of ENMs, and NOCs may have great impacts on the fate and transport of ENMs. In this research, we studied kaolinite, one of the most common clay minerals.

At the molecular level, kaolinite has a layer-by-layer structure. It is composed of stacked layers of one silica tetrahedral and one alumina octahedral sheet linked by oxygen atoms\textsuperscript{26,27}. The thickness of the stacks is typically 0.05-2 μm and can be up to 4000 μm\textsuperscript{30}.

Surface properties of kaolinites vary depending on source and water chemistry. For kaolinites obtained from different sources, the isoelectric points have been reported as less than 2 to up to 4\textsuperscript{28,31}. The surface zeta potentials were also reported to be dependent on the relative composition of silica and alumina\textsuperscript{32}.
2.3 Fate and transport of engineered nanomaterials (ENMs)

To prevent adverse effects of ENMs on human and ecosystem health, it is important to understand not only ENMs’ toxicity, but also their human exposure pathways and bioavailability in the environment. Particularly, in aquatic systems, the human exposure and bioavailability of ENMs are dependent on their fate and transport behavior. Suspended particles can either settle down to the streambed after homo/hetero-aggregation or flow back to the aqueous phase through pumping flows from the water-sediment interface. Some of the settled particles can transport through the sediment via the filtration process. Human exposure can take place through food chains starting from the ENMs bio-uptake in the sediments or in the aqueous phase. Among these processes, aggregation, deposition and sedimentation play important roles. Over the past few decades, not only have theories in colloidal chemistry been applied in order to describe and predict these processes, but investigations on the fate and transport of ENMs have also been making great contributions in testing these theories. In this section, relevant theories on particle interaction processes will first be introduced. Then, studies on the transport and filtration of ENMs and their co-transport with natural colloids will be further reviewed. Finally, how this present study moves forward from the previous ones will be explained.

2.3.1.1 Particle interactions in aqueous phase

In order to estimate the interactions between charged colloidal surfaces, Derjaguin–Landau–Verwey–Overbeek (DLVO) theory acts as a framework for researchers. The DLVO theory
assumes an additive total interaction energy \( V_T \) between particles, combining electrical double layer repulsion \( V_R \) and Van der Waals attraction \( V_A \) as a function of particle separation distance.

\[
V_T = V_R + V_A
\]  
(1)

The shape of the interaction energy profile can directly affect particle stability, which influences colloidal aggregation and deposition. Colloid particles tend to more easily aggregate at the dips (e.g. primary minimum and secondary minimum in figure 2.1) in the profiles and be more stable at the peaks (energy barriers), where more kinetic energy is required to overcome the high potential energy.

In particular, the total potential energy for spherical particles can be described as:

\[
V_T(h) = \pi R \left\{ -\frac{H_{121}}{12\pi} \frac{1}{\hbar} + \frac{64kTn_0\Gamma_0^2}{\kappa^2} \exp(-\kappa h) \right\}
\]  
(2)
Where, R is the particle radius, \( h \) is the separation distance, \( H_{12} \) is the Hamaker constant for medium 1 separated by a liquid 2 (Hamaker constant is only dependent on the characteristics of the particles and the medium), \( k \) is Boltzmann’s constant, \( n_0 \) is the number concentration of ions, T is temperature, \( \Gamma_0 \) is defined as \( \tanh(z \epsilon_0 \Phi_0 / 4kT) \) where \( z \) is the charge valence of the electrolyte ions and \( \Phi_0 \) is particle surface potential, \( \kappa \) is the inverse Debye length which depends on the dielectric strength and ionic strength of the solvent.

Experimental results often show sharp changes on the stability of colloidal suspension at a certain point of electrolyte concentration, the critical coagulation concentration (CCC). The governing factors of the CCC are characterized by the Schulze-Hardy rule.

\[
CCC \propto 1 \times 10^4 \left( \frac{\varepsilon_\epsilon \epsilon_0}{z^6} \right)^5 \frac{(kT)^5}{\Gamma_0^4} H_{12}^2
\]

The rule is derived from eqn. (2) by setting the maximum of total interaction energy to be zero. In high surface charge conditions, CCC is proportional to the negative sixth power of the ion valence. This relationship has been extensively used to evaluate the applicability of DLVO theory\textsuperscript{33}.

2.3.1.2 ENMs stability (homoaggregation) described by the DLVO theory

The DLVO theory has been shown to adequately describe the colloidal stability of various types of nanoparticles such as nC\textsubscript{60}\textsuperscript{34} and nano-Au\textsuperscript{35}. However, unlike spherical nanoparticles, CNTs, have several special properties. For example, CNTs have a cylindrical shape with a very large aspect ratio. This contradicts one of the assumptions of eqn. (2). So far, whether the equations
that calculate the electric double layer and van der Waals forces in DLVO theory are correct for CNTs has not been determined conclusively. Though efforts have been made to modify DLVO theory, such as the derivation of potential energy between spherical and cylindrical particles\textsuperscript{36}, most of the studies on CNTs test the DLVO theory by applying the Schulze-Hardy rule on experimental results. In a study by Giordano et al. on the aggregation of CNTs in N, N-dimethylforamide, it was argued that the DLVO theory was not able to describe CNT aggregation because of the small Debye length and the large aspect ratio of CNTs. This contradicted a former study by Sano et al. that indicated that the coagulation of single-walled carbon nanotubes in electrolyte solutions followed the Schulze-Hardy rule\textsuperscript{37}. More recently, this conclusion of Sano et al. was supported by Smith et al., in whose research the approximation of spherical shape for oxidized multi-walled carbon nanotubes was capable of describing the particle coagulation, also by fitting data based on the Schulze-Hardy rule\textsuperscript{38}. Generally, the applicability of DLVO theory on CNTs remains controversial; thus, more efforts might need to be made before a universal agreement is reached.

In addition to DLVO forces (electrical double layer repulsion and Van der Waals attraction), some short-range forces should be taken into consideration as well. These non-DLVO forces include Born repulsion, hydration effects, hydrophobic interactions, steric interactions, polymer bridging, etc.\textsuperscript{36}. Polymers and natural organic matters (NOM) existing in the solvent or natural water are very likely to have sorption with nanoparticle surfaces. They can either act as coagulants by polymer bridging or steric interaction, in which particles approaching each other are hindered depending on the surface coverage.

Among the non-DLVO forces, steric interaction has been reported as the mechanism of stabilization effect of NOM for colloid particles\textsuperscript{53}. This is a repulsive force that stems from the
penetration of adsorption layers of polymers/NOM which increase the free energy. In natural water conditions, the ubiquitous NOM play an important role on the aggregation and deposition behavior of NOM. In most cases, NOM is reported to increase the stability of ENM via steric hindrance. However, in the presence of Ca\(^{2+}\), NOM can enhance the aggregation behavior via Ca\(^{2+}\) bridging effect. In terms of heteroaggregation and co-transport of ENM with other ENMs and NOCs, research on the effect of NOM is still in a preliminary stage. Findings and limitations of current literature in this area will be introduced in sections 3.3.1.2 and 3.3.1.6.

2.3.1.3 Heteroaggregation

Compared with homoaggregation, heteroaggregation of ENMs with the ubiquitous NOCs is expected to be more likely to occur in aquatic systems and thus has recently drawn greater attention from researchers. Related studies can be classified in terms of ENM-ENM and ENM-NOC heteroaggregation. In the current study ENM-NOC heteroaggregation was analyzed. By facilitating or hindering aggregations depending on physical and chemical properties of particles and solution conditions, NOCs can significantly alter particle size distribution of the original ENM system. Since particle size is a governing factor of sedimentation, the presence of NOC plays an important role on the removal of ENMs. In this section, relevant studies were critically reviewed for typical cases of ENM-NOC heteroaggregation, factors that impact heteroaggregation and methods to analyze heteroaggregation.

Many previous research on ENM-NOC interactions focuses on two materials that are of opposite charge under most naturally-occurring pH conditions. In these cases, electrostatic attraction serves as one of the main mechanisms that induces heteroaggregation. One typical type is the heteroaggregation of positively surface-charged ENMs (e.g. metal oxide nanoparticles and
surface modified carbon-based nanomaterials) with negatively charged natural clays\textsuperscript{39-42,46}. Zhou et al. reported that at pH=4 positively charged TiO\textsubscript{2} was destabilized by montmorillonite while further coagulation was reduced in low ionic strength conditions.\textsuperscript{42} Praetorius et al. investigated heteroaggregation of TiO\textsubscript{2} nanoparticles and SiO\textsubscript{2} particles by applying novel modeling for determining attachment efficiency. They concluded that the attachment efficiencies for heteroaggregation were close to 1 when the two types of particles were oppositely charged (pH=5).\textsuperscript{44} Using previous modeling, the same research group showed heteroaggregation of TiO\textsubscript{2} with smectite\textsuperscript{43}. In another study investigating the effect of natural colloids and NOM particles in Rhine and Meuse water samples on the stability of CeO\textsubscript{2} particles, heteroaggregation between natural colloids and nanoparticles were shown through the sedimentation experiments\textsuperscript{43}. Han et al. described the stability of cationic, anionic and nonionic surfactant-coated multi-walled CNT suspensions (CTAB-MWCNT, SDBS-MWCNT and TX-100-MWCNT) in the presence of clay minerals (kaolinite and montmorillonite). Both clay minerals enhanced the CTAB coated CNT sedimentation while little effect of kaolinite was shown on the stability of SDBS- and TX-100-coated CNT. The addition of montmorillonite did not change the SDBS-MWCNT stability but caused partial deposition for TX100-MWCNT. The enhanced sedimentation scenarios were attributed to either the bridging effect of surfactants on CNT and clays or the sorption of surfactants by clays, which caused the aggregation processes\textsuperscript{45}. Except for the common cases where the NOCs are negatively charged in natural water conditions, several kinds of positively charged minerals (e.g. goethite and hematite) were reported to have interaction with the negative surfaces of ENMs. Smith et al. studied heteroaggregation between citrate-stabilized gold nanoparticles and hematite colloids and found that heteroaggregation occurred at the pH at which the two materials were oppositely charged\textsuperscript{44}. Chen et al. reported heteroaggregation of carbon
nanotubes and hematite particles at an unadjusted pH of 5.2 ± 0.2. Heteroaggregation resulting from electrostatic attractions between GO and positively charged goethite was shown by Zhao et al.

Although the above studies revealed the heteroaggregation behavior induced by charge attraction, it should be noted that ENMs with positively charged surfaces may not transport a long distance since most NOCs are negatively charged in aquatic systems: charge attraction will lead to sedimentation of ENMs, thus preventing their transport. When released into the environment, ENMs that are able to transport a longer distance may have more chances to interact with NOCs, resulting not only in their complex human exposure pathways but also in alteration of their toxicity. Therefore, heteroaggregation of negatively charged ENMs with negative NOCs is highly worth investigating. However, related research is limited. Carbon-based nanomaterials are primarily focused on in current studies: their heteroaggregation with negative NOCs were indeed revealed in a number of studies. Apart from DLVO forces, complex sorption mechanisms including hydrophobicity, hydrogen bonding and Lewis acid-base were reported.

In Yang et al., the effectiveness of GO as flocculants to remove water contaminants was investigated. The charge repulsion between GO and particulate kaolin was believed to be overcome by hydrogen bonding and Lewis acid-base interaction followed by the removal of kaolin through sweeping flocculation. Similarly for carbon nanotubes, Zhang et al. characterized the interactions of 14C-labeled MWCNT and kaolinite through a batch sorption test and showed that hydrophobicity and Louis acid-base interaction played the dominant role on the sorption process. In addition to the cases where charge repulsion can be overcome by other types of interactions in ENP-NOC heteroaggregation, Zhou et al. demonstrated coagulation and sedimentation processes of Ag and montmorillonite at pH=4, which is below the isoelectric point.
of sites on montmorillonite edge\textsuperscript{42}. This implies that negatively charged ENMs might attach to clay particles with surface charge heterogeneity, although the overall electrostatic force is repulsive. The current study focuses on heteroaggregation of kaolinite and COOH-MWCNT, both of which are of negative surface charge. Different from the studies by Zhang et al.\textsuperscript{9}, which studies MWCNT-kaolinite heteroaggregation via a sedimentation experiment, the current study additionally utilized a separation method which determined concentration of attached CNTs, free CNTs and kaolinite in the suspension.

As was stated, surface charge plays an important role in heteroaggregation. It is largely affected by solution chemistry factors (pH, ionic strength, ion type and natural organic matter, etc). Solution pH and ionic strength affect attachment efficiency by altering zeta potential of the particle surface. Higher pH results in deprotonation of surface functional groups and thus more negative surface charge. Increase in ionic strength lead to thinner electric double layer of the particle. Variations in pH across the point of zero charge (PZC) may cause charge reversal effects on the particle surface, significantly changing attachment efficiency. Reports on these effects in heteroaggregation studies were all as expected and can be found in some of the references mentioned previously\textsuperscript{44, 46, 47}. Another important variable is ion species. Based on DLVO theory, ions with higher valence screen surface charge more efficiently, thus lowering the interaction energy and enhancing heteroaggregation. Among limited studies about heteroaggregation, only calcium ion was tested as typical divalent/multivalent ion in one article by Afrooz et al., showing the increase of aggregation between Au nanoparticles and plunic acid-modified single-walled carbon nanotubes in the presence of Ca\textsuperscript{2+}\textsuperscript{48}. Besides the above factors, the universal existence of NOM makes it vital in controlling heteroaggregation. Most previous studies on mono-typed ENM systems reported that NOM stabilized the particles via steric
hindrance. However, in the presence of Ca\textsuperscript{2+}, Ca\textsuperscript{2+} bridged NOM molecules and largely enhanced homoggregation\textsuperscript{49}. Occasionally, in medium NOM concentrations, NOM can bridge colloids, resulting in an increase in aggregation. Regarding heteroaggregation studies, three articles reported similar steric effects of NOM in binary systems of TiO\textsubscript{2}/SiO\textsubscript{2}\textsuperscript{44}, gold nanoparticle/pluronic acid-coated SWNT\textsuperscript{52} and citrate-stabilized gold nanoparticle/hematite\textsuperscript{47}. Among them, Afrooz et al. reported that the co-existence of Ca\textsuperscript{2+} and humic acid increased heteroaggregation of gold nanoparticle and pluronic acid-coated SWNT via bridging between humic acid by Ca\textsuperscript{2+}. One study by Taujale et al. showed via sedimentation experiments that as alginate concentration increased, the heteroaggregation rate of Al\textsubscript{2}O\textsubscript{3} (positive at pH=5) and MnO\textsubscript{2} (negative at pH=5) particles increased, then decreased. At the same time, pyromellitic acid had no effect on the heteroaggregation, possibly because of its much smaller molecule size than alginate\textsuperscript{50}. In general, the presence of NOM and Ca\textsuperscript{2+} can greatly affect ENM-ENM and ENM-NOC aggregation with complex mechanisms and results. More efforts need to be made in order to predict fate and transport of ENMs in highly environmentally relevant conditions. The current study is expected to provide fundamental insight on the impact of NOM and Ca\textsuperscript{2+} in CNT-kaolinite system (Task 2). To the best of our knowledge, this is the first study that systematically studies this topic.

Aggregation of ENMs is followed by the stage of transport. Variations in the shape, configuration and surface properties of ENMs will likely alter their mobility in subsurface porous media. Thus, apart from the stability of the colloidal systems, the properties of the heteroaggregates are another topic that is highly worth investigating. Unfortunately, studies in this area are fairly scarce. Configurations and morphology of heteroaggregates can be impacted by multiple factors. Current studies show the influence of environmental factors, including
solution condition (pH, ionic strength, ion type, and NOM) and ENM/NOC concentration ratio. In the previously mentioned research by Praetorius et al., regarding SiO$_2$ and TiO$_2$ heteroaggregation, secondary heteroaggregates were formed at both pH=8 and pH=5 with 1 mM NaCl. At pH=5, TiO$_2$ bridged SiO$_2$ particles via electrostatic attraction, forming more stable structures than those of SiO$_2$ homoaggregates. When pH increased to 8, the repulsive charges of TiO$_2$ and SiO$_2$ resulted in loose TiO$_2$ bridges inside the secondary aggregates and therefore less dense structures compared with SiO$_2$ homoaggregates. The differences led to higher maximum heteroaggregation rates than those of homoaggregation$^{44}$. 

In the other research focusing on TiO$_2$ heteroaggregation, but with smectite, ionic strength and relative concentration/surface area ratio largely affected particle configuration. Under 0.001 M NaCl, secondary heteroaggregation occurred through TiO$_2$ bridging at a critical nanoparticle/clay number concentration ratio. However, when NaCl was more than 0.1 M, smectite platelets were destabilized before interacting with TiO$_2$ and forming homoaggregates. In the latter case secondary heteroaggregation was driven by surface area ratio of the two materials$^{46}$. Yi et al. studied heteroaggregation of CeO$_2$ with nanoparticles of pyrohenic carbonaceous material (n-PCM). At pH=5.3 (attractive charges), as n-PCM concentration increased, primary aggregation followed by secondary heteroaggregation and then re-established CeO$_2$ covered by n-PCM layer was reported. Changes in the structure resulted from charge screening and charge reversal. At pH=7.1, CeO$_2$ particles were neutral and n-PCMs were negative. n-PCMs form a shell on CeO$_2$. The size of the core-shell heteroaggregates increases with the decrease in n-PCMs/CeO$_2$ concentration ratio$^{51}$. Morphology of the constituent particles could also play an important role on the configuration. Through SEM, TEM and AFM images, Zhao et al. speculated the possible structures of GO-goethite heteroaggregates. Goethite free particles/aggregates could be wrapped.
by one GO sheet or adsorbed between two GO sheets. In the current study, CNT and kaolinite took on tube and platelet shapes, respectively, which is extremely usual. The configurations of their aggregates can be different from those studied in the other binary systems.

In order to prove and describe heteroaggregation process, methods can be generally classified into three types. One is to determine the attachment kinetics derived from particle size results obtained by dynamic light scattering. Since only one refractive index could be applied in the measurement, studies using this method tested binary systems with a much larger concentration of one composition (usually NOC) than the other, of which the light scattering can be ignored. Sedimentation experiments are another approach, in which a large concentration difference is required in the mixture in order to get accurate concentration results of the supernatant (usually gained based on UV-vis light absorbance). Additionally, several studies performed adsorption/desorption tests to obtain isotherms to characterize ENM-NOC interaction.

Reasonable separation methods such as centrifugation are crucial in the adsorption/desorption experiments, and thus the particle size difference between mixture compositions was narrowly restricted. A method to characterize heteroaggregation that allows a wide range of ENM/NOC particle sizes and concentration ratios is the most challenging part of this type of research and needs development.

Among the available methods, flow cytometry is a promising approach that can possibly distinguish heteroaggregates from homoaggregates and free particles. Flow cytometry is a rapid method that can detect and quantify light scatter and relative fluorescence intensity of a single particle/cell. Different from a DLS or fluorescence spectrophotometer which output average values of the whole particle suspension, a flow cytometer applies hydrodynamic focusing which restricts the cells to the center of the stream to accomplish a one-by-one particle flow. There are
two kinds of signal generation in flow cytometry: light scatter signals and florescence. Light scatter consists of forward scatter (FSC) and side scatter (SSC). Forward scatter is detected along the axis of incident light in the forward direction, while side scatter is detected at 90° to the laser beam. In the size range of most cells (1 – 150 µm), FSC is sensitive to particle size and SSC is more related to cell granularity and internal complexity. However, for smaller particles such as some ENMs, SSC might be more sensitive to particle size according to Mie scattering theories. Thus, in binary systems, if one type of particle is fluorescently labelled, various compositions in the system can be distinguished by performing gating analysis of the output plots. Since cytometry is primarily applied in biological fields, heteroaggregation studies using flow cytometry are seldom found. Rollie et al. determined the cluster composition in heteroaggregation of polystyrene (PS) particles and Rhodamine-B labeled melamine-formaldehyde (MF-RhB) particles\(^5^3\). This is the only study that used a flow cytometer for particle aggregation issues.

For ENM aggregation studies, flow cytometry has several limitations. First, the size of most nanoparticles (1-1000 nm) is below the resolution of a flow cytometer, making the single particle hard to recognize. Also, flow cytometry provides information on the distribution of various compositions in the suspension, but it is not a tool for determining concentrations of various compositions.

In this work, the application of flow cytometry on CNT-kaolinite system is studied. We found that flow cytometry was able to quantitatively determine the degrees of homo- and heteroaggregation. This provides useful insight in terms of techniques to study ENM-NOC heteroaggregation as well as the application of flow cytometry to submicron materials such as ENMs.
2.3.1.4 Classical colloid filtration theory

In addition to particle-particle aggregation or particle-collector deposition, transport behaviors of CNT and kaolinite in porous media also depend on other factors such as hydrodynamic condition and particle size. The well-known classical colloid filtration theory (CFT) provides a description of colloidal behaviors in transport through saturated porous media. In a filtration system, based on mass balance calculation and assumptions of spherical collectors and a first-order deposition, the normalized breakthrough concentration of the colloid particles at steady state can be described using eqn. (4)

$$eqn.$$
\[
\frac{C}{C_0} = \exp \left( -\frac{3}{2} \frac{(1 - \theta_w)}{d_c} \right) \alpha \eta_0 L
\]  

(4)

where \( C \) is the steady state effluent concentration, \( C_0 \) is influent concentration, \( \theta_w \) is the porous media volumetric water content, \( d_c \) is the diameter of the collector, \( \alpha \) is particle-collector attachment efficiency, \( \eta_0 \) is single collector efficiency and \( L \) is the transport distance.

Single collector efficiency, \( \eta_0 \), is a combination of the efficiencies from Brownian diffusion (\( \eta_D \)), gravity sedimentation (\( \eta_G \)) and interception (\( \eta_I \)): 

\[
\eta_0 = \eta_D + \eta_G + \eta_I
\]

(5)

After introducing several dimensionless parameters, Tufegki and Elimilech present a correlation equation for \( \eta_0 \):

\[
\eta_0 = 2.4A_S^{1/3} N_R^{-0.081} N_{Pe}^{-0.715} N_{vdW}^{0.052} + 0.55A_S N_R^{1.675} N_A^{0.125} + 0.22N_R^{-0.24} N_G^{1.11} N_{vdW}^{0.053}
\]

(6)

Dimensionless parameters in eqn. (6) are defined in reference\textsuperscript{55}.

It can be seen from CFT that the mobility of particles in the filtration media is controlled by \( \alpha \) and \( \eta_0 \). The impacting factors for \( \alpha \) that have influence on the interaction energy between particles and collectors include ionic strength, pH, and Hamaker constant. For \( \eta_0 \), particle size and hydrodynamic conditions are governing factors.

In column experiments, based on experimental results of particle breakthrough curves, attachment efficiency was generally obtained by two methods. One method is through
calculation from eqn. (4) in CFT. Another is to set up advection-dispersion model equations and fit the attachment coefficient value.

2.3.1.5 Transport modelling

In a 1-D column system, a 1-D advection-dispersion-reaction equation can be used to describe particle transport and retention in the porous media:

\[
\frac{\partial C}{\partial t} + \frac{\rho_b}{\theta_w} \frac{\partial S}{\partial t} = D_H \frac{\partial^2 C}{\partial x^2} - v_p \frac{\partial C}{\partial x}
\]

(7)

where \(C\) is aqueous phase concentration, \(S\) is solid phase concentration, \(\rho_b\) is bulk density of the porous media, \(\theta_w\) is water saturation content, \(t\) is time, \(D_H\) is hydrodynamic dispersion coefficient, \(v_p\) is pore velocity, and \(x\) is the distance travelled after time \(t\).

Assumptions for this equation include 1) first order attachment rate and 2) site blocking, ripening and detachment are negligible, with \(\frac{\rho_b}{\theta_w} \frac{\partial S}{\partial t}\) expressed as follows:

\[
\frac{\rho_b}{\theta_w} \frac{\partial S}{\partial t} = k_{att}C
\]

(8)

where, \(k_{att}\) is the first order attachment rate, which is related to the product of \(\alpha\) and \(\eta_0\) in CFT:

\[
k_{att} = \frac{3\alpha \eta_0 (1 - \theta_w)}{2d_c}
\]

(9)
With the initial and boundary conditions in pulse input, eqn. (7) and eqn. (8) make the following solution for solid and aqueous concentration as a function of time\textsuperscript{56,57}.

\begin{equation}
    c(x, t) = \frac{M}{Q} e^{-kt} \frac{x}{2\sqrt{\pi} t^3 D} e^{-\frac{(x-\nu t)^2}{4Dt}}
\end{equation}

where M is the total mass of the injected particles and Q is the volumetric flux.

Though CFT and the 1D-advection-dispersion model showed successful interpretations of colloid filtration in many studies, there are cases where they are not suitable for application. The classical filtration theory assumes 1) negligible blocking, ripening and detachment, 2) first order deposition rate, and 3) spherical particles and spherical collectors in the system in trajectory analysis. Size exclusion is a phenomenon where particles are prevented from traveling through narrow pore channels in the media because of the particle shape or size, leading to a smaller effective pore volume and a shorter breakthrough time. Straining effects happen when particles are blocked at the pores. In CNT transport, a lot of studies have shown evidences of straining effects. Jaisi et al. demonstrated a lower mobility of SWNT in the soil compared with fullerene under the same solution chemistry, indicating a straining effect in SWNT transport\textsuperscript{10}. Wang et al. reported a tube-length dependency of the straining effect in the column test of MWNT by fitting a modified model considering physical straining\textsuperscript{58}.

Also, several previous column test studies using step input showed no plateaus in the breakthrough curves\textsuperscript{64,59,70}, indicating site blocking processes during the injection. In addition, CNT is tube-shaped. The interception efficiency could vary a lot depending on whether it makes
side contact or end contact with the collector surface. Therefore, modification to the CFT-based models is needed for CNT transport.

Considering the tube-shaped CNT, Liu et al. developed a new expression for single collector efficiency with expressions for side and end contact that showed good agreement with their experimental results\(^{65}\). To account for blocking and detachment, the term \(\frac{\rho_b \partial S}{\theta_w \partial t}\) in the 1-D advection-dispersion model can be redefined by adding a dimensionless correcting factor, \(\psi\):

\[
\frac{\rho_b \partial S}{\theta_w \partial t} = k_{att}\psi C - \frac{\rho_b}{\theta_w}k_{det}S
\]

(11)

\[
\psi = \frac{S_{max} - S}{S_{max}}
\]

(12)

where, \(k_{det}\) is the detachment rate constant, \(S_{max}\) is the retention capacity, and \(\psi\) equals zero when the maximum retention is reached\(^{64}\). Another expression is

\[
\psi = \left(\frac{S_{max} - S}{S_{max}}\right)\left(\frac{d_c + \chi}{d_c}\right)^{-\beta}
\]

(13)

where \(\beta\) is an empirical variable controlling the shape of the retention profile\(^{60}\).

By incorporating site blocking combined with a straining effect into 1-D advection-dispersion model, the governing equation becomes:
\[
\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} + v_p \frac{\partial C}{\partial x} - k_{att} \varphi_{att} C - \frac{\rho_b}{\varepsilon} k_{det} S_{att} - k_{str} \left( \frac{d_{50} + x}{d_{50}} \right)^\beta
\]  

(14)

2.3.1.6 Fate and transport of ENMs

The mobility of ENMs through porous media is impacted by various factors, including both physicochemical and hydrodynamic parameters, such as ionic strength, solution pH, solution ion types, collector size, media material, pore water velocity, natural organic matter and influent concentration.

For some of these factors, CFT combined with the DLVO theory has successfully explained and predicted how they influence the mobility of the particles by showing consistency with a large number of column experiment results for various types of ENMs.

Particularly, in terms of the CNTs, several studies on their transport in porous media have been reported. The influence of ionic strength has been most extensively studied. Generally, experimental results are consistent with DLVO theory, showing an increase in CNT mobility with decreasing ionic strength. For example, Jaisi et al. assess the deposition of single walled CNTs in clean quartz sand with an influent SWNT concentration of 87 (±4) mg/L and at pH 7.0 under various KCl solution concentrations. Results showed that the normalized concentration decreases from 0.78 at 3 mM to 0.09 at 55 mM of KCl\textsuperscript{63}. This trend agrees with previous studies\textsuperscript{61}. In another study evaluating transport of SWNTs in natural soil columns, also consistent with filtration theory, the deposition rate increased with salt concentration, and the increase was more significant in the presence of divalent ions rather than monovalent ions\textsuperscript{63}.
Solution pH influences particle deposition by changing the surface charge of both particles and collectors through ion sorption at the interface. An important parameter to evaluate the surface charge is the point of zero charge (PZC). Particles will typically be negatively charged when solution pH is higher than PZC while positively charged when pH is lower than PZC. For example, Tian et al. investigated transport and retention of MWNTs and SWNTs in three types of porous media (acid-cleaned, baked, and natural sand) under unfavorable conditions. With pH increasing from 8 to 10, MWNT breakthrough concentration increased from 0.23 to 0.78 for natural sand and from 0.24 to 0.75 for baked sand, with similar results for SWNT\textsuperscript{62}. However, opposite results were obtained for a solution containing Ca\textsuperscript{2+} because of the calcium bridging between surface functional groups. Research on release of deposited MWNT from silica surfaces using QCM-D shows that at 1.5 mM CaCl\textsubscript{2}, when pH changes from 7.1 to 4.0, a large fraction of MWNT can be released since the bridges between CNT and silica surfaces were broken\textsuperscript{63}.

In addition to the factors above, research shows that other parameters such as pore water velocity, grain size, presence of NOM, straining, and media heterogeneity also play significant roles on the transport of CNTs. For example, Liu et al. demonstrated a strong influence of high MWNT mobility at pore water velocities higher than 4.0 m/d\textsuperscript{65}.

Mattison et al. reported an increase in normalized concentration of 60%, with media size ranging from fine sand to silt\textsuperscript{64}. Kasel, et al. proved the changes in the MWNT retention profile at high influent concentrations, which cause site blocking/filling\textsuperscript{65}. Khan et al. described a strong mobility of SWNTs in glass and weak mobility in paper\textsuperscript{66}.

Regarding the influence of NOCs on CNT transport in porous media, except for several articles on the influence of NOM, a single study on CNT and bacteria co-transport was reported.
The universal existence of NOM and its interaction with colloid particles makes it significant to evaluate the effect of NOM on the fate and transport of nanoparticles. NOM can either enhance the mobility by a hindrance effect or enhance the deposition by a bridging effect.

As is shown, solution conditions have significant influence on the transport of colloidal particles. In natural aqueous systems, solution conditions such as pH and ionic strength vary in wide ranges at different spatial and temporal conditions. In this study, a range of pH and ionic strength values were applied to investigate the co-transport of CNT and kaolinite.

2.3.1.7 Co-transport of ENM and NOC

Studies that focused on the co-transport of CNT with other colloids in porous media are very limited. As a result, related research in terms of the whole ENM group is critically reviewed in this section. In contrast with studies on the transport of individual ENMs, literature on co-transport of ENMs and other colloids or non-colloid contaminants was much less common. In natural aquatic systems, naturally occurring colloids (e.g. NOM, clay, and bacteria, etc.) may greatly influence the transport of engineered nanoparticles. The level of impact is mainly related to two factors: 1) the extent of ENM and NOCheteroaggregation, which depends on both particle surface chemistry and solution chemistry, and 2) Deposition rate of ENM-NOC heteroaggregates on media collectors. The ENM-NOC heteroaggregates can facilitate ENM transport or hinder the breakthrough by straining. Decades before the wide application of nanomaterials, colloid facilitated transport had been studied in co-transport conditions in environmental media where colloidal particles facilitated breakthrough of the contaminants by acting as carriers. For example, Simunek et al. studied the facilitated transport of cadmium by bacteria. Recently, several studies have shown ENM-facilitated transport of contaminants including fullerene-
facilitated transports of PCB and phenanthrene\textsuperscript{68}, CNT facilitated transport of TBBPA\textsuperscript{69}, hydroxyapatite nanoparticles-facilitated transport of copper\textsuperscript{70} and MWCNT-facilitated transport of phenanthrene\textsuperscript{71}. However, colloidal particles do not always facilitate the breakthrough of their co-transporting substances. In the study of Fang et al. on the co-transport of TiO\textsubscript{2} nanoparticles with Cu in four types of soils, TiO\textsubscript{2} enhanced the mobility of Cu through colloid facilitated transport in three of the four different soils but reduced the recovery of Cu in the fourth type of soil, indicating the influence of soil properties\textsuperscript{72}. Also, Vasiliadou et al. showed the inhibition of \textit{Pseudomonas Putida} transport by kaolinite in a glass bead column caused by their attachment to kaolinite particles that had previously adsorbed onto the glass\textsuperscript{73}. A study on the effect of CNTs on \textit{E. coli} transport in saturated porous media showed that under low ionic strength conditions, CNTs did not affect the retention and transport of \textit{E. coli}. However, under high ionic strength, the mobility of \textit{E. coli} was hindered, which was attributed to heteroaggregation between \textit{E. coli} and CNTs and the CNT-cell bridging effect\textsuperscript{74}.

There are four studies on ENM-ENM and ENM-NOC co-transport. Prof. Tong and Prof. Jaisi are the main investigators in this field. Prof. Tong’s group focused on the co-transport of TiO\textsubscript{2} with nC\textsubscript{60} in 2013\textsuperscript{75} and with clay (kaolinite and bentonite) in porous media in 2014\textsuperscript{76}. Environmental factors of ionic strength, pH and ion valence were studied. In the past two years, Prof. Jaisi’s group published two studies regarding the co-transport of hydroxyapatite nanoparticles with goethite under various pH, natural organic matter\textsuperscript{77}, ionic strength and flow rate conditions\textsuperscript{78}. Cai et al. studied the co-transport of nTiO\textsubscript{2} and nC\textsubscript{60} nanoparticles in saturated quartz sand under different ionic strength and pH conditions\textsuperscript{81}. Transport of nTiO\textsubscript{2} did not show significant changes at pH=5 but increased at pH=7 because of the site competition with nC\textsubscript{60} on the surface of the sand. The transport of nTiO\textsubscript{2} was demonstrated to be retarded under all solution conditions. The
straining of heteroaggregates and site ripening were considered to be the reasons for low mobility at pH=5 whereas at pH=7 the straining was not a contributing factor in decreasing nTiO\textsubscript{2} mobility\textsuperscript{81}. In terms of the co-transport of nTiO\textsubscript{2} and clay, column experiments were performed in quartz sand with fixed pH and different ionic strength and ion valences. The results showed the facilitated transport of TiO\textsubscript{2} by betonite in both NaCl and CaCl\textsubscript{2} solutions and by kaolinite in NaCl, but a decreased recovery by kaolinite in CaCl\textsubscript{2} through the straining of kaolinite-TiO\textsubscript{2} heteroaggregates\textsuperscript{82}. Wang et al. reported that the co-transport of negatively charged hydroxyapatite nanoparticles with positively charged goethite showed higher mass recovery than for individual transport of hydroxyapatite at pH 10.5 due to the competition of adsorption sites. The results were the opposite at pH 7.5 when the enhanced homo- and heteroaggregation caused an increase in particle size, which resulted a straining effect in the porous media\textsuperscript{83}. As to the effect of hematite on the transport of hydroxyapatite, the mobility of the ENM is decreased by the clay mainly due to increased homo- and heteroaggregation.

Consistent with research on individual ENM transport, the retention of hydroxyapatite particles in porous media was enhanced by decreasing flow rate and pH, and increasing ionic strength\textsuperscript{84}. However, the effect on co-transport under the co-existence of divalent ions and NOM has not been shown in ENM-NOC studies to date, except for one study from Prof. Tong’s group on the co-transport of bacteria cells and hematite. This study proved that 0.1 mg/L humic acid enhances the transport of cells but has the opposite effect in the co-preservation of Ca\textsuperscript{2+}. When humic acid concentration increased to 1 mg/L, mobility was enhanced in both the presence and absence of Ca\textsuperscript{2+} \textsuperscript{79}.

It should be noted that in the above studies, ENMs (TiO\textsubscript{2} and hydroxyapatite) were positively charged under most pH conditions of natural water (5 - 9). Little studies for negatively charged
ENM has been performed. In addition, methods used to quantify the ENMs and NOCs were for the total concentrations and were unable to distinguish the attached and free ENM compositions. Table 2-1 lists the method used for quantitatively analyze ENM/NOC in ENM-ENM and ENM-NOC co-transport studies.

To the author’s knowledge, this study is the first to explore the co-transport behavior of COOH-MWCNT and kaolinite in saturated porous media. Mechanisms for the mutual effects of COOH-MWCNT and kaolinite will be discussed based on the breakthrough curves of all the components of the kaolinite-CNT mixture (total kaolinite, attached CNT and free CNT). The combined effect of ionic strength and pH on the co-transport will be systematically studied. In terms of the injection method, all the co-transport studies described in the literature have used step input. In step input cases, most fluent samples have to be sonicated periodically in order to maintain stability during the entire time period of a column experiment$^{83,84}$. To avoid the inconsistency of the particle stability that may be caused by the periodic sonication, pulse input was applied in the current study instead of step input.

Table 2-1 Methods used to quantitatively analyze ENM/NOC in ENM-ENM and ENM-NOC co-transport studies

<table>
<thead>
<tr>
<th>Reference number</th>
<th>Material A</th>
<th>Material B</th>
<th>Method to analyze the amount of ENMs/NOCs in the effluent and/or porous media (retention)</th>
</tr>
</thead>
<tbody>
<tr>
<td>81</td>
<td>nC$_{60}$</td>
<td>TiO$_2$</td>
<td>In the effluent samples and porous media, total nTiO$<em>2$ (including individual and attached particles) concentration were measured using UV spectrophotometer. nC$</em>{60}$ were analyzed using TOC.</td>
</tr>
<tr>
<td>82</td>
<td>TiO$_2$</td>
<td>Kaolinite, bentonite</td>
<td>Total nTiO$_2$ was determined using UV spectrophotometer at 600 nm where absorbance of clay particles was negligible.</td>
</tr>
<tr>
<td>83</td>
<td>Hydroxyapatite nanoparticles</td>
<td>Goethite</td>
<td>Grain sand in the column was extracted in NaOH solution. Total phosphorus was determined by phosphomolybdate blue method. The amount of total goethite was analyzed using a UV-spectrophotometer.</td>
</tr>
<tr>
<td>84</td>
<td>Hydroxyapatite</td>
<td>Hematite</td>
<td>Phosphomolybdate blue method was used to determine total phosphorus concentration in the effluent and retained in the column. A UV spectrophotometer was used to measure the hematite.</td>
</tr>
</tbody>
</table>
Chapter 3

Stability of multiwalled carbon nanotubes in natural aquatic environments: interactions with naturally occurring colloids

Abstract

When released into the environment, the fate of engineered nanomaterials will be affected by many environmental factors including the presence of naturally occurring colloids (NOCs). This study characterized the interactions of carboxylated multi-walled carbon nanotubes (COOH-MWCNTs) with a model NOC, kaolinite, and investigated their impact on the colloidal stability of the COOH-MWCNTs. Sedimentation and aggregation kinetics were measured under a range of solution conditions and carbon nanotube (CNT)-to-kaolinite ratios. Results show that interactions between the COOH-MWCNTs and kaolinite can form both primary and secondary heteroaggregates under different solution conditions. The effect of heteroaggregation on COOH-MWCNT stability, however, strongly depends on the CNT-to-kaolinite ratio; it can either increase or decrease the stability of the suspension depending on the structure of the heteroaggregates formed. In natural surface waters, CNT in natural surface waters exhibits decreased stability, a notable portion of which is attributed to the naturally occurring colloids. The effect of other water quality parameters (e.g., ionic composition) also contributes to the decreased stability.
3.1 Introduction

Engineered nanomaterials (ENMs) are manufactured and contain particles in unbound, aggregate or agglomerate state with more than 50% of the number in the size range from 1 nm to 100 nm at one or more external dimension\textsuperscript{90}. Due to their excellent properties on mechanics\textsuperscript{81}, surface area\textsuperscript{82} and electrocatalysis\textsuperscript{83}, etc., ENMs have been increasingly produced and applied in medicine\textsuperscript{84}, cosmetics\textsuperscript{85}, microelectronics\textsuperscript{86} and water treatment\textsuperscript{87-89}, etc. over the past decades. However, despite its excellent performance and wide application, it was found that ENMs were toxic on living organisms since their nano-sized structure could penetrate cell walls and do harm to biological structures\textsuperscript{90, 91}. It was also found that ENMs could disperse abundantly in water and transport widely in aquatic systems, which might cause serious environmental issues once released from industry to natural water systems. Therefore, learning the fate (e.g. aggregation and sedimentation) and transport of ENMs in aquatic systems is of great significance to assess their environmental risks.

In aqueous phase, homoaggregation, which refers to aggregation among ENMs particles, and its effect on ENMs stability has been extensively studied. Environmental pH, ionic strength\textsuperscript{92}, ion valence and natural organic matters (NOM)\textsuperscript{93} were found to significantly affect aggregation kinetics. However, compared with ENMs, naturally occurring colloids (NOCs) have a much higher concentration range in natural waterbodies, which was more likely to cause heteroaggregation between ENM and NOC rather than ENM-ENM homoaggregation.

According to the very limited amount of published research, the mechanisms of heteroaggregation were much more complicated than homoaggregation. These studies reported electrostatic attraction as the main mechanism for ENM-NOC heteroaggregation. Among them,
most studies focused on NOC and ENM that are negatively charged in the solution condition\textsuperscript{39-43}. In addition to electrostatic attraction, hydrogen bonding and Louis acid-base interaction between oxygen groups and hydroxyl groups were considered to be the reason for the seemingly charged heteroaggregation\textsuperscript{47}. In terms of heteroaggregation rate, it was also largely affected by solution chemistry (pH, ionic strength, ion valence and NOM, etc.). These impacting factors along with the ENM/NOC concentration ratio can lead to various structures of ENM-NOC heteroaggregates\textsuperscript{51, 94, 95, 104}, which can alter the size, stability and surface physical-chemical properties of the ENM. Consequently, the bioavailability of ENM in surface water and its mobility in the subsurface porous media would be affected.

As the most common NOC and ENMs, heteroaggregation between kaolinite and carbon nanotubes (CNT) has seldom been studied. In these limited studies, Han et al. found that clay minerals enhanced the sedimentation of cationic cetrimonium bromide coated CNT, which was attributed from either bridging effect of surfactants between CNT and clays or the sorption of surfactants by clays\textsuperscript{103}. Zhang et al investigated the interaction of \textsuperscript{14}C labeled multi-walled CNT with kaolinite, smectite and shale, and reported that DLVO (Derjaguin–Landau–Verwey–Overbeek) forces and hydrogen bonding were main mechanisms of the attachment of CNT on kaolinite\textsuperscript{9}. These studies investigated the adsorption behavior of the CNT-NOC systems, and put forward some possible mechanisms of their interaction. However, they were not conducted in real natural water, which was more environmentally relevant and could better reveal the actual aggregation and transport behavior of CNTs in the environment. In addition, these studies did not discuss the structure of formed heteroaggregates, which differed greatly in various solution conditions and could greatly affect their stability in aquatic systems.
To address these knowledge gaps, in this study, the heteroaggregation of carboxylated multi-walled carbon nanotubes (COOH-MWCNT) with kaolinite and its influence on the stability of COOH-MWCNT were systematically investigated. A range of natural water pHs was applied to reveal the role of solution chemistry on the sedimentation of the binary system. Various structures of the heteroaggregates formed under different background solutions were proposed based on the sedimentation behavior. In addition, the stability of COOH-MWCNT was also characterized in filtered and unfiltered natural water to investigate the heteroaggregation with natural colloids.

3.2 Materials and methods

Solution preparation. COOH-MWCNTs were obtained from Nanolab, Inc. (Newton, MA). They were 15 ± 5 nm in average diameter and 1 – 5 µm in length. As outlined by the manufacturer, the COOH-MWCNTs were produced by treating MWCNTs synthesized by chemical vapor deposition with a HNO₃/H₂SO₄ mixture; the resulting COOH-MWCNTs contain 2-7 wt% COOH as determined by titration.

The aqueous stock suspension of COOH-MWCNT was prepared by adding 5 mg COOH-MWCNT to 100 mL ultrapure water and sonicating in an ice bath for 30 min at 100 W using a sonicating probe (Vibra-Cell, VCX 500, Sonics and Material, Newton, CT). After preparation, the CNT stock suspension was kept at room temperature in a glass bottle wrapped with aluminum foil.

Kaolinite. Kaolinite powder was obtained from EMD Chemicals (Gibbstown, NJ), which had an intensity mean diameter of 400 ± 15 nm when suspended in deionized water measured by Dynamic Light Scattering (DLS). Before use, 50.3 g kaolinite powder was poured in 300 mL
ultrapure water and the suspension was pre-settled for 45 hours. Top 85% of the supernatant was then collected, and its concentration was determined by drying the remaining kaolinite and calculating weight difference. After that, the supernatant was diluted to obtain a kaolinite stock suspension of 100 mg/L, and it was stored in the same way as that of CNT.

Natural water samples of Trinity River and Lake Houston were collected from respective inlet at the City of Houston’s East Water Purification Plant (EWPP), and they were stored in the dark at 4 ºC before use. Reagent-grade NaCl, HCl, NaOH with a purity of 90% or above were used throughout the experiments for ionic strength and pH adjustments. Deionized water generated by a Barnstead Epure water system (Dubuque, IA) was used in the preparation of all solutions.

**Characterization of COOH-MWCNT, kaolinite and natural water samples.** A high-resolution dual beam UV-Vis spectrophotometer (UV-2550, Shimadzu Scientific Instruments, Columbia, MD) was used to determine the concentration of COOH-MWCNT and kaolinite with 1 cm Quartz crystal cuvettes. The absorbance was measured at 255 nm due to the peak absorbance found in the COOH-MWCNT spectra and the absorptivity of the COOH-MWCNTs relative to kaolinite (Figure 3-S2, supporting information), and satisfactory linear relationships ($R^2>0.999$) were obtained between light absorbance and concentration of COOH-MWCNT or kaolinite (Figure 3-S3).

A Zen 3600 Zetasizer Nano (Malvern, Worestershire, UK) with a He-Ne laser of 633 nm was used to measure particle size of COOH-MWCNT and kaolinite by dynamic light scattering (DLS) and electrophoretic mobility by phase analysis light scattering (PALS). Each DLS measurement consisted of 20 runs with an equilibration time of 5 minutes. Refractive indexes applied for COOH-MWCNT and kaolinite were 2.56 and 1.59, respectively. 30-min DLS
measurement of 5 mg/L kaolinite and COOH-MWCNT suspensions in deionized water were also performed to check the stability. The intensity mean diameter was measured every 15 seconds. PALS measurements were performed over pH range of 1.5 - 7.5. Each sample was measured three times at 25 °C.

To characterize the morphology of COOH-MWCNT and kaolinite, a transmission electron microscope (TEM) at 100 kV (JEM-2010F FasTEM, JEOL USA, Peabody, MA) was used. 5 mg/L COOH-MWCNT and 5 mg/L kaolinite suspensions were prepared by diluting stock solutions to 5 ppm with deionized water. For each sample, one droplet of the diluted suspension was applied on a 400-mesh, carbon lacey film supported copper grid (Ted Pella, Redding, CA) and dried in room temperature before the imaging process.

The natural water samples were analyzed for basic water quality parameters within 24 hours of sampling. The EWPP conducts daily testing of both Trinity River and Lake Houston waters using standard EPA testing methods. Relevant water quality parameters provided by the water treatment plant were reported in Table 3-S1.

**Sedimentation Experiments.** Sedimentation experiments were performed to investigate the colloidal stability of COOH-MWCNT under various solution conditions and different CNT-to-NOC ratios. In each experiment, 20 mL aqueous suspensions of COOH-MWCNT, kaolinite or mixture of COOH-MWCNT and kaolinite were prepared by adding stock suspensions to background solutions in 20 mL scintillation vials. The concentration of kaolinite was held constant at 5 mg/L in all experiments, while the CNT concentration was varied to obtain CNT-to-NOC ratios of 1, 0.2, 0.04, 0.02 and 0.01. The ionic strength of all samples was kept constant at 1 mM NaCl, and the pH was adjusted to 3.0 ± 0.2, 5.5 ± 0.2, and 7.0 ± 0.2 using HCl and
NaOH solutions. Control kaolinite and COOH-MWCNT suspensions were prepared at the same kaolinite or COOH-MWCNT concentrations used in the mixture samples.

Once prepared, the suspensions were mixed by a magnetic stirrer for 1 hr at 400 rpm and left to settle for 42 h. Samples were taken immediately after preparation of the suspensions, after 1 h mixing, and at 20 and 42 h during quiescent sedimentation. Each sample of 3 mL was withdrawn from the center of the scintillation vial and measured 3 times for UV/Vis absorbance at 255 nm using a high-resolution dual beam UV-Vis spectrophotometer (UV-2550, Shimadzu Scientific Instruments, Columbia, MD). The absorbance of the kaolinite is not negligible at 255 nm (Figure 3-S1, supporting information). Therefore, a kaolinite control was analyzed for all experiments to account for the kaolinite’s contribution to the CNT-NOC mixture’s absorbance. 50 µg/L was the lowest concentration of MWCNT that could be detected by the instrument (± 0.002 abs).

**Aggregation experiments.** Aggregation experiments were performed to track the changes in particle size distribution as a function of time. Because DLS does not distinguish CNTs from kaolinite, the concentration of the COOH-MWCNTs was reduced to 5 or 10 µg/L so that the COOH-MWCNTs did not produce any detectable light scattering signal. While the Huynh group was able to use concentrations of MWCNTs that were low enough to result in a scattered light intensity 20 times lower than the accompanying hematite particles, this study was limited by the detection limit of the UV-Vis, which limited the concentrations of MWCNTs. More details were discussed in the supporting information (Section 1). The kaolinite concentration used was 0.5 mg/L, yielding a CNT-to-kaolinite ratio of 0.01 and 0.02. Only these two lowest CNT-kaolinite ratios of 0.01 for pH 5.5 and 0.02 for pH 3, 5.5 and 7 were analyzed in the aggregation experiments. At these concentrations, the particle size change observed were attributed to kaolinite homoaggregation and heteroaggregation between COOH-MWCNTs and kaolinite.
Samples of 1 mL were taken from the particle suspensions immediately after preparation, after 1 h mixing, and at different time points during the 42 h sedimentation, and analyzed for particle size distribution. The suspensions were gently hand shaken before sampling to re-suspend settled particles.

**Experiments with Natural Water.** The sedimentation experiments were also performed using the two natural water samples before and immediately after filtration through 0.2 µm cellulose acetate membrane (Whatman). The same experimental protocol used in the sedimentation experiments in synthetic water was followed.

### 3.3 Results and discussion

**Characterization of COOH-MWCNT and kaolinite.** Morphologies of COOH-MWCNT (black powder, Figure 3-1 (A1)) and kaolinite (white powder, Figure 3-1(B1)) were demonstrated by the TEM. As shown in Figure 1A, COOH-MWCNT clearly showed its large aspect ratio, which had a tube diameter of 15 ± 5 nm and length of 1 -5 µm. Kaolinite demonstrated a platy structure (Figure 3-1 (B)), which was formed by silica tetrahedral and alumina octahedral sheets. Size distributions of kaolinite revealed in the TEM images is consistent with the DLS results (400.4 nm ± 15.9 nm). DLS of COOH-MWCNT showed the intensity mean diameter of 393.3 ± 24.0 nm, though the TEM imaging did not provide size distribution of single COOH-MWCNT due to the tangling of the tubes. When dispersed in water, particle size of kaolinite and CNT suspensions did not show increasing or decreasing trends during 30 min aggregation (Figure 3-S4), indicating that the two materials were stable in deionized water. Electrophoretic mobility (EPM) of COOH-MWCNTs and kaolinite was also compared at various solution pH. As shown in Figure 3-1(C), EPM of CNT and kaolinite stayed
negative from -4.79 to -1.19 \mu m/(s-V/cm) and no isoelectric point was shown through the studied pH ranging from 3 to 9, indicating that CNT and kaolinite were both negatively charged, and kaolinite surface charge was more negative than CNT. Solution pH also revealed expected influence on surface charge of the materials: EPM tended to be more negative at higher pH, which was consistent with previous CNT and kaolinite studies\textsuperscript{96}.

To further evaluate the effect of homoaggregation on CNT and kaolinite colloidal stability, sedimentation experiments were conducted for CNT and kaolinite for 42 h at three pH of 3.0, 5.5 and 7.0. As shown in Figure 1(D), normalized absorbance (absorbance/absorbance at 0 h) of both materials decreased with time, indicating that homoaggregation happened causing sedimentation of CNTs and kaolinites. It was also observed that the normalized absorbance of kaolinite decreased more (63% at pH 7) compared to CNT (98% at pH 7) after 42 h of sedimentation, indicating that CNT was more stable than kaolinite, which might be due to its lower density (2.1 g/cm\textsuperscript{3}) compared with kaolinite (2.65 g/cm\textsuperscript{3}). In addition, with the decrease of pH from 7 to 3, the normalized absorbance of both CNT (from 98% to 87%) and kaolinite (from 63% to 13%) illustrated significant decrease after 42 h of operation, indicating that low pH reduced stability of control suspensions. The destabilization effect was consistent with the electrophoretic mobility results shown in Figure 1(C). As pH decreased, CNT and kaolinite were less negatively charged, thus particles were destabilized due to decreased repulsive electrostatic forces.

Notably at pH 3, large amounts of kaolinite settled down from the suspension with absorbance of only 13% at 42 h, which was greatly related to its unique structure. As presented in Figure 1(A) and previous study, kaolinite was composed of stacked layers of one silica tetrahedral and one alumina octahedral sheet linked by oxygen atoms\textsuperscript{97}, the isoelectric points of the kaolinite phase and edge different, leading to surface charge heterogeneity. It was reported that point of zero
charge (PZC) at kaolinite edge sites were about 7 while its overall PZC was about 4\textsuperscript{98}. Therefore, the edge of kaolinite was positively charged and could easily attach to phases of other kaolinite particles, resulting in large homoaggregates and sedimentation\textsuperscript{39}. At pH 5.5 and 7, no change on the light absorbance of CNT and kaolinite was shown before and after 1-hr mixing. This is consistent with the assumption that light absorbances is solely dependent on mass concentration in the suspension. However, at pH 3, light absorbance decreased by 8\% for CNT and 27\% for kaolinite. In this lower pH, intensive homoaggregation happened and particle size increased remarkably at 0 h and during the 1 h of mixing. For example, at pH 3 the size of kaolinite increased from 1116 nm at 0 h to 1552 nm at 1 h while it was in the range of 500-800 nm at pH 5.5 and 7. It is speculated that this great increase in particle size decreased the light absorbances even though the mass concentrations were kept the same.

Figure 3-1 Representative TEM images and solid powder of COOH-MWCNT (A, A1) and (B, B1) kaolinite; (C) Electrophoretic mobility of COOH-MWCNTs and kaolinite; (D) Normalized absorbance (absorbance/absorbance at 0 hr) as a function of sedimentation time for COOH-MWCNT and kaolinite control samples under 1 mM NaCl and pH 3, 5.5 and 7. CNT: COOH-MWCNT; Kao: kaolinite; CLF: carbon lacey film.
Effect of heteroaggregation on kaolinite and COOH-MWCNT colloidal stability. Particle sedimentation in the suspension of COOH-MWCNT and kaolinite mixture was characterized at various CNT-to-kaolinite ratios: 1, 0.2, 0.04, 0.02, and 0.01 at pH of 3, 5.5 and 7.

As shown in Figure 3-2, heteroaggregation of COOH-MWCNTs and kaolinite occurred at all CNT-to-kaolinite ratios, and the impact of heteroaggregation on colloidal stability of the suspension can be very different depending on the CNT-to-kaolinite ratio. At CNT-to-kaolinite ratio of 1, the absorbance of the COOH-MWCNT/kaolinite mixture remained relatively stable throughout the 42 h experiment: 90% of original absorbance was maintained at 42 h. The sum of the absorbance of the COOH-MWCNT and kaolinite controls was comparable to the total absorbance of the mixture. This implies that the kaolinite and COOH-MWCNTs interaction did not significantly affect each other’s colloidal stability. At CNT-to-kaolinite ratio of 0.2, the mixture become less stable and the normalized absorbance was 78% compared with 90% at the ratio of 1. The sum of the controls, however, showed similar absorbance to the mixture: the difference of the normalized absorbance was less than 5% throughout the 42 h settling, indicating that the heteroaggregation did not affect each other’s stability either at this ratio. At CNT-to-kaolinite ratio of 0.04, 0.02 and 0.01, however, the absorbance of the mixture was notably higher (~15%) than that of mixture, implying that heteroaggregation with CNT increased the stability of kaolinite.

Particle size data in aggregation experiment was obtained in order to further testify this effect. Figure 3-2 also compared particle size distribution of kaolinite control and the mixture at a CNT-to-kaolinite ratio of 0.02 immediately after preparation, 1 h mixing, and 42 h settling. Because the concentration of COOH-MWCNTs was chosen to have non-detectable light scattering, the DLS measurements of the COOH-MWCNT and kaolinite mixture samples were attributed to
kaolinite homoaggregates and kaolinite-CNT heteroaggregates. The measured intensity mean diameters of the kaolinite and the COOH-MWCNT/kaolinite mixture both show notable increase in the period of 42 h, suggesting formation of aggregates. At 0 h (i.e., immediately after sample preparation), the two samples had the same intensity based mean diameter, consistent with the negligible light scattering of the COOH-MWCNTs at 5 μg/L. Over time, however, the mixture exhibited slower aggregation as suggested by the smaller intensity mean particle size after 1 h mixing ($p$ of student t-test = 0.09) and 42 h sedimentation ($p$ = 0.02). The decreased aggregate size was consistent with the slower sedimentation shown in Figure 3-2, suggesting that the presence of COOH-MWCNTs and their interaction with kaolinite particles resulted in heteroaggregation, and the heteroaggregates were smaller than the kaolinite homoaggregates formed in the absence of the COOH-MWCNTs, i.e., COOH-MWCNTs stabilized kaolinite particles.

Figure 3-2 (A) Normalized absorbance at pH 5.5 and ionic strength 1 mM over 42 hours of COOH-MWCNT-Kaolinite mixture (“Mix”, solid lines), and the sum of CNT and Kaolinite control absorbance (“Sum”, dash lines) for CNT:NOC ratios of 1, 0.2, 0.04, 0.02 and 0.01. Bottom columns indicated the intensity mean diameter of kaolinite control and mixture for 0.02 CNT:kaolinite ratio at pH 5.5. CNT: COOH-MWCNT; Kao: kaolinite.
At pH 3, the COOH-MWCNTs begin to show evidence of enhanced sedimentation at specific ratios (Figure 3-3 (A)). The combined absorbance of the mixture and the controls are very similar throughout the settling period for ratio 1 and at 0 h and 42 h for ratio 0.2. There is no evidence of enhanced sedimentation or aggregation due to the presence of kaolinite. When the CNT-to-kaolinite ratio decreased to 0.01, 0.02 and 0.04, the COOH-MWCNT/kaolinite mixture exhibited faster decrease in light absorbance than the additive sum of the COOH-MWCNT and kaolinite controls, suggesting faster sedimentation of the mixture suspension than that predicted based on the individual sedimentation behaviors of COOH-MWCNT and kaolinite. These results strongly suggested that heteroaggregation between COOH-MWCNTs and kaolinite occurred and the heteroaggregation reduced the colloidal stability of the suspending, leading to accelerated sedimentation. This is supported by the particle size measurement at the same CNT-to-kaolinite ratios. As is shown in Figure 3-3 (B), after 42 h the intensity means of the mixture with 0.02 and
0.01 ratio were significantly higher than that of the kaolinite control (p of student t-test = 0.03), indicating the enhanced aggregation in the mixture at this CNT/NOC ratio after 42 hours.

Figure 3-3 (A) Normalized absorbance at pH 3 and ionic strength 1 mM NaCl over 42 hours of COOH-CNT-Kaolinite mixture (“CNT:kaolinite”, solid lines), and the sum of CNT and Kaolinite control absorbance (dash lines) for CNT:NOC ratios of 1, 0.2, 0.04, 0.02 and 0.01. (B) Intensity mean diameter (nm) of kaolinite control and mixture for 0.02 and 0.01 CNT:Kaolinite ratios at pH 3. CNT: COOH-MWCNT; Kao: kaolinite.

At higher pH 7, the sums of CNT and kaolinite controls were very close to the absorbance of the mixture (Figure 3-S5 (A)) at the same CNT-kaolinite ratios. After 42 hours, the difference of normalized absorbance between the mixture and the sum of controls were 8% for ratio 0.2 and <5% for the rest ratios. Particle size at ratio of 0.02 was measured and found remaining stable.
through the sedimentation process and there was no significant difference between the mixture and the kaolinite controls (Figure 3-S5 (B)). This implied that the particles were not interacting very often, preventing aggregation from occurring.

**Proposed mechanisms of heteroaggregation.** Based on the electrophoretic mobility, sedimentation and aggregation results, we proposed the following mechanisms of the CNT-kaolinite heteroaggregation.

At pH 5.5, the stabilization effect at ratios from 0.01-0.2 is attributed to the formation of primary heteroaggregates (i.e., aggregates formed directly between individual particles or homoaggregates). The coverage of CNT on kaolinite surface impedes the formation of larger aggregates due to the repulsive force among CNT particles. This agrees with the sedimentation result of controls in Figure 3-1 (D) that CNT particles settled much slower than kaolinite at pH 5.5. It is also supported by electrophoretic mobility results in Figure 3-1 (C), showing more negative surface potential of CNT over kaolinite. As is discussed above, heteroaggregation occurred at all ratios tested although both CNT and kaolinite were negatively charged. One possible reason is that the repulsive electrostatic force can be overcome by the attractive Van der Waals force, leading to the attachment of CNT to kaolinite. In addition to DLVO forces, the oxygen groups of COOH-MWCNT and hydroxyl groups of kaolinite can be Louis acid and base, respectively. The attractive Louis acid-base interaction and hydrogen bonding between these surface groups also attributed to the heteroaggregation. Similar mechanisms of CNT-kaolinite heteroaggregation was discussed by Han et al\(^9\).

At pH 3, due to the surface charge heterogeneity of kaolinite mentioned in section 3.1, the electrostatic attraction of the edge and phase of kaolinite platelets destabilizes NOC particles,
resulting in the increase of particle size and decrease of light absorbance after sedimentation. In the mixture, negatively charged COOH-MWCNT can attach to kaolinite edge. The -COOH groups on these primary complexes then form bridges between kaolinite edges, developing secondary heteroaggregates with even larger particle sizes than kaolinite homoaggregates.

At pH 7, this relatively high pH caused increase in the negative surface potential of CNT and kaolinite, leading to greater repulsive forces. Thus, little CNT-kaolinite heteroaggregation occurred and the stability of CNT and kaolinite was not significantly affected by each other. Schematic of the particles in the mixture under the studied ranges of pHs and CNT-to-kaolinite ratios was demonstrated in Figure 3-4.

Effect of pH and COOH-MWCNT: kaolinite ratio on the stability of the binary systems.

Figure 3-4 summarizes the percent impact of pH and CNT-to-kaolinite ratio on the stability of COOH-MWCNT/kaolinite mixture. The percentage impact on stability is described by equation (1):

\[
\% \text{ impact on stability} = \frac{\text{Abs}_m - (\text{Abs}_c + \text{Abs}_k)}{\text{Abs}_c + \text{Abs}_k} \times 100\%
\]  

(1)

Here Abs\(_m\), Abs\(_c\) and Abs\(_k\) are the absorbances of the CNT-kaolinite mixture, COOH-MWCNT control and kaolinite control after 42 hours of sedimentation, respectively. Positive values indicate enhanced colloidal stability of the mixture, while negative values indicate decreased stability and accelerated sedimentation.

In Figure 3-4, the percent impact on stability is plotted verses the five ratios tested (1, 0.2, 0.04, 0.02, and 0.01) at pH 3, 5.5, and 7. As discussed previously, heteroaggregation occurred at pH of 3 and 5.5 but was not significant when pH increased to 7 throughout CNT-to-kaolinite ratios.
tested. For pH 7, there was generally no ratio-dependent effect on stability, all ratios showed little indication of any impact on stability, with slight negative values calculated (1% - 10%). In the cases when heteroaggregation happened (pH 3 and 5.5), the impact of stability was largely depended on the CNT-to-kaolinite ratio. At high ratios of 1 and 0.2, stability of the CNT and kaolinite was not significantly affected by each other. One possible reason was that concentration of CNT was too high at these ratios, and the non-aggregated CNTs could impede any extensive aggregation. Similar phenomenon was reported by Huynh et al. on the interaction of multi-walled carbon nanotube and hematite. When CNT:kaolinite ratios decreased to 0.02 and lower, stabilizing effect (up to 40% of impact) was demonstrated at pH 5.5 while at pH 3 the mixture was greatly destabilized. When the ratio decreased at pH 3, the impact on stability increased in magnitude. The values became more negative, until all the mixture of the CNT and kaolinite completely settled out of solution at the 0.01 ratio due to the formation of large secondary heteroaggregates. This demonstrates that low pH leads to enhanced sedimentation as the proportion of kaolinite increases.

The above results suggested that the configuration of the CNT-kaolinite heteroaggregates and the stability of the mixture were largely dependent on the CNT-to-kaolinite ratio and pH. When in high ratios, stability was not significantly affected and there was little effect of the solution pH. In lower ratios, i.e. higher proportion of kaolinite, pH played dominate role on the stability. The CNT-kaolinite mixture can either be stabilized or destabilized depending on the structure of heteroaggregates formed under the certain pH, which is largely dependent on CNT-to-kaolinite ratio.
Natural water samples. Natural Water Samples from the Houston EWPP drinking water plant were analyzed to determine the impact natural colloids present in unaltered natural water samples effected the sedimentation behavior of the CNT. A portion of the natural water samples were filtered using a 0.2 µm filter to determine the effects larger colloidal matter has on the sedimentation behavior of the CNT. The UV-Vis absorbance of the CNT in filtered and unfiltered Trinity River and Lake Houston water samples was measured for 5 mg/L and 100 µg/L CNT suspensions.

Figure 3-5 plotted the filtered and unfiltered samples against the absorbance of COOH-CNT of the corresponding concentrations dispersed in DI water at pH 7. Here, y axis was relative absorbance of the samples compared with their corresponding controls (i.e., DI water, filtered and unfiltered water samples without CNT). From the figure, it was clear that the constituents present in the natural sample resulted in increased sedimentation of CNT when compared to the
controlled synthetic sample prepared in DI water. However, the filtered and unfiltered samples behave differently depending on the concentration of CNT present.

Both natural water samples behaved quite similarly as they were taken at similar locations. For the 5 mg/L sample, the filtered and unfiltered sedimentation behavior is very similar. Both settle out of solution by 20 hours and have similar rates of change. The unfiltered sample shows slight indication of enhanced sedimentation, as it decreases in absorbance at a slightly faster rate than the filtered sample.

At the lower concentration of 100 μg/L, the filtered and unfiltered samples behave quite differently. Both samples registered at a negative absorbance at 0 hr (immediately after preparation), which implies that components of the natural water are removed along with the CNT through sedimentation. The filtered sample, although less stable than the DI water-dispersed sample, does not settle out of solution to the extent of the unfiltered sample. This implies that the unfiltered sample is much less stable than the filtered sample.

As reported in Table 3-S1 in supporting information, the significant concentration of Ca²⁺, silica, and the relatively high reported conductivity all could potentially aid in the destabilizing of CNT in the Lake Houston and Trinity River Systems. While distilled water has an average conductivity of 0.5 to 3 µmhos/cm, the two natural water samples have a conductivity of approximately 400 µmhos/cm (EPA). This increase in ionic strength could result in increased potential for homoaggregation/heteroaggregation of the CNT. As is discussed previously, the Ca²⁺ can also aid in heteroaggregation, as it can result in a bridging effect between particles.

The difference of sedimentation rates between 100-μg/L CNT samples in filtered and unfiltered natural water suggested that large naturally occurring colloids in natural waters greatly
contributed to the accelerated sedimentation of CNT via heteroaggregation. This result is within our expectation and it clearly demonstrated the important role of naturally occurring colloids present in natural surface water on the fate and transport of CNT.

![Graph A](image)

![Graph B](image)

Figure 3-5 Changes in absorbance of filtered and unfiltered 5 mg/L and 100 µg/L COOH-CNT in (A) Trinity River and (B) Lake Houston water sample compared with the same concentrations dispersed in DI water at pH 7. X axis represents the sedimentation time at 0 hr, after 1-hr mixing, 20 hr and 42 hr. Y axis (ΔAbsorbance) is the difference of absorbance between samples and their corresponding controls, i.e., DI water, filtered and unfiltered water samples without CNT.

### 3.4 Environmental implications

Our results revealed that COOH-MWCNT can from different structures of heteroaggregates with kaolinite depending the solution pH and CNT-to-kaolinite ratio, which then significantly
influence the stability of COOH-MWCNT. When in natural water samples, MWCNTs stability was decreased mainly due to aggregation with naturally occurring colloids. Other water quality parameters such as ion compositions also attributed to the sedimentation. In natural surface waters, a wide range of natural water constituents is possible and will vary with each source (Table 3-S2). The makeup of the natural water will also change seasonally and with changing amounts of precipitation. These changes could drastically affect CNT fate, transport and environmental risk. For example, under high pH, CNT particles can be mostly free and stabilized, which is more bioavailable than that in acidic waters, where most of the CNT would be destabilized and settled as large aggregates with naturally occurring colloids (NOC). The stabilizing effect of CNT on NOC may indicate CNT’s potential to transport other contaminants. In future studies, the impact of natural water constituents such as natural organic matter and Ca$^{2+}$ should be systematically studied. The co-transport of CNT with NOC in subsurface porous media should also be characterized to better predict environmental hazards in the fate and transport of nanomaterials in the environment.

3.5 Supporting Information

The reason for lowering COOH-MWCNT concentrations for the DLS measurement. While the Huynh group was able to use concentrations of MWCNTs that were low enough to result in a scattered light intensity 20 times lower than the accompanying hematite particles$^{99}$, this study was limited by the detection limit of the UV-Vis, which limited the concentrations of MWCNTs. Figure shows the scattered light intensities of the kaolinite and MWCNTs in a 1 mM NaCl solution at the same laser intensity. Although the scattered light intensity of the MWCNTs was still lower than the kaolinite particles (approximately 6 times lower for 200 µg/L) the contribution of the MWCNTs to the light scattering will not be as negligible as in previous
studies. Therefore, in this study the concentration of the COOH-MWCNTs was reduced to 5 or 10 μg/L for the DLS measurement of CNT-kaolinite mixtures so that the COOH-MWCNTs did not produce any detectable light scattering signal.

![Figure 3-S1 Derived count rate (kcps) of 5 mg/L kaolinite and 200 μg/L COOH-MWCNT in 1 mM NaCl solution.]

**Characterization of COOH-MWCNT and Kaolinite**
Figure 3-S2 Light absorbance spectrum of COOH-MWCNT (10 mg/L) and kaolinite (5 mg/L).

Figure 3-S3 Light absorbance vs. concentration curves of COOH-MWCNT and kaolinite. Samples of different concentrations were prepared by diluting stock suspensions with deionized water (pH=6.5, unadjusted).
Figure 3-S4 Intensity mean diameter of (A) kaolinite and (B) COOH-MWCNT as a function of time. Kaolinite and COOH-MWCNT samples were both 5 mg/L prepared by diluting the stock suspensions with deionized water.

Water quality characteristics of natural water samples and a comparison of other water bodies.

Table 3-S1 Water quality characteristics for the Trinity River and Lake Houston inflow to the EWPP plant.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Source</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trinity River</td>
<td>Lake Houston</td>
</tr>
<tr>
<td>----------------------</td>
<td>---------------</td>
<td>--------------</td>
</tr>
<tr>
<td><strong>Alkalinity</strong></td>
<td>90</td>
<td>84</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>7.4 ± 0.5</td>
<td>7.4 ± 0.5</td>
</tr>
<tr>
<td><strong>Bromide</strong></td>
<td>0.16</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td><strong>Calcium</strong></td>
<td>39</td>
<td>22</td>
</tr>
<tr>
<td><strong>Chloride</strong></td>
<td>38</td>
<td>41</td>
</tr>
<tr>
<td><strong>Conductivity</strong></td>
<td>412</td>
<td>348</td>
</tr>
<tr>
<td><strong>Iron (Fe)</strong></td>
<td>0.57</td>
<td>0.16</td>
</tr>
<tr>
<td><strong>Magnesium</strong></td>
<td>3.9</td>
<td>2.6</td>
</tr>
<tr>
<td><strong>Manganese</strong> (Mn)</td>
<td>0.06</td>
<td>0.11</td>
</tr>
<tr>
<td><strong>Nitrate</strong></td>
<td>0.11</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td><strong>Nitrite</strong></td>
<td>&lt;0.04</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td><strong>Potassium</strong></td>
<td>6.1</td>
<td>5.7</td>
</tr>
<tr>
<td><strong>Silica (SiO₂)</strong></td>
<td>5.2</td>
<td>3</td>
</tr>
<tr>
<td><strong>Sodium</strong></td>
<td>23</td>
<td>27</td>
</tr>
<tr>
<td><strong>Sulfate</strong></td>
<td>43</td>
<td>12</td>
</tr>
<tr>
<td><strong>Total Hardness (CaCO3)</strong></td>
<td>115</td>
<td>66</td>
</tr>
<tr>
<td><strong>TOC</strong></td>
<td>5.7</td>
<td>6.5</td>
</tr>
<tr>
<td><strong>Total Phosphate</strong></td>
<td>0.12</td>
<td>0.28</td>
</tr>
<tr>
<td><strong>Total Residual Chlorine</strong></td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>TSS</strong></td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td><strong>TDS</strong></td>
<td>227</td>
<td>191</td>
</tr>
<tr>
<td><strong>Turbidity</strong></td>
<td>11</td>
<td>4.88</td>
</tr>
<tr>
<td><strong>UV 254</strong></td>
<td>0.132</td>
<td>0.163</td>
</tr>
</tbody>
</table>

Table 3-S2 Comparative water quality values from different source waters. Rain water (adapted from Ottofuelling).

<table>
<thead>
<tr>
<th></th>
<th>Rain water</th>
<th>Lake Water</th>
<th>River Water</th>
<th>Sea Water</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Na⁺</strong></td>
<td>1.1</td>
<td>0</td>
<td>3.1</td>
<td>11145</td>
<td>mg/L</td>
</tr>
<tr>
<td><strong>K⁺</strong></td>
<td>0.3</td>
<td>0</td>
<td>0.9</td>
<td>414</td>
<td>mg/L</td>
</tr>
<tr>
<td><strong>Mg²⁺</strong></td>
<td>0.4</td>
<td>6</td>
<td>8.7</td>
<td>1339</td>
<td>mg/L</td>
</tr>
<tr>
<td><strong>Ca²⁺</strong></td>
<td>1</td>
<td>45.6</td>
<td>43</td>
<td>429</td>
<td>mg/L</td>
</tr>
<tr>
<td><strong>Cl⁻</strong></td>
<td>1.1</td>
<td>2.5</td>
<td>2.8</td>
<td>20065</td>
<td>mg/L</td>
</tr>
<tr>
<td><strong>HCO₃⁻</strong></td>
<td>1.2</td>
<td>12.6</td>
<td>9.4</td>
<td>1787</td>
<td>mg/L</td>
</tr>
<tr>
<td><strong>SO₄²⁻</strong></td>
<td>4.2</td>
<td>15</td>
<td>53</td>
<td>230</td>
<td>mg/L</td>
</tr>
</tbody>
</table>

Sedimentation experiment at pH 7
Figure 3-S5 (A) Normalized absorbance at pH 7 and ionic strength 1 mM NaCl over 42 hours of COOH-CNT-Kaolinite mixture (“CNT:kaolinite”, solid lines), and the sum of CNT and Kaolinite control absorbance (dash lines) for CNT:NOC ratios of 1, 0.2, 0.04, 0.02 and 0.01. (B) Intensity mean diameter (µm) of kaolinite control and mixture for 0.02 CNT:kaolinite ratio at pH 7.
Chapter 4

Heteroaggregation of carboxyl-functionalized multi-walled carbon nanotubes with kaolinite under environmentally relevant conditions

Abstract

Fate and transport of the engineered nanomaterials (ENMs) in aquatic systems has been increasingly concerned due to their potential human exposure and reported toxicity to the living organisms. The ubiquitous naturally occurring colloids (NOC) is an important impacting factor controlling the aqueous stability of ENMs mainly via ENM-NOC heteroaggregation. This work studied the heteroaggregation of carboxyl-functionalized multi-walled carbon nanotubes (COOH-MWCNT) with kaolinite under environmentally relevant solution conditions by synthesizing background solutions with a range of solution pH (3-9), ionic strength (1 mM – 100 mM NaCl), NOM (0, 10, 50 mg/L) and Ca$^{2+}$ (0.33 – 3.3 mM CaCl$_2$) concentrations. In assessing the extent of heteroaggregation, attached CNT was separated from the total CNT in the CNT-kaolinite mixture through a series of ultra-sonication and centrifugation methods. Particle size and morphology of the aggregates were characterized using dynamic light scattering and TEM imaging, respectively. By analyzing the plots of attached CNT percentage over total CNT as functions of the environmental impacting factors, we concluded that the increase of ionic strength and decrease of pH enhanced the heteroaggregation. At the extremely low pH of 3, large secondary
heteroggregation (attachment among the aggregates that were primarily formed) occurred and the particles were strongly destabilized. Ca$^{2+}$ and NOM played opposite roles on the stability of CNT. The addition of Ca$^{2+}$ induced the heteroaggregation through bridging effect and charge screening while NOM hindered the aggregation via steric effect in low Ca$^{2+}$ conditions. When Ca$^{2+}$ reached 3.3 mM, the effect of Ca$^{2+}$ dominated and induced large heteroaggregates, destabilizing the CNT-kaolinite system. This study is the first one that quantitively assess the heteroaggregation of CNT with naturally occurring colloids under environmentally relevant conditions. Results from this work provide useful information for the prediction of CNT fate and transport in natural water systems.

4.1 Introduction

As an important class of the emerging environmental pollutants, release of the engineered nanomaterials (ENMs) in natural systems has been discussed$^{100}$. When released into aquatic environments, the aggregation/dispersion of ENMs plays an important role on their transport and bioavailability. Dispersed ENMs can be more stable in surface water and more bioavailable than the aggregated, while the latter will be settled faster and possibly become a source of pollutants in the future$^{101}$. The aggregation behaviors of ENMs will strongly be affected by the solution chemistry, including pH, ionic strength and ion valence. Characteristics of the aggregates or individual particles such as the stability, morphology, surface charge, etc. will also be altered through interactions with naturally occurring colloids and dissolved natural organic matters (NOM).

Among carbon-based nanomaterials, the typical 1-D carbon nanotubes (CNT) is one of the most produced ENMs. It has experienced an increasing commercial application in various fields, such
as composite materials, microelectronics, energy storage and biotechnology\textsuperscript{102}. At the same time, the toxicity of CNT has been increasingly reported. It has been shown that CNTs can cause inflammatory effects in rat lungs, DNA damages, bacterial cell membrane damage and pathological effects in mice. Also, the toxicity was dependent on CNT properties such as size, surface charge and shape\textsuperscript{103}. These properties were reported to be strongly affected by solution pH, ionic strength and ionic valence in natural waterbodies. Many studies on CNT aggregation reported that the aggregation was induced when ionic strength increased and multivalent ions (e.g. Ca\textsuperscript{2+} and Mg\textsuperscript{2+}) were added due to shrinking of the electrical double layers. Increasing pH resulted in decreasing aggregation kinetics because of the deprotonation of surface functional groups and thus more negative surface charge\textsuperscript{104}.

The ubiquitous naturally occurring colloids (NOC) and dissolved organic matters (NOM) in natural waterbodies are also important factors affecting the aqueous stability of ENMs. Substantial amounts of studies have reported that NOM stabilized the ENM particles via steric hindrance\textsuperscript{34}. However, in the co-presence of Ca\textsuperscript{2+} and NOM, Ca\textsuperscript{2+} was found to bridge NOM molecules and largely enhanced aggregation. The number of studies about the ENM-NOC aggregation (heteroaggregation) was relatively limited. The mechanisms of how pH and ionic strength impact ENM-ENM aggregation as is stated above was found the same for ENM-NOC heteroaggregation in studies of TiO\textsubscript{2}-natural clays\textsuperscript{41} and gold nanoparticles- hematite binary systems\textsuperscript{43}. Only two studies have investigated the combined effect of NOM and NOC on ENM aggregation and demonstrated the steric effects of NOM in binary systems of TiO\textsubscript{2}/SiO\textsubscript{2}\textsuperscript{122}, and citrate-stabilized gold nanoparticle/hematite\textsuperscript{105}. More efforts need to be made in order to predict fate and transport of ENMs in highly environmentally relevant conditions.
To our best knowledge, there is no published research that systematically investigated the aggregation and aqueous stability of ENM under the combined effect of solution pH, ionic strength, ion valence, NOC and NOM in natural water conditions.

The objective of this study is to investigate the stability of carboxylized multi-walled carbon nanotubes (COOH-MWCNT) in the presence of NOC under a range of solution pH, ionic strength, NOM and Ca$^{2+}$ concentrations. Kaolinite, one of the most common natural clay minerals, is chosen as the model NOC. kaolinite has a platy morphology and at the molecular level, it has a layer-by-layer structure. It is composed of stacked layers of one silica tetrahedral and one alumina octahedral sheet linked by oxygen atoms$^{106}$. Because of the special structure, the isoelectric points of the kaolinite phase and edge are different which may lead to surface charge heterogeneity: point of zero charge (PZC) for kaolinite edge sites were reported at about 7 while the overall PZC was about 4$^{107}$. The proportions of attached CNT over total CNT were obtained through a series of ultra-sonication and centrifugation processes. Sizes of the CNT-kaolinite mixture were measured by dynamic light scattering and configurations of the heteroaggregates were characterized by TEM imaging. By combining the attached CNT portion and particle size results, the heteroaggregation of CNT with kaolinite and the stability of the mixtures under various solution conditions were determined. A 3-D surface graphs of the attached CNT proportion as functions of environmental factors were plotted for understanding heteroaggregation mechanisms and formations of the heteroaggregates.

**4.2 Materials and methods**

**Carbon nanotubes.** Carboxyl-functionalized MWCNT powder (2-7 wt% -COOH) was purchased from NanoLab Inc. (Waltham, MA) with average diameter of 15 ± 5 nm and length of
1-5 µm. In making the CNT stock suspension, a vibra-Cell VCX 500 sonicating probe was
applied to a 500 mL-beaker with 200 mg CNT powder and 200 mL deionized water. Barnstead
Epure water system (Dubuque, IA) was used to produce the deionized water used in the
preparation of all stock solutions and samples in this study. The sonication was processed in an
ice bath at 50 W for 10 min, 75 for 10 min and 100 W for 10 min (total 30 min). CNT suspension
was kept in a 500-mL glass bottle and wrapped in aluminum foil at room temperature within one
year. Every time before use, the stock suspension was sonicated in a cup horn (Vibra-Cell) for 5
min at 100 W.

Kaolinite. Kaolinite powder was purchased from EMD chemicals (Gibbstown, NJ). The
kaolinite stock suspension was made by adding 50.3 g kaolinite powder (EMD chemicals,
Gibbstown, NJ) into 300 mL DI water followed by sonication at 100 W for 30 min in an ice bath
using a Vibra-Cell VCX 500 sonicating probe. Ultrasonicated suspension was then presettled for
45 hours and top 85% of the supernatant was collected as kaolinite stock suspension. The
suspension was then stored at room temperature in an amber glass bottle before use within six
months. Similar to CNT suspension, the stock suspension was sonicated in a cup horn (Vibra-
Cell) for 5 min at 100 W every time before use. The particle size of kaolinite in the stock
solution was characterized by TEM imaging and periodical dynamic light scattering. The
intensity mean result by dynamic light scattering was 599 nm ±27 nm. TEM imaging (Figure
4-1) showed that kaolinite particles were in the form of platelets and they had a large size
distribution range.
Figure 4-1 TEM image of kaolinite particles.

**Natural Organic Matter.** Suwannee River NOM (RO isolation) (SRNOM, International Humic Substances Society, Atlanta, Georgia) was used as the surrogate for aquatic NOM. SRNOM stock solution was made following the protocol of Qu et al. for Suwannee River Humic Acid solution\textsuperscript{108}. Briefly, SRNOM powder (100 mg) was dissolved in DI water overnight in dark and pH 8.5 followed by filtering through a 0.45 μm-pore-size filter to remove undissolved SRNOM. The concentration of the stock solution was determined by total organic carbon (TOC) analysis and the carbon content of SRNOM\textsuperscript{127}. Reagent-grade HCl and NaOH with a purity of 90% or above were used throughout the experiments for pH adjustments.

**Preparation of kaolinite-CNT mixtures and control samples.** The objective of this study was to determine the effect different ionic strength, pH, Ca\textsuperscript{2+} (in the form of CaCl\textsubscript{2}) and NOM concentration had on the heteroaggregation behavior of COOH-MWCNT. The concentration of kaolinite and CNT were kept at 900 mg/L and 4.5 mg/L, respectively, for all samples in a series of solution condition matrix. In order to determine both the effect of pH and ionic strength, pH
was varied at 3, 5 and 9 when in 1 mM NaCl but kept at 9 for 10 mM and 100 mM NaCl conditions. To determine the effect of Ca\(^{2+}\), another group of samples were prepared where NaCl were totally or partly replaced with CaCl\(_2\) that had the same ionic strength as the corresponding NaCl solutions. Thus 0.33 mM and 3.3 mM CaCl\(_2\) were applied as the surrogate for 1 mM NaCl and 10 mM NaCl respectively. To avoid extreme sedimentation, 100 mM NaCl was replaced with 3.3 mM CaCl\(_2\) + 90 mM NaCl instead of 33 mM CaCl\(_2\). NOM varied at 0 mg/L, 10 mg/L and 50 mg/L to simulate conditions of no NOM, medium and high NOM in the environment. Kaolinite control samples were also prepared under the same solution conditions. To induce heteroaggregation, 10 mL of each sample was prepared in a 20- mL scintillation bottle and stirred for 1 hour at approximately 400 rpm. After the mixing, concentrations of different components (attached CNT, free CNT and kaolinite) were determined for mixture samples and particle size was obtained for mixture and control samples.

**Concentration measurement of CNT-kaolinite mixtures.** To obtain the concentration of various compositions (free CNT, attached CNT and kaolinite) in the CNT-kaolinite mixture samples, consecutive procedures of ultra-sonication (1 min, 100 W) and centrifugation (5,000 rpm, 3 min – 5 min, according to the solution conditions) were performed to examine optimal centrifugation conditions which effectively separated total CNT from the mixture. After the centrifugation conditions were determined, collected samples were centrifuged and 800-nm light absorbance of the supernatant was measured as that of the free CNT (X1). NOM has no absorbance at 800 nm wavelength. Afterwards, total 800-nm light absorbance of attached CNT and kaolinite (X2) was measured through washing out the remaining supernatant by sequentially adding background solution, centrifugation, extracting supernatant and adding deionized water followed by 1-min ultra-sonication at 100 W. Finally, 800-nm light absorbance of attached CNT
(X3) was determined by adding solutions back from the cuvette to the microtube after the measurement of X2 and then centrifugation at optimal separation conditions. Each measurement was performed three times. With the above procedures, light absorbance of various compositions in the mixture including free CNT (X1), attached CNT (X3), total CNT (X1+X3) and kaolinite (X2-X3) can be obtained. To certify that free CNT can be effectively removed when obtaining X2, quality control experiments were run by determining the mass of free CNT through centrifugation and measuring light absorbance of the supernatant. Results showed that 94%-95% of free CNT was washed out, thus the washing method was reasonable.

Particle size measurement. Dynamic light scattering (DLS) was used to measure particle size of mixture and kaolinite control samples. A Zen 3600 Zetasizer Nano (Malvern, Worcestershire, UK) was used for all measurements. The intensity of the light scattering was measured at 173°. Since CNT/kaolinite ratio is extremely small (0.005), count rate results showed that CNT contribute little (<5%) to the total light scattering of the mixture (Table 4-1). Thus, the DLS method can be used to measure particle size distribution of CNT-kaolinite mixture in our case. Each sample was measured three times at 25°C. Each measurement duration consisted of 20 runs with an equilibration time of 5 minutes. For each sample, particle size was expressed as intensity mean (nm) and was measured before and after 1 hour mixing.

Table 4-1 Count rate (kcp/s) of 4.5 mg/L CNT and 900 mg/L kaolinite in selected solution conditions.

<table>
<thead>
<tr>
<th>Condition</th>
<th>CNT</th>
<th>Kaolinite</th>
<th>CNT/kaolinite</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 mM NaCl, pH=9</td>
<td>556.2</td>
<td>24301.1</td>
<td>2.3%</td>
</tr>
<tr>
<td></td>
<td>30.1</td>
<td>3018.5</td>
<td></td>
</tr>
</tbody>
</table>
TEM imaging. In order to study the configuration of the CNT-Kaolinite heteroaggregates, a protocol for the imaging of the samples was developed. A transmission electron microscope (TEM) operating at 100 kV (JEM-2010F FasTEM, JEOL USA, Peabody, MA) was used to examine the kaolinite and CNT-NOC heteroaggregate structures. Carbon lacey TEM grids (Ted Pella, Redding, CA) were used for all experiments. Suspensions were prepared using the same protocol as the separation experiments. The CNT and kaolinite concentrations were diluted 100 times from those previously used in order to optimize the number of heteroaggregates seen on each grid. The samples were mixed for 1 hr at approximately 400 rpm.

4.3 Results and discussion

4.3.1 In the absence of NOM: Effect of solution condition on the stability of CNT-kaolinite mixture.

In the absence of NOM, solution conditions including pH, ionic strength and Ca\(^{2+}\) played important roles on the aggregation behavior of CNT-kaolinite system. As is seen in Figure 4-2 (a), at the low pH of 3, more than 80% of CNT formed heteroaggregates with kaolinite in 1 mM NaCl. As pH increased from 3 to 5, this percentage dramatically decreased to 22.53% and went down slightly to 15.3% when the pH further increased to 9. In general, heteroaggregation is enhanced at low pH. This is attributed to the fact that lower pH results in less negative surface potential of CNT and kaolinite. Thus, the electrostatic repulsive force is decreased. A similar trend was shown for the mixture in 0.33 mM CaCl\(_2\). It should be noted that in the presence of
calcium ions, attached CNT was more than that in the NaCl solution. This is due to the charge screening and bridging effect of Ca\(^{2+}\).

To better understand the stability and formation of the heteroaggregates, particle size results after 1-hr mixing were presented in Figure 4-2 (B). As is expected, intensity mean diameter of both samples in NaCl and CaCl\(_2\) solutions was significantly higher (>1500 nm) at pH =3 than that at pH 5 and 9 (~500 nm). In 1 mM NaCl solutions, at low pH of 3 the system was significantly destabilized. It is noted that particle size in the mixture is much higher than that in kaolinite control, indicating that CNT induced secondary heteroaggregation. This is reasonable because kaolinite edges are positive at pH=3 and the clay particles homoaggregated through phase-edge attraction. In the mixture, negatively charged CNT can combine multiple clay particles together by attaching to the edges of kaolinite platelets. Thus, secondary heteroaggregation formed and the size is even much larger than the large kaolinite homoaggregates in the control group. At pH=5 and 9, particle size of the mixture kept close to that of the kaolinite control, indicating the system was more stable in these conditions. The small percentage of attached CNT and the high NOC/CNT mass ratio resulted in no increase of particle size. Also, unlike that at pH=3, surface potential of the particles was more negative, and the CNT-kaolinite interaction was decreased. Primary instead of secondary heteroaggregates could be the main form of the attached CNT.

In terms of the mixture in 0.33 mM CaCl\(_2\), particle sizes were close to that in 1 mM NaCl at pH 5 and 9. Again, in these conditions attached CNT were considered mainly in the form of primary heteroaggregates. However, different from that in NaCl, particle size of the mixture at pH=3 (2245 nm) were close to that of the kaolinite control (2082 nm). The charge screening and bridging effect of Ca\(^{2+}\) enhanced homoaggregation in kaolinite control groups. Little increase in the particle size of the mixture from that of the kaolinite control suggests that there may be not
notable amount of secondary aggregates developed from pre-aggregated kaolinite with sizes close to kaolinite control. It is speculated that the main structures of heteroaggregates at pH=3 in 0.33 mM CaCl₂ could be 1) similar to the secondary heteroaggregates in 1 mM NaCl or 2) CNT attached on the surface of kaolinite homoaggregates.

Overall, when the ionic strength is equal to 1 mM NaCl and there is no NOM, the CNT-kaolinite system is strongly destabilized under extremely low pH in both solutions with and without calcium ions. However, at medium (5) to high (9) pHs, both systems in NaCl and CaCl₂ are stable and there is stable attached CNT in aqueous phase in the presence of Ca²⁺.
Figure 4-2 (A) attached CNT percentage and (B) intensity mean diameter of the mixture and NOC control samples under various pHs (3, 5 and 9) at 1 mM NaCl/0.33 mM CaCl$_2$. All samples were tested after 1 hr mixing.

To study the effect of ionic strength, attached CNT percentage and particle size results in 1 mM NaCl/0.33 mM CaCl$_2$, 10 mM NaCl/3.3 mM CaCl$_2$ and 100 mM NaCl/3.3 mM CaCl$_2$ + 90 mM NaCl at pH 9 are presented in Figure 4-3. It is clearly revealed that attached CNT increased with the increase of ionic strength and samples with Ca$^{2+}$ had higher attached CNT percentage compared to that in NaCl throughout the ionic strengths tested. This is because higher ionic strength decreases electrostatic repulsive force between particles by shrinking their electric double layers. And Ca$^{2+}$ has charge screening effect that more effectively reduced the negative surface potential of particles than Na$^+$ even though the ionic strength remains constant. In
addition, as is stated previously, Ca\(^{2+}\) may also enhance heteroaggregation by bridging CNT and kaolinite particles.

![Graph showing Attached CNT percentage and intensity mean diameter of the mixture and NOC control samples under various ionic strengths at pH=9. All samples were tested after 1 hr mixing.](image)

Figure 4-3 (A) Attached CNT percentage and (B) intensity mean diameter of the mixture and NOC control samples under various ionic strengths at pH=9. All samples were tested after 1 hr mixing.

From the particle size results shown in Figure 4-3 (B), unlike in 10 mM NaCl and 100 mM NaCl where particle size only slightly increased with the increase of ionic strength, Ca\(^{2+}\) largely
destabilized the systems under the same ionic strengths. The dramatic increase of particle size is largely attributed to the bridging effect of Ca$^{2+}$. The size is as large for the corresponding kaolinite control samples, indicating strong kaolinite homoaggregation under these conditions. As is seen in Figure 4-4, TEM images clearly showed large heteroaggregates and strong

Figure 4-4 TEM images of CNT-kaolinite mixture. Solution condition: 3.3 mM CaCl$_2$ and pH=9. There is no NOM in the solution. The scale bar represents 200 nm.
kaolinite-kaolinite aggregation. This is consistent with the particle size results. However, it is hard to tell from Figure 4-4 whether the heteroaggregates were mainly primary.

4.3.2 In the presence of NOM: Effect of pH on the stability of CNT-kaolinite mixture

Figure 4-5 attached CNT percentage (left) and intensity mean diameter (nm) (right) of CNT-kaolinite mixtures in various NOM concentrations and pHs. The ionic strength is 1 mM NaCl/0.33 mM CaCl$_2$.

In the ionic strength of 1 mM NaCl/0.33 mM CaCl$_2$, heteroaggregation decreased with the increase of NOM (Figure 4-5). This is expected because NOM can hinder the heteroaggregates and stabilize the system via steric effect, which is consistent with the particle size results: intensity mean diameter significantly decreased as NOM concentration increased. Similary to that without NOM, in the presence of NOM, heteroaggregations were still enhanced by Ca$^{2+}$. As is showed that the dashed curves for CaCl$_2$ were all above the same-coloured solid lines of NaCl at a fixed NOM concentration, indicating that the charge screening and bridging mechanisms of Ca$^{2+}$-enhanced aggregation were also applicable in the presence of NOM. However, under this low ionic strength, samples were not distablized except for at pH 3 where strong
heteroaggregation exists and particle sizes were all above 1000 nm in the presence of Ca\(^{2+}\). When without Ca\(^{2+}\), 10 mg/L NOM was strongly enough to stabilize the system at pH 3. These results imply that at low ionic strength conditions (1 mM NaCl), NOM greatly increases the stability of CNT-kaolinite system while Ca\(^{2+}\) played the opposite role.

4.3.3 Effect of Ca\(^{2+}\), NOM and ionic strength on the stability of CNT-kaolinite mixture

![Graph showing attached CNT percentage and intensity mean diameter (nm) of CNT-kaolinite mixtures in various NOM concentrations and ionic strengths under pH 9.](image)

Figure 4-6 attached CNT percentage and intensity mean diameter (nm) of CNT-kaolinite mixtures in various NOM concentrations and ionic strengths under pH 9.
In pH 9 and no Ca\(^{2+}\) conditions, the solid lines in the two graphs of Figure 4-6 showed consistent results on the stability of the mixture. At 0 and 10 mg/L NOM concentration, attached CNT and particle size increased with the increase of ionic strength (though at 10 mg/L NOM there is no significant shift of the size in 1 mM NaCl from 10 mM NaCl). At 50 mg/L NOM the heteroaggregation was very low in all three ionic strengths and the particles were all below 1000 nm. Thus, the effect of ionic strength was not notable at 50 mg/L NOM.

In terms of the effect of NOM, it is clearly showed that attached CNT proportion decrease as the increase of NOM. Also, except for some samples which were already stable without NOM (in 1 mM and 10 mM NaCl), Particle size decreased when in higher NOM concentration. Combining results of these samples (vary in ionic strength) with that in the last section (vary in pH), it can be concluded that in the absence of Ca\(^{2+}\), NOM hinder heteroaggregation and stabilize the system. Again, steric hindrance is the main mechanism for this impact.

Samples with Ca\(^{2+}\) are presented as the dashed lines. Results clearly shows that Ca\(^{2+}\) enhanced heteroaggregation throughout the NOM concentration ranges. Attached CNT in samples with 3.3 mM Ca\(^{2+}\) increased more significantly (up to ~80%) than that with 0.33 mM Ca\(^{2+}\) (~30% at most). This implies that the more Ca\(^{2+}\), the more efficiently surface charges of CNT and kaolinite particles are screened. Also, more Ca\(^{2+}\) could provide more bridges that combine the particles. This is in consistent with the particle size result: particles of samples with 3.3 mM Ca\(^{2+}\) is much bigger (1000 nm to more than 3000 nm) than those in only NaCl under the same ionic strength (500 - 800 nm). However, for samples with 0.33 mM Ca\(^{2+}\) the increase is not as significant (all under 1000 nm).
The combined effect of NOM and Ca$^{2+}$ is more complex compared with previous environmental factors discussed. For 0.33 mM CaCl$_2$, the attached CNT decreased with the increase of NOM because of the steric effect. Particle sizes were ~500 nm in various NOM concentrations and the stability of the samples was not affected. However, in 3.3 mM CaCl$_2$, attached CNT increased from 77% to 96% when NOM increased from 0 to 10 mg/L. Both CNT and NOM contains carboxyl groups, to which Ca$^{2+}$ has preferential affinity. Ca$^{2+}$ adsorbed on CNT and NOM, including Kaolinite-NOM and CNT-NOM, can act as bridges and thus enhance homo/heteroaggregation. Particle sizes did not show notable differences according to the student t-test results ($p=0.46$). This implies that the heteroaggregates formed by this mechanism may not be concentrated enough to alter the mean particle size or there was no further bridging to form secondary aggregates. As NOM further increased to 50 mg/L, attached CNT was not decreased notably ($p=0.45$) but the particles were smaller compared with that in 10 mg/L NOM, indicating that the homo/heteroaggregates turned smaller due to the enhanced steric effect but still most of the CNT were attached to kaolinite.

Attached CNT results for 3.3 mM CaCl$_2$ plus 90 mM NaCl was very similar to that in only 3.3 mM CaCl$_2$. Particle size results were also very close in 0 and 10 mg/L NOM. Interestingly, the mean particle size greatly dropped from 2800 nm at 10 mg/L NOM to 1139 nm at 50 mg/L NOM, much smaller than that for only 3.3 mM CaCl$_2$ (2153 nm). It reflected that at 50 mg/L NOM, the sample in 3.3 mM CaCl$_2$ can be more stable when the solution contains 90 mM more of NaCl. Theoretically more NaCl would result in destabilization of the system. The mechanism for this phenomenon is undetermined.

In summary, the effect of NOM on CNT-kaolinite heteroaggregation is largely affected by Ca$^{2+}$. There is an optimal Ca$^{2+}$/NOM ratio which maximize the heteroaggregation. When NOM further
increases, the binary system can be stabilized but remained the same amount of attached CNT. It is also possible that heteroaggregation was strongly hindered when the amount of NOM keeps increasing (applicable in natural water with extremely high NOM concentration), but that concentration range is beyond the target solution conditions in this study.

4.3.4 3D surface graphs in summarizing the formation of particles in CNT-kaolinite mixtures under the studied solution conditions

Figure 4-7 3D surface graphs of attached CNT/total CNT as functions of 1) NOM and pH at 1 mM NaCl (upper left), 2) NOM and pH at 0.33 mM CaCl$_{2}$ (upper right), 3) NOM and ionic strength at pH=9 w/o Ca$^{2+}$ (bottom left) and 4) NOM and ionic strength at pH=9 w/ Ca$^{2+}$ (bottom right).
To provide an overview of differing pH, ionic strength and NOM had on the aggregation of CNT, 3D surface graphs of attached CNT percentage in total CNT as functions of the solution chemistry variables were reported in Figure 4-7 and the speculated formations of the heteroaggregates are presented at the corresponding solution condition areas in the graph. It is demonstrated in the graph that heteroaggregation was enhanced as the ionic strength increased and the NOM concentration decreased. In general, the same trend was applied to the stability of the samples, as is indicated by the formations of the heteroaggregates. At low Ca\(^{2+}\) concentration, NOM effectively hinders heteroaggregation and stabilize both CNTs and Kaolinite; At higher Ca\(^{2+}\) concentration (3.3mM or 3.3 mM+ 90 mM NaCl), the heteroaggregation process was dominated by the effect of Ca\(^{2+}\), which cause large secondary aggregation.

4.4 Conclusions

In this work, the influence of natural organic matters and naturally occurring colloids on the aggregation of carboxyl multi-walled carbon nanotubes in natural aquatic environments were studied. Formations of the aggregates and stability of the CNTs were determined by measuring proportions of the attached CNT and the particle size of the aggregates in a range of solution pH, ionic strengths and NOM concentrations. Based on the experimental results, the following conclusions were made:

1) Low pH and high ionic strength conditions enhanced the heteroaggregation of CNT and kaolinite. Extremely low pH conditions caused large secondary heteroaggregates and destabilized CNT.
2) Calcium ions were more effective in inducing heteroaggregation than sodium ions due to the charge screening and bridging effects.

3) In low to no calcium ions, NOM hindered heteroaggregation through steric effect and stabilized the CNT.

4) When in the high calcium ion concentration range, despite the NOM concentration, CNT formed extremely large heteroaggregates and was totally destabilized due to the dominating impact of calcium ions.
Chapter 5


Abstract

The increasing production and release of the engineered nanomaterials (ENMs) has posed potential environmental risk of surface water and groundwater pollution. The fate of ENMs in aqueous phase can be affected by natural occurring colloids (NOCs) and solution chemistry. Flow cytometry, which is a common technique for cell analysis in biological field, may provide useful size and fluorescence information in ENM-related aggregation studies. In this study, the applicability of flow cytometry in analyzing aggregations in Alexa Fluor 633 dyed carbon nanotubes (AFCNT) and kaolinite mixture was investigated under various pH and ionic strength conditions. Dot plots and relative frequency histograms of size and fluorescence signal intensities were reported to demonstrate population distributions of the particles in each sample. The relative extent of 1) homoaggregation (aggregation among the same type of particle) in AFCNT and kaolinite controls, respectively and 2) heteroaggregation in the mixtures among differing solution chemistries were defined through calculating areas of difference on the relative frequency histograms from those of the corresponding non-aggregated controls. Results showed that single
submicron-sized AFCNT and kaolinite particle can be detected by the flow cytometer although their sizes were close to the resolution of the instrument. Significant homo- and heteroaggregation were determined under extremely low pH (3) and highly ionic (100 mM NaCl) solutions while little aggregation was found in medium (5) and high pHs (9) under 5 mM NaCl. Findings in this study indicated that flow cytometry is a promising tool in analyzing submicron colloidal particles. It can be applied on future aggregation studies of ENMs-NOC and provide meaningful information for risk management of ENMs in aquatic environments.

5.1 Introduction

The environmental risk of engineered nanomaterials (ENMs) has attracted more and more attention by scientific communities due to ENMs’ greatly increasing production\textsuperscript{109} and reported toxicity on living organisms\textsuperscript{110}. To examine the risk, it is crucial to learn the human exposure pathways of ENMs via natural waterbodies. Once released into surface water, the ENMs will interact with themselves (homoaggregation) or other types of colloids (heteroaggregation) such as natural clay particles. These aggregations will probably alter the fate and transport of ENMs in aqueous media by changing their size, structure and surface physical-chemical properties, etc. As a result, there is an urgent need of systematic investigations on impacting factors and developed analytical tools for the ENMs aggregation behavior.

Over the past decades, ENMs homoaggregation has been extensively studied. In fact, the predicted concentrations of ENMs in real aquatic systems are much lower compared with the contents of naturally occurring colloids (NOC). For example, in freshwater, carbon nanotubes are predicted to be in nano grams per liter level (0.005 ng/L to 0.008 ng/L) while concentrations of colloidal matter are typically in a range of 1 mg/L to 10 mg/L\textsuperscript{111}. Thus, the probability of ENMs homoaggregation
may be lower relative to the heteroaggregation of ENMs with NOC. Several studies on the ENMs-NOC systems have shown that solution pH and ionic strength greatly impacted the aggregation status of ENMS for TiO$_2$-smectite$^{111}$, TiO$_2$-montmorillonite and graphene oxide-goethite$^{30}$ binary systems through affecting surface charges of the particles. Though being increasingly focused on, works regarding ENMs-NOC heteroaggregation is still limited. One of the main reasons is the lack of established analytical techniques that can accurately describe both the homo- and heteroaggregation processes.

Current methods of investigating heteroaggregations can be generally classified into three types. One is to determine the attachment kinetics derived from particle size results obtained by dynamic light scattering. Since only one refractive index could be applied in the measurement, studies using this method tested binary systems with a much larger concentration of one composition (usually NOC) than the other, of which the light scattering can be ignored$^{34}$. Sedimentation experiments are another approach, in which a large concentration difference is required in the mixture in order to get accurate concentration results of the supernatant (usually gained based on UV-vis light absorbance)$^{112,132}$. Additionally, several studies performed adsorption/desorption tests to obtain isotherms to characterize ENM-NOC interaction$^{132}$. Reasonable separation methods such as centrifugation are crucial in the adsorption/desorption experiments, and thus the particle size difference between mixture compositions was narrowly restricted.

Flow cytometry, a standard tool of cell analysis in biological research, can be used as a more efficient and accurate way to quantitatively determine the extents of homo- and heteroaggregation. Unlike the traditional DLS and fluorescence spectrophotometer approaches which output average values of colloidal suspensions, a flow cytometer rapidly collects real-time size and fluorescence information of each particle. Consequently, it allows very low particle concentrations which are
close to that in real waterbodies and provides detailed information of the aggregation types. Since the flow cytometer is designed primarily for biological cells in micrometer dimensions, its application on nano-sized materials has been rarely investigated. In biological field, Van der Pol et al. and Nolte-'t Hoen et al. performed flow cytometric analysis of nanosized cell-derived vesicles and found that the vesicles can be resolved provided by appropriate signal channels and thresholds settings\textsuperscript{113, 114}. For pure physical aggregation, Rollie et al. determined the cluster composition in heteroaggregation of polystyrene (PS) particles and Rhodamine-B labeled melamine-formaldehyde (MF-RhB) particles\textsuperscript{115}. This is the only previous study that focused on aggregations of non-biological and nano-sized particles.

To the best of our knowledge, this work is the first study that utilizes flow cytometry to analyze aggregations in the ENMs-natural inorganic colloid system. Homo- and heteroaggregation behaviors in the mixtures of Alexa-Fluor 633 dyed multi-walled carbon nanotubes (AFCNT) and kaolinite, one of the most common clay minerals were characterized using flow cytometry. Carbon nanotube is chosen to study as the typical 1-D ENM. Also, fluorescent labeled carbon nanotubes have been used in increasing number of studies for drug delivery systems\textsuperscript{116,117}. AFCNT, kaolinite and the mixture samples were tested under a range of solution pHs (3, 5, 9 and 10) and ionic strengths (0 mM, 5 mM and 100 mM NaCl). The relative extent of homo- and heteroaggregation was quantitatively determined through analyzing data from dot plots of fluorescence vs. light scattering intensities and relative frequency histograms. It is concluded that flow cytometry can evaluate the impact differing solution conditions had on the aggregation behavior in AFCNT-kaolinite mixtures.
5.2 Materials and methods

**Carbon nanotubes.** COOH-MWCNT with diameter of 15 ± 5 nm and length of 1 – 5 µm were obtained from Nanolab, Inc. (Newton, MA). As outlined by the manufacturer, the COOH-MWCNTs were synthesized by chemical Vapor deposition (VCD) using a NH₄OH/HSO₄ mixture. The COOH-MWCNT are 2-7% wt COOH by titration, as reported by the manufacturer. An aqueous stock suspension of COOH-MWCNT was prepared by adding 5 mg COOH-MWCNT to 100 mL ultrapure water and dispersed by a sonicating probe (Vibra-Cell, VCX 500, Sonics and Material, Newton, CT) at 50 W for 10 min, 75 for 10 min and 100 W for 10 min (total 30 min). An ice bath was used to prevent overheating.

**Kaolinite.** The kaolin clay (kaolinite) was purchased from EMD Chemicals (Gibbstown, NJ). The average diameter of the kaolinite was 0.5 µm as reported by the manufacturer. Before use, the kaolinite powder was poured in ultrapure water and the suspension was presettled for 24 hours. The top 80% of the supernatant was collected from the mixture and a 100 mg/L solution was prepared by diluting the concentrated solution. The particle size after settling was 599 nm ±27 nm measured by dynamic light scattering. Barnstead Epure water system (Dubuque, IA) was used to produce the deionized water used in the preparation of CNT and kaolinite stock solutions.

**Fluorescent labeling of COOH-MWCNT.** A water-soluble dye, Alexa Fluor 633 hydrazide (Thermo Fisher Scientific, catalog number A30634) was used to fluorescently label COOH-MWCNT. The dye has maximum light absorption and emission at 632 nm and 647 nm, respectively. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) was used to conjugate Alexa Fluor 633 to carboxyl groups on CNT. 0.01 ml 1000-ppm CNT stock suspension
immediately followed by 0.1 mg Alexa Fluor 633 and 3 mg EDC was added to 0.89 mL MES buffer solution (pH=5.5) in a 1.5-mL microtube. The suspension containing CNT, EDC and dye molecules were shaken on a platform shaker at ~400 rpm for 16 hrs in dark. the tube was then centrifuged at 15 k rpm for 90 min and supernatant was replaced with Millipore water from a Super-Q water purification system (Catalogue No. ZFSQ115P4) to remove excessive dye, EDC and MES. The washing process was repeated at least 3 times till the supernatant showed no detectable fluorescence intensity according to 3-D scanning results measured by the fluorescence meter (F-2500 fluorescence spectrophotometer (Hitachi, Tokyo, Japan). The 3-D scanning showed fluorescence intensity throughout excitation wavelength from 500 to 700 nm and emission wavelength from 400 to 800 nm. After the labeling process, the fluorescently labeled CNT (AFCNT) was ultra-sonicated for 10 min at 100 W to break any aggregates and then stored in dark at -20ºC. The AFCNT particle size in the stock solution was 512 nm ± 76 nm measured by dynamic light scattering. The concentration was 4.6 mg/L AFCNT determined by the light absorbance at 800 nm using a high-resolution dual beam UV-Vis spectrophotometer (UV-2550, Shimadzu Scientific Instruments, Columbia, MD). The Alexa Fluor 633 hydrazide dye does not have light absorbance at 800n.

Electrophoretic mobility. Surface charges of AFCNT and kaolinite were characterized by phase analysis light scattering (PALS) using a Zen 3600 Zetasizer Nano (Malvern, Worestershire, UK). Considering the long aspect ratio of AFCNT, electrophoretic mobility was reported instead of zeta potential. The concentration of AFCNT and kaolinite samples was 10 mg/L in solution conditions of 1) 5 mM NaCl and pH=3, 2) 5 mM NaCl and pH=5 3) 5 mM NaCl and pH=9 and 4) 100 mM NaCl and pH=9. All samples were measured using disposable capillary cells (DTS
Each sample was measured 3 times and 10 runs were performed each time.

**Sample preparation.** AFCNT controls (0.0625 ppm), kaolinite controls (0.625 ppm) and their mixtures were prepared by adding concentrated stock solutions to synthesized background solutions of 5 conditions: 1) Millipore water with no NaCl added and pH=10 as the non-aggregated condition, 2) 5 mM NaCl and pH=3, 3) 5 mM NaCl and pH=5 4) 5 mM NaCl and pH=9 and 5) 100 mM NaCl and pH=9. For the Millipore water and pH=10 condition, the sample was measured immediately after 5 min of 100 W ultra-sonication to avoid aggregation. The background solution was prepared using Millipore water and reagent-grade NaCl, HCl, NaOH with a purity of 90% or above were used for ionic strength and pH adjustments. To minimize the noises during flow cytometer measurements, the background solutions were filtered with 1 µm PPVP membrane filter. AFCNT control, kaolinite control and the mixture samples under the 3 solution conditions were prepared in 1.5 mL-microtubes and shake for 1 hr on a platform shaker at about 400 rpm. Number of events in the filtered background solutions obtained by the flow cytometer was lower than 1% of any of the AFCNT, kaolinite or mixture samples. Each sample was then well shaken and poured in a 5-mL polystyrene round-bottom tube (Corning Scientific, Corning, NY, catalogue number 352058) for flow cytometry measurements.

**Measurement of aggregation by flow cytometry.** A FACS Canto II flow cytometer (BD biosciences, San Jose, CA) was used to simultaneously measure light scattering and fluorescence intensity of each particle in the prepared samples. The excitation laser 633 (red) which collected signals from 650 nm to 670 nm was chosen. The channel is labeled ‘APC’, which is the intended dyes for this laser. For “APC-A” (A stands for signal area) signals, the instrument considered particles as noises because of their small size (diameter <1000 nm in most solution conditions)
and thus made the measurements distribute symmetrically around zero through automatic correction. Therefore, “APC-H” (H stands for signal height) was applied instead of the more commonly used area signals (APC-A) to represent the fluorescence intensity caused by Alexa Fluor 633. In selecting signals that can reflect particle size information, histograms of particle number vs. area signals of FSC (FSC-A) and SSC (SSC-A) were plotted using standard polystyrene with sizes of 50 nm, 100 nm and 200 nm (Figure 5-1). Results showed that only histograms for SSC-A had distinctive distribution among the three types of polystyrene particles. 

According to Mie theory, the larger the refractive index, the more possible the particle is detectable. Refractive index of polystyrene (1.59) is close to that of kaolinite (1.56) and smaller than that of CNT (2.59). Therefore, SSC-A can reflect size of the AFCNT and kaolinite particles.

Based on the above analyses, SSC-A and APC-H were determined to reflect size and fluorescence intensity of the particles, respectively. An SSC threshold of 200 was set. The PMT voltage for SSC and APC were both 500V. To narrow the differences of the injected volume
from one sample to another, fixed measuring time of 20 s and the low flowrate of 10 µL/min were applied for all measurements. 2 parallel experiments were made for each sample. Since the particle size of kaolinite and AFCNT is lower than the resolution of the cytometer (2 µm), it is possible that swarm detection occurs where a count is generated by multiple particles rather than a single particle\(^{135}\). To investigate whether swarm detection occurred in this study, linear trend lines of event number as a function of nonlabeled-CNT and kaolinite concentrations (ranging from 0.0078 to 0.25 mg/L) with equations and \(R^2\) values were analyzed. These samples were prepared in Millipore water with unadjusted pH (~6) and measured under the same cytometer settings as is mentioned above. Swarm detection can be considered negligible when \(R^2 > 0.995\).

**Standard fluorescent particles.** Before the AFCNT, kaolinite and mixture samples were measured, performance of the cytometer was examined using 0.2 µm Fluoro-Max Dyed Green Aqueous Fluorescent Particles (G200) (catalogue number G200, Thermal fisher Scientific). The particles were spherical and made of polystyrene (Refractive Index =1.59). 0.0625 mg/L G200, 0.625 mg/L kaolinite and their mixture samples were tested in 5 mM NaCl at pH 9 and 3. The signal heights collected by the ‘FITC’ channel (‘FITC-H’) was chosen to determine fluorescence intensities of these green particles. Other cytometer settings were the same as is mentioned above.

**Data analysis.** For each sample, three graphs were obtained: 1) dot plots of APC-H Vs. SSC-A, 2) relative frequency histograms of SSC-A indicating size distribution and 3) relative frequency histograms APC-H\(^3\)/SSC-A indicating the fluorescence over surface area assuming fluorescence intensity is proportional to the square of particle diameter (d) and light scattering is proportional to \(d^6\). The decrease of APC-H\(^3\)/SSC-A indicates more kaolinite proportion in one
particle/aggregate. For example, a decrease in APC-H^3/SSC-A in the mixture from that of the CNT control implies CNT-kaolinite heteroaggregation. Dot plots and gating analysis (determining areas of specific populations on the plots) were performed using FCS Express 6 Plus (De Novo Software, Los Angeles, CA).Histograms were plotted using Python 2.7.

5.3 Results and discussions

5.3.1 Detection of swarm effect

Figure 5-2 event number of CNT and kaolinite as a function of their concentrations.

Curves for event number of CNT and kaolinite as a function of their concentrations are shown in Figure 5-2. R^2 values for CNT and kaolinite are 0.9972 and 0.9988, respectively. The linear
trends indicate that swarm detection is negligible for both CNT and kaolinite particles: the flow cytometer is capable of detecting single particles or aggregates in this study.

5.3.2 Performance of the flow cytometer examined by spherical standard fluorescent particles (G200).

Figure 5-3 Dot plots of G200 and mixture at 5 mM NaCl, pH=9 (A), (B)) and 5 mM NaCl, pH=3 (E), (F). (C), (D): stacked histogram of FITC-H and SSC-A in 5 mM NaCl and pH=9.
Figure 5-3 presents the dot plots and histograms of G200 and the G200-kaolinite mixtures in 5 mM NaCl at pH 3 and 9. In Figure 5-3 (A), events for the 200 nm G200 were distributed in a narrow SSC-A range. A small population at the upper right area indicates the G200 homoaggregation. In the mixture (Figure 5-3 (B)), the lower band presents kaolinite particles which has large size distribution and little fluorescence. At pH 9, histograms of the mixture for FITC-H and SSC-A (blue curves in Figure 5-3 (C) and (D)) presents close to the sum of histograms for G200 and kaolinite controls (red and black curves in Figure 5-3 (C), suggesting little heteroaggregation. This is expected since the high pH can prevent particle interactions due to electrostatic repulsion. As the pH decreased to 3, the G200 homoaggregation area was larger (blue gate in Figure 5-3 (E)). For the mixture, a band-shaped population (red gate in Figure 5-3 (F)) at the right of the primary G200 gate was shown as the heteroaggregates. In summary, the above results presented in Figure 5-3 was consistent with the size, fluorescence intensity and the aggregation status expected for the standard G200 and kaolinite particles. Thus, the flow cytometer is considered applicable in the following aggregation analyses for AFCNT, kaolinite and their mixture samples.
5.3.3 Fluorescent labelling of CNT

Figure 5-4 3D scanning fluorescence spectrum of Alexa Fluor 633 labeled COOH-MWCNT (AFCNT). The vertical axis stands for fluorescence intensity. EM and EX axis stands for emission and excitation wavelengths, respectively.

In Figure 5-4, 3D scanning fluorescence spectrum of Alexa Fluor 633 labeled COOH-MWCNT (AFCNT) was presented. The AFCNT particles showed significant fluorescence intensity up to 17.38 a.u. in the presented EM and EX ranges, where no fluorescence was detected for the non-labeled CNT. It can also be seen that there is significant fluorescence (from 6 a.u. to 15.5 a.u) at
the APC laser excitation wavelength of 633 nm and the collecting emission wavelength from 650 to 670 nm. This implies that the fluorescence of AFCNT is detectable under the APC channel.

5.3.4 Electrophoretic mobilities

![Electrophoretic mobilities of AFCNT and kaolinite](image)

Figure 5-5 Electrophoretic mobilities of AFCNT and kaolinite in 5 mM NaCl, pH=3, 5 and 9 and in 100 mM NaCl, pH=9.

Electrophoretic mobilities (EPMs) of AFCNT and kaolinite under the target solution conditions were shown in Figure 5-5. Both AFCNT and kaolinite had negative surface charge throughout the pH range. The two materials have similar mobilities except at pH=9, where the mobility of AFCNT (-2.8 µm cm/Vs) is much lower than that of kaolinite (-1.7 µm cm/Vs). It is expected that the mobility tends to be more negative as pH increases. As the ionic strength increased from 5 mM to 100 mM NaCl at pH 9, the EPMs became less negative: -1.8 µm cm/Vs for AFCNT and -
1.3 µm cm/Vs for kaolinite. This is consistent with double layer theory that the electrical double layers of the particles get shrunk as ionic strength increases.

5.3.5 In Millipore water and pH=10

![Figures 5-6](image)

Figure 5-6 Dot plots of fluorescence (APC-H) Vs. size (SSC-A) signals of AFCNT control, kaolinite control, AFCNT and kaolinite mixture and the overlay plot of the three samples in millipore water at pH=10.

In Millipore water and pH=10, AFCNT and kaolinite controls and the mixture serve as the non-aggregated controls for samples in other conditions. Their dot plots obtained via flow cytometer were shown in Figure 5-6. The dot plot of AFCNT control showed two branches. The upper branch had a linear trend under the log-scale axis, which is expected because the fluorescence is
theoretically in proportional to the surface area. The lower branch could be generated by not well-labeled AFCNT and the noises. This is the first study showing dots of submicron, long-aspect ratio particles on fluorescence vs. light scattering plots. For kaolinite control, the APC-H intensities were all under 100 a.u. and the SSC-A ranges widely for $10^2$ to over $10^5$. This is consistent with the little fluorescence and large size distribution of kaolinite particles. In the mixture, the bottom edge of the dots shifted upward compared with that of the kaolinite control. This may because of either heteroaggregation or the auto-fluorescent impurities in the mixture that covered at the surface of kaolinite particles. The auto-fluorescent impurities such as dye aggregates was reported not efficient to be washed off by ultracentrifugation\textsuperscript{136}. In addition, the SSC-A histograms of the control sum and the mixture was almost overlapped in this condition (data not shown), indicating little heteroaggregation. Therefore, the fluorescent impurities were considered the reason for the upward shifting of dots in the mixture. These dot plots verified that the flow cytometer can resolve submicron particles can show reasonable fluorescence intensities. In the following discussion, the effect of solution condition on the aggregation in AFCNT and
kaolinite mixture was analyzed by comparing the graphs of each sample with that measured in this non-aggregated condition.

5.3.6 Statistics of the particles in various solution conditions

Figure 5-7 SSC-A mean values and number of events of AFCNT, kaolinite, mixture and control sum in various solution conditions.

To generalize the trend of aggregation in various solution conditions, the number of events and SSC-A mean for AFCNT, kaolinite, mixture and the sum of AFCNT and kaolinite control were shown in Figure 5-7. A larger SSC-A mean and smaller count indicate stronger aggregation. Figure 5-7 clearly shows a trend that the effect of solution conditions on the aggregation extent is in the order of 5 mM NaCl and pH=3>100 mM and pH=9>5 mM NaCl and pH=5>5 mM NaCl and pH=9, which is consistent with the electrophoretic mobility results. For example, at pH=3 the number of events decreased by 94%, 71%, 82% and 78% for AFCNT control, kaolinite control, mixture and control sum, respectively compared with that in the non-aggregated condition. This indicates that significant aggregation occurred at pH=3. For other conditions, the
percentages were much fewer: 2%-31%. The trend in SSC-A mean results were consistent with that of the number of events.

It is determined from these statistical results that flow cytometer can be applied as a fast and accurate tool in providing information to predict the stability of ENM and ENM-NOC systems. In addition to stability, surface chemistry of the free particles and aggregates can also significantly affect their mobility in subsurface porous media and finally alter their human exposure pathways. Thus, it is necessary to study the constituents of aggregates and their proportions. In the following sections, the presence of varies types of aggregates, namely AFCNT homoaggregates, kaolinite homoaggregates and AFCNT-kaolinite heteroaggregates was investigated in each solution condition.

5.3.7 In 5 mM NaCl and pH=3

Although the number of events in Figure 5-7 indicated strong AFCNT homoaggregation in 5 mM NaCl and pH=3, the dot plot of AFCNT did not show significant increase in fluorescence intensity (Figure 5-8). This is attributed to the aggregation-caused quenching (ACQ) during the CNT homoaggregation. The ACQ issue were frequently found in the development of luminescent materials\(^{118}\).

For kaolinite particles, it is hard to determine the presence of homoaggregates from dot plots in pH=3 and non-aggregated conditions due to the wide size distribution of kaolinite. Therefore, the overlay relative frequency histograms of SSC-A were plotted to compare size distribution of kaolinite control samples (Figure 5-9 (A)). It can be seen in Figure 5-9 (A) that particles with SSC-A between \(10^2\) and \(3 \times 10^3\) at pH=3 is less than that in non-aggregated condition while the fraction of particles in larger SSC-A range (\(>3 \times 10^3\)) was higher. The area of difference was 9.4%
as is pointed by the red arrow, indicating that particle sizes of 9.4% of the total kaolinite population at pH=3 was increased due to homoaggregation.

In the mixture, the dot plots presented significant increase in APC-H from that in AFCNT control (Figure 5-8). In fact, the total APC-H increased by 116% (data not shown) compared with that of the control sum. This implies that kaolinite reduced the AFCNT homoaggregation and thus recovered the fluorescence intensity via heteroaggregation. Apart from dot plots, the overlay relative frequency histograms of APC-H^3/SSC-A in pH=3 and the non-aggregated conditions were plotted. A smaller APC-H^3/SSC-A indicates larger kaolinite proportion in one aggregate. In Figure 5-9 (C), the histogram of the mixture at pH=3 significantly shifted left from that of the non-aggregated mixture: the area of difference is as high as 21%, implying significant kaolinite-related aggregation (kaolinite homoaggregation and/or heteroaggregation). To confirm the presence of heteroaggregation, the relative frequency histograms of APC-H^3/SSC-A for the control sum with the overlay of the non-aggregated control should be plotted to exclude homoaggregation. However, in this condition of pH 3, AFCNT homoaggregation cannot be analyzed by the APC-H^3/SSC-A histogram because of the fluorescence quenching. Thus, the overlay histogram of kaolinite controls was plotted (Figure 5-9 (B)) instead of the control sum. It is demonstrated in Figure 5-9 (B) that the kaolinite homoaggregation was not significant since the area of difference is only 5.4%. Therefore, the shifting in the mixture histogram was mainly attributed to heteroaggregation. In fact the impact AFCNT homoaggregation was not included although its presence was determined thorough the dot plot and event number statistics. The influence of kaolinite on the APC-H^3/SSC-A was larger than shown in Figure 5-9 (C) considering the AFCNT homoaggregation. To summarize, in this extremely low pH condition, significant heteroaggregation occurred and kaolinite took up a large proportion in each single
heteroaggregate. This is expected and consistent with the speculation in our previous studies that at pH=3, the edge of kaolinite platelets had positive surface charge since its edge point of zero charge is larger than 3. At the same time, the phases of the particles are negative and thus tend to form large aggregates via phase-edge attachment. In addition, AF-CNT may bridge the kaolinite particles by attaching to the edge of the platelets. These two effects resulted in large proportion
of kaolinite in single heteroaggregates. Similar speculation was proposed on the aggregation of kaolin clays and TiO$_2$ nanoparticles$^{119}$. 

Overall, in 5 mM NaCl and pH=3, the presence of homo-and heteroaggregation can be determined by flow cytometry. The limitation is that it cannot provide information of the configuration in each aggregate (e.g. primary or secondary aggregation).

Figure 5-8 Dot plots of fluorescence (APC-H) Vs. size (SSC-A) signals of AFCNT control, kaolinite control, AFCNT and kaolinite mixture and the overlay plot of the three samples in 5 mM NaCl at pH=3.
Figure 5-9 Overlay relative frequency histograms in 5 mM NaCl, pH=3 and in millipore water, pH=10 of (A) SSC-A for kaolinite controls (B) APC-H\(^3\)/SSC-A for kaolinite controls (C) APC-H\(^3\)/SSC-A for mixtures. The red arrows and labels indicate the percentage of area of difference (not overlaid parts).
5.3.8 In 100 mM NaCl and pH=9

In high ionic strength (100 mM NaCl) and high pH 9 condition, the dot plots for the mixture and AFCNT control with overlays of non-aggregated samples were shown in Figure 5-10 (A) and (B). Gate 1-4 were created to study the distributions in the upper and lower branches of the plots in the mixtures and AFCNT controls, respectively. Percentage of difference in a specific gate was defined as follows:

\[
\frac{\text{counts within the gate}}{\text{total counts}} \times 100% 
\]

A positive percentage of difference indicates that the sample has more fraction of particles located in the gate than that in the non-aggregated condition. From Figure 5-10 (C), the percentage of difference in Gate 1 is negative (3%) and equally positive in Gate 2 for the mixtures. This may be attributed to the attachment of kaolinite on AFCNT particles in 100 mM NaCl and thus reduced the fluorescence intensity. On the contrary, the fraction of AFCNT in gate 3 is 15% more than that in the non-aggregated control sample, suggesting that as the ionic
strength increased to 100 mM NaCl, part of the AFCNT moved from the lower branch (Gate 4) to the upper one (Gate 3). This reveals significant AFCNT homoaggregation.

Figure 5-10 Overlay dot plots of APC-H Vs. SSC-A in 100 mM NaCl, pH=9 and in Millipore water of (A) mixtures and (B) AFCNT controls. (C) Differences of the population percentage in each gate drawn in (A) and (B) between the two solution conditions.
In terms of heteroaggregation, overlay relative frequency histograms of APC-H\(^3\)/SSC-A for control sums and mixtures were plotted in Figure 5-11 to investigate the presence and extent of heteroaggregation.

Figure 5-11 Overlay relative frequency histograms of APC-H\(^3\)/SSC-A for CNT + kaolinite control sum (A) and mixtures(B). Solution conditions: 1) 100 mM NaCl, pH=9 and 2) Millipore water, pH=10.

In Figure 6-11 (A), histograms of the control sum in 100 mM NaCl has more area at the large APC-H\(^3\)/SSC-A range compared with that in Millipore water and the area of difference is 9.6%. This is caused by the AFCNT homoaggregation in this high ionic strength conditions, which is consistent with the gating analysis. However, it is reported in Figure 6-11 (B) that the mixture shifted left from its non-aggregated control and the area of difference is 16.3%. This implies that although the AFCNT homoaggregation increased the fluorescence over size signal in the absence of kaolinite, the kaolinite can offset the change and further reduced the fluorescence and
increased particle size. Therefore, it is determined that heteroaggregation occurred under the high ionic strength condition.

5.3.9 In 5 mM NaCl, pH=5 and pH=9

As the solution pH increases to medium (pH 5) and high range (pH 9), it can be seen from the overlay dot plots of mixtures (Figure 5-12) that almost all dots in both conditions overlapped with that in the non-aggregated condition. Under each condition, no distinct populations were shown in the AFCNT control dot plot from that in the corresponding mixture (Figure 5-13). To further investigate the extent of homo- or heteroaggregation, overlay relative frequency histograms of SSC-A and APC-H₃/SSC-A were plotted for the control and mixture samples in Figure 5-14. Similar to the dot plots results, the histogram of each sample totally overlapped with its corresponding controls (SSC-A histogram) or mixture (APC-H₃/SSC-A histogram) in the Millipore water: the areas of difference were up to 5.4%. These plots indicate that there is no significant aggregation of CNT and kaolinite mixture in conditions of low salinity and medium to high pHs. Nevertheless, the number of events and mean SSC-A statistics in Figure 5-7 implies that aggregation occurred in these conditions. Thus, it can be inferred that aggregations happened under these conditions but were not significant. The detailed relative extent of homo- and
heteroaggregation compared with other conditions was defined via the percentage of impact
calculation, which is discussed in section 5.3.10.

Figure 5-12 Overlay dot plots of fluorescence (APC-H) Vs. size (SSC-A) signals for mixtures.
Solution conditions: (A) 5 mM NaCl, pH=5 and Millipore water, pH=10; (B) 5 mM NaCl, pH=9
and Millipore water, pH=10.

Figure 5-13 Overlay dot plots of fluorescence (APC-H) Vs. size (SSC-A) signals for kaolinite,
AFCNT and mixture samples in (A) 5 mM NaCl, pH=5 and (B) 5 mM NaCl, pH=9.
Figure 5.14 Overlay relative frequency histograms in conditions of 1) Millipore water pH=10, 2) 5 mM NaCl, pH=5 and 3)5 mM NaCl, pH=9. (A) SSC-A histogram of AFCNT control; (B) SSC-A histogram of kaolinite control; (C) APC-H^3/SSC-A histogram of mixture samples. Areas of difference were all under 6% (values not shown).
5.3.10 Impact of solution condition on the homo- and heteroaggregation

To quantify the impact differing pHs and ionic strength had on the aggregation of controls and mixtures under the solutions tested throughout this study, the percent impact on aggregation was calculated using the following methods in Table 5-1.

Table 5-1 Calculation equations of the percent impact on AFCNT homoaggregation, kaolinite homoaggregation and AFCNT-kaolinite heteroaggregation

<table>
<thead>
<tr>
<th>Percent impact on aggregation</th>
<th>Calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>% impact on AFCNT homoaggregation</td>
<td>Area of difference on SSC-A relative frequency histograms between AFCNT controls in the studied solution condition and that in the non-aggregated condition (Millipore water, pH=10)</td>
</tr>
<tr>
<td>% impact on kaolinite homoaggregation</td>
<td>Area of difference in SSC-A relative frequency histograms of kaolinite controls in the studied solution condition and that in the non-aggregated condition</td>
</tr>
<tr>
<td>% impact on AFCNT-kaolinite heteroaggregation</td>
<td>Area of difference in APC-H^3/SSC-A relative frequency histograms of mixtures in the studied solution condition and that in the non-aggregated condition + area of difference of the control sums (kaolinite controls for pH 3) in the two solutions</td>
</tr>
</tbody>
</table>
Figure 5-15 Percentage impact of solution conditions on aggregation. At pH=3, the dashed marker indicates that the actual value is higher. % impact on AFCNT homoaggregation at pH=3 is not available due to the fluorescence quenching.

The calculated percentage of impact on aggregation is shown in Figure 5-15. It should be noticed that the % impact can only be compared among different solution conditions within one type of aggregation. At pH 3, the result for AFCNT homoaggregation is not available due to the ACQ effect. As is discussed in section 3.5, the % impact on heteroaggregation at pH 3 (dashed marker) did not exclude the impact of AFCNT homoaggregation, though dot plots implied significant homoaggregation. Thus, the theoretical impact on heteroaggregation at pH 3 should be higher than the value shown on the plot.

Except for the fact that the impact of AFCNT homoaggregation at 5 mm NaCl, pH 3 is not available, Figure 5-15 demonstrated that there were significant aggregations under 100 mM NaCl and pH=9 but little aggregation was found at 5 mM NaCl, pH 5 and 9. % impacts of various solution conditions on heteroaggregation and kaolinite homoaggregation follow the order of 5 mM NaCl, pH 3 > 100 mM NaCl, pH=9 > 5 mM NaCl, pH 5 > 1 mM NaCl, pH 9. This is
consistent with the SSC-A and number of events statistics (Figure 5-7) and electrophoretic mobility (Figure 5-5) results. The results indicate that solution pH and ionic strength can greatly alter the aggregation of the AFCNT: the extremely low pH or high salinity can greatly induce the AFCNT homoaggregation and their heteroaggregation with kaolinite. When released in natural water of similar solution chemistry, changes may occur in the particle size and surface properties of AFCNT and thus its stability and bioavailability in surface water could be reduced. When in low ionic strength and medium to high pHs, AFCNT are highly likely to be in the form of free particles and thus directly exposed to the living organisms in aqueous phase.

In summary, this is the first time that the relative extents of homo- and heteroaggregations of ENM with natural occurring colloids are determined using flow cytometry. The impact of solution conditions on the extent of aggregation obtained from the flow cytometry data was consistent with the hypothesis that aggregations become stronger as pH decreases and ionic strength increases. Therefore, flow cytometry can be used as an efficient tool for the determination of aggregation status regarding ENMs in natural water environments.

### 5.4 Conclusions

This study investigated the applicability of flow cytometry as a tool in determining the aqueous aggregation of fluorescently labeled multi-walled carbon nanotubes (AFCNT) under natural pH and ionic strength conditions in the presence of natural clay colloids (kaolinite). By systematically analyzing the flow cytometry data of AFCNT, kaolinite controls and their mixtures in various pHs and ionic strengths, we draw the conclusions as follows:

1) The BD FACS Canto II flow cytometer used in this study can resolve carbon nanotubes and kaolinite colloids, even though their size (mostly <1 µm) are much smaller than that of the
biological cells. Noise signals from the colloidal suspensions can be sufficiently removed by filtering the background solutions.

2) Flow cytometer can detect the presence and extent of AFCNT homoaggregation and its heteroaggregation with kaolinite. The aggregation of AFCNT is largely affected by natural occurring colloids, solution pH and ionic strength conditions. The differing aggregation status suggest changes on AFCNT size and surface physical-chemical properties, which can greatly affect its bioavailability.

3) Flow cytometry is a promising and efficient tool in predicting the aggregation and stability of the engineered nanoparticles in natural water environments. It is limited in figuring out the configurations of aggregates especially for the non-spherical and polydispersed CNT and kaolinite suspensions.
Chapter 6

Co-transport of Carboxyl-functionalized Multi-walled Carbon Nanotubes and Kaolinite in Saturated Porous Media

Abstract

When released into the environment, the transport of engineered nanomaterials (ENMs) will be affected by various environmental factors including the presence of naturally occurring colloids (NOCs). However, the role of NOCs in ENM transport in natural aquatic environments remains a major knowledge gap, preventing accurate prediction of ENM concentrations in aquatic systems. In this study the effect of a model NOC, kaolinite, on the transport of carboxylated multi-walled carbon nanotubes (COOH-MWCNTs) through saturated porous media was investigated under a wide range of solution conditions. Results show that the presence of kaolinite has important impact on the transport of COOH-MWCNTs through the formation of heteroaggregates, as well as competition for and blockage of attachment sites on mineral surfaces even under conditions where bulk physicochemical properties of the COOH-MWCNTs and kaolinite suggest repulsive interactions. The formation and structure of the heteroaggregates depend strongly on pH and ionic strength. Primary heteroaggregates form at high ionic strength and high pH, while low pH leads to the formation of very large, secondary heteroaggregates due to the charge heterogeneity.

1 Contents of this chapter was submitted to Environmental Science and Technology.
of kaolinite. The transport of primary heteroaggregates is dominated by the transport properties of kaolinite, while secondary heteroaggregates are removed by straining due to their very large size. Under conditions when heteraggregation is minimal, kaolinite competes for and blocks attachment sites, greatly enhancing COOH-MWCNT mobility in the porous media. These findings suggest that interactions with NOCs are important processes affecting ENM environmental fate and transport.

6.1 Introduction

The unique properties of engineered nanomaterials (ENMs) and the novel functions they enable have motivated significant investment in research and development of nanotechnology. At the same time, research on environmental risk of ENMs is greatly outpaced by the development of nanomaterials and nanotechnologies. Reported evidence on ENMs toxicity\textsuperscript{120} and their release to the environment\textsuperscript{111,121} indicates that ENMs can potentially impose adverse effect on ecosystem and human health through diverse exposure pathways\textsuperscript{122,142-144}.

The unique mechanical, optical and electrical properties of carbon nanotubes (CNTs) have led to strong interests in their applications in many fields, including composite materials, biotechnology and water treatment, etc\textsuperscript{123-125}. In 2015, the global market of CNT was reported to be $2.26 billion\textsuperscript{124}. However, recent studies also found biological toxicity of CNTs\textsuperscript{3,126-129}, which depends on their size\textsuperscript{128}, chemical composition and shape\textsuperscript{110}, and hence may be affected by solution chemistry and naturally occurring colloids (NOCs).

Studies on transport of various ENMs have shown that ENM size and shape\textsuperscript{130}, pore velocity\textsuperscript{131}, ENM concentration and solution chemistry\textsuperscript{132} are important factors affecting ENM mobility in porous media. The influence of ionic strength on CNT transport has been extensively studied,
and shown in general agreement with the DLVO theory, i.e., CNT mobility increases with decreasing ionic strength.\textsuperscript{61,133} Due to its large aspect ratio, CNT transport studies have shown evidences of straining as an important removal mechanism.\textsuperscript{10,61,130} However, transport of CNTs alone without the presence of NOCs does not represent realistic exposure conditions.

When released into natural aquatic environments, nanoparticles can aggregate among themselves (homoaggregation) or with other particles (heteroaggregation). Because NOCs are much more abundant than ENMs, heteroaggregation between ENMs and NOCs are highly likely to occur. This can lead to important changes in particle properties (e.g., size, shape and surface chemistry), and consequently transport behaviors of the ENMs. Few studies have investigated the interactions between ENMs and NOCs and their effect on ENM fate and transport in the environment.\textsuperscript{75,76} A number of studies have invested ENM-NOC interactions. Electrostatic attraction was reported to be the main mechanism of heteroaggregation. This occurred at pH between the point of zero charges (PZCs) of the NOC and the ENM, when their surfaces were oppositely charged.\textsuperscript{39-43} Hydrogen bond formation\textsuperscript{9,47} and Louis acid-base interaction between the oxygen functional groups of the ENM and hydroxyl groups of the NOC\textsuperscript{170} have also been suggested. Heteroaggregation rate was greatly influenced by solution chemistry, and ENM can form various structures with NOC depending on the solution chemistry and the ENM/NOC concentration ratio.\textsuperscript{54,134,168} Very few studies, however, have investigated the co-transport of ENMs and NOCs. Cai et al. reported that the transport of TiO$_2$ nanoparticles through saturated packed columns of quartz sand were retarded in the presence of kaolinite due to straining of TiO$_2$-kaolinite heteroaggregates, but was enhanced in the presence of betonite through betonite-facilitated transport.\textsuperscript{75,76} Another study reported that goethite enhanced mobility of
hydroxyapatite nanoparticles at pH 10.5 by competing for adsorption sites, but increased hydroxyapatite retention at pH 7.5 when homo- and hetero-aggregation led to straining.\textsuperscript{77}

In this study, we investigate the transport of carboxylated multi-walled carbon nanotubes (COOH-MWCNT) in saturated porous media in the presence of a common clay, kaolinite. The 1-D structure and very large aspect ratio of CNTs make them an interesting nanomaterial to study transport properties as much of the knowledge on ENM transport has been built upon spherical particles. Furthermore, the negative charges on the surface of COOH-MWCNTs stabilize them in the aqueous phase\textsuperscript{135}, potentially making them highly mobile in natural aqueous environments. Considering the abundance of NOCs and their role in the transport of contaminants\textsuperscript{75-77,136}, understanding the role of NOCs in the transport of highly mobile ENMs such as COOH-MWCNTs is very important in predicting the environmental fate and transport of these ENMs. Kaolinite was selected as the model NOC because it is ubiquitous in the aqueous environment, and has unique charge heterogeneity that leads to interactions with both positively and negatively charged particles.

To our knowledge, this is the first study on the co-transport behavior of CNTs and kaolinite. Important insights were obtained on the different mechanisms through which kaolinite affects COOH-MWCNT transport through saturated porous media. The specific role of heteroaggregation between COOH-MWCNTs and kaolinite in their respective transport was examined.

6.2 Materials and methods

**Carbon nanotubes.** Carboxyl-functionalized MWCNT powder (2-7 wt% -COOH) was purchased from NanoLab Inc. (Waltham, MA). The CNTs have an average diameter of 15 ± 5
nm and length of 1-5 µm. In making the CNT stock suspension, a Vibra-Cell VCX 500 sonicating probe was applied to a 500-mL beaker containing 200 mg CNT powder and 200 mL deionized water. The sonication was processed in an ice bath at 100 W for 30 min. The CNT suspension was kept in a 500-mL glass bottle covered with aluminum foil at room temperature. Before every use, the stock suspension was sonicated in a Vibra-Cell cup horn for 5 min at 100 W.

**Kaolinite.** Kaolinite powder was purchased from EMD chemicals (Gibbstown, NJ). The kaolinite stock suspension was prepared by adding 50.3 g kaolinite powder (EMD chemicals, Gibbstown, NJ) into 300 mL DI water followed by sonication at 100 W for 30 min in an ice bath using a Vibra-Cell VCX 500 sonicating probe. The ultrasonicated suspension was then presettled for 45 hours, and the top 85% of the supernatant was collected as kaolinite stock suspension. The suspension was stored in an amber glass bottle at room temperature. The stock suspension was sonicated in a Vibra-Cell cup horn for 5 min at 100 W before every use.

**Porous media (quartz sand).** Quartz sand obtained from Sigma-Aldrich Co. was sieved to a size range of 250-300 µm, and soaked in 1.5 M HNO₃ solution for 24 h followed by another 24 h in 1 M NaOH. The sand was then rinsed with deionized water till the rinsing water reached neutral pH, and oven-dried at 100 °C for 24 h.

**Electrophoretic mobility.** Electrophoretic mobility of the CNT, kaolinite, and quartz sand used in column experiments was measured by phase analysis light scattering (PALS) using a Zen 3600 Zetasizer Nano (Malvern, Worcestershire, UK). The concentration of CNT and kaolinite samples was 10 mg/L. Because the size of the quartz sand (250-300 µm) is much larger than the upper size limit of the instrument (10 µm), the fine fraction of the sand (159 ± 27.4 nm in
diameter) was used for electrophoretic mobility measurement. The fine fraction of sand was obtained by soaking the treated sand in DI water, letting stand for 30 min, and collecting the supernatant. Measurements were made at pH 3, 5 and 9 with NaCl concentration varying from 1 to 100 mM as the background electrolyte. Each sample was measured 3 times with 10 runs in each measurement.

**Column experiments.** Column experiments were performed using suspensions of CNT, kaolinite, and kaolinite-CNT mixture under various solution conditions. The concentrations of CNT and kaolinite used were 90 mg/L and 18,000 mg/L, respectively, i.e., a kaolinite to CNT concentration ratio of 200. This was chosen to represent an environmentally relevant kaolinite to CNT ratio. Influent suspensions were prepared immediately before the experiments, and mixed for 1h at ~ 400 rpm before injection.

A schematic of the column setup is shown in Figure 6-S1. A glass column (Omnifit, Cambridge, UK) of 1.5 cm in diameter was wet packed with 14.5 g clean sands, resulting in a pore volume ranging from 2.13 to 2.41 cm³. In each experiment, 10 pore-volume of DI water and 10 pore-volume of the background solution were sequentially passed through the column using a syringe pump at a flow rate of 2 mL/min. Then, the influent suspension was injected into the column though a 0.28-ml injection loop controlled by two four-way valves (Figure 6-S1) at a flow rate of 0.84 mL/min (Darcy velocity of 0.47 cm/min). Three pore-volume of the background solution was then injected at the same flow rate. Two parallel column experiments were performed for each testing condition.

The column effluent was monitored for respective analytes in each experiment. For the CNT control and NaNO₃ tracer experiments, effluent concentration was determined by real time light
absorbance measurement at 800 nm and 302 nm, respectively, using a UV-Vis spectrophotometer (Shimadzu, UV 2550) equipped with a sub-micro flow-through cell (Precision Cells, Type 513M). Measurement was taken every 9 seconds for a duration of 15 min. For the kaolinite control and kaolinite-CNT mixture experiments, effluent samples were collected continuously in 1.5-mL microtubes using a fraction collector (Pharmacia Frac-100) at 0.32 min intervals. Effluent samples from the kaolinite control experiments were diluted 2.5 times using the corresponding background solution, and analyzed for light absorbance at 800 nm in a 0.7 mL microcuvette (30ES10, NSG Precision Cells, Inc, NY).

Composition of the effluent samples from the CNT-kaolinite mixture experiments was analyzed to determine the concentrations of free CNT, attached CNT and kaolinite. The samples were first centrifuged, and supernatant collected to determine the concentration of free CNTs by measuring light absorbance at 800 nm. The sample was then washed with the background solution 3 times by centrifugation to remove all free CNTs. The remaining materials were re-suspended in deionized water, and ultra-sonicated at 100 W for 1-min to detach CNTs from kaolinite. Light absorbance at 800 nm was measured, which represents the contribution from both attached CNTs and kaolinite. The detached CNT was then removed by centrifugation to determine the total concentration of kaolinite, from which the concentration of attached CNTs can be calculated. Control experiments were run to confirm that the washing process by centrifugation effectively removes free CNT from the mixture sample. Details of the experimental protocol are described in the Supporting Information. Samples were analyzed in duplicates.

Particle size distribution of each influent suspension before and after the 1 hr mixing was determined by dynamic light scattering using a Zen 3600 Zetasizer Nano (Malvern, Worcestershire, UK). Samples were diluted 20 times using the corresponding background
solutions before measurement. Triplicate measurements were performed for samples taken before mixing, each measurement consisting of 20 runs. For samples taken immediately after mixing, particle size was monitored continuously with measurements taken every 10 s for 20 min. The aggregation rate was calculated as \( \frac{d(d_p)}{dt} \) to examine the stability of these influent suspensions. This provides information on the aggregation state of the particles during the 15 min column experiments.

**DLVO calculation.** In order to compare experimental results of heteroaggregation and co-transport with theoretical expectations, DLVO interaction energy under various solution conditions were calculated. Details of the calculation and the results were shown in the Supporting Information.

**Calculation of attachment efficiency.** Particle transport in the porous media was described using a 1-D advection-dispersion model with deposition (Eq. 6.1).

\[
\frac{\partial C}{\partial t} + k_{\text{att}}C = D_H \frac{\partial^2 C}{\partial x^2} - v_p \frac{\partial C}{\partial x}
\]  

Eq. 6.1

Here, \( C \) is the aqueous phase concentration, \( t \) is time, \( D_H \) is hydrodynamic dispersion coefficient, \( v_p \) is pore velocity, and \( x \) is the distance. Deposition is modeled as a first order reaction with a first order attachment rate constant of \( k_{\text{att}} \), and site blocking, ripening and detachment are assumed negligible. Based on the classical filtration theory, \( k_{\text{att}} \) can be calculated by Eq. 6.2 assuming spherical particles.

\[
k_{\text{att}} = \frac{3\alpha \eta v_p (1 - \theta_n)}{2d_c}
\]  

Eq. 6.2

130
Here, $d_c$ is the diameter of the collector; $\alpha$ is particle-collector attachment efficiency, and $\eta_0$ is the single collector efficiency determined using the correlation equation proposed by Tufenkji and Elimelech\textsuperscript{35}.

For a pulse input into a fresh column, the solution to Eq. 2 provides aqueous concentration of particles as a function of column depth $x$ and time $t$ (Eq. 3)\textsuperscript{57},

$$c(x,t) = \frac{M}{Q} e^{-k_{att}t} \frac{x}{2\sqrt{\pi t D_H}} e^{-\frac{(x-\gamma t)^2}{4Dt}}$$  \hspace{1cm} (6.3)

where $M$ is the total mass injected, and $Q$ is the volumetric flow rate.

Attachment efficiency ($\alpha$) for CNT, kaolinite and CNT-kaolinite heteroaggregates onto quartz sand was calculated by fitting the column experiment breakthrough curves using Eq. 6.3. To determine the $\alpha$ values for CNT-kaolinite heteroaggregates, breakthrough data of attached CNT were used.

6.3 Results and discussion

6.3.1 Surface properties of CNT, kaolinite, and quartz sand.

Electrostatic forces are expected to play an important role in the interactions between the CNT, kaolinite and quartz sand surface. Figure 6-1 presents the electrophoretic mobility (EPM) of CNT, kaolinite and quartz as a function of pH and ionic strength. In the presence of 1 mM NaCl, all three surfaces were negatively charged over the pH range of 3 to 9. As expected, EPM of all three particles became more negative as pH increased (Figure 6-1 (A)) and ionic strength decreased (Figure 6-1 (B)), with kaolinite having the highest negative EPM values. This suggests
that double layer repulsion between kaolinite and quartz sand would be stronger than that between CNT and quartz sand. The EPM of the CNTs decreased from -2.71 µm/(s-V/cm) to -3.66 µm/(s-V/cm) when pH increased from 3 to 9, consistent with previous CNT studies\(^9\).

![Electrophoretic mobility of CNT, kaolinite and quartz sands as a function of (A) pH (in 1 mM NaCl solution) and (B) ionic strength (pH 9).](image)

**Figure 6-1** Electrophoretic mobility of CNT, kaolinite and quartz sands as a function of (A) pH (in 1 mM NaCl solution) and (B) ionic strength (pH 9).

**Influent suspension stability.** The intensity based mean particle size of all influent suspensions was monitored as a function of time to characterize their colloidal stability. All influent suspensions were very stable except for the CNT control and the CNT-kaolinite mixture at pH 3 (Table S2). The CNT control suspension exhibited an aggregation rate (measured by d(d\(_p\))/dt) of 0.60 nm/s at this low pH, while the kaolinite control was much more stable (d(d\(_p\))/dt = 0.30
nm/s) even though kaolinite and CNT had similar EPM values at pH 3 (Figure 6-1). This was attributed to the unique 1-D morphology of the CNTs as well as the strong van der Waals force between CNT surfaces\textsuperscript{137}. These observations were consistent with the DLVO interaction energy calculation (Figure 6-S3). At pH 3, the energy barrier between kaolinite particles (94 kT) is higher than that between the CNTs (82 kT) due to the larger particle size and smaller Hamaker constant of kaolinite. At this low pH, heteroaggregation between CNTs and kaolinite led to sedimentation of large aggregates over time, resulting in a decrease in intensity based mean particle size of the CNT-kaolinite mixture with time (d(d\_p)/dt = -1.41 nm/s).

Comparison between experimental data and DLVO calculation also points to significant limitation of the DLVO theory when applied to the system studied. For example, the energy barriers for CNT-CNT, kaolinite-kaolinite and CNT-kaolinite interactions in 1 mM NaCl at pH 3 were greater than or similar to those in 100 mM NaCl at pH 9; the secondary minimum was large in 100 mM NaCl at pH 9, but negligible in 1 mM NaCl at pH 3. However, both homoaggregation of CNT and heteroaggregation between CNT and kaolinite were much more prominent in 1 mM NaCl at pH 3. This is largely attributed to the charge heterogeneity of kaolinite, which is not considered in the DLVO calculation. Kaolinite contains permanent negative charges from the Si-O face, but point of zero charge (PZC) of the Ai-O lamella edge is generally 5-8\textsuperscript{138-140}. Therefore, kaolinite surface is all negatively charged at pH 9, but contains negative charges on the face and positive charges at the edges at pH 3. This leads to aggregation with the negatively charged CNT. Another limitation of the DLVO calculation is the assumption of spherical shape. CNTs have 1-D morphology and kaolinite is plane-shaped. Similar inconsistencies were reported in studies of oxidized MWCNTs\textsuperscript{141} and UV-irradiated nC\textsubscript{60}\textsuperscript{108}. More discussion on the DLVO calculation is provided in the Supporting Information.
6.3.2 Transport of CNTs.

Figure 6-S104) show a concentration peak at 1.05 pore volume with a mass recovery, percent of analyte mass exiting the column, of 105%. These results confirm that the column was properly setup, and the 1-D advection dispersion model applies.

Figures 6-2 (A) and 6-2 (B) shows the average breakthrough curve of the CNT control experiments and the calculated mass recovery, respectively. CNT was largely retained by the column under all conditions tested despite the strong negative charge of the CNT and the quartz sand. The mass recovery ranged from 3 to 68.8%, corresponding to the calculated attachment efficiency $\alpha$ (Table 6-S4) from 4.8 (100 mM NaCl, pH 9) to 0.84 (1 mM NaCl, pH 9). In general, CNT transport through the column increased with increasing pH and decreased with NaCl concentration. However, the calculated energy barrier between CNT and quartz sand was the largest in 1 mM NaCl at pH 5 (239 kT) followed by pH 3 (222 kT) and pH 9 (202 kT) (Figure 6-S3 (C)) because the influent particle size decreased as pH increased. The inconsistency between CNT transport and DLVO calculation as well as the very high attachment efficiency values suggest that straining, which was not considered in the transport model, might have contributed
to the retention of the CNT. The hydrodynamic diameter of the CNT increased notably from 347.5 ± 99.78 nm in 1 mM NaCl at pH 9 to up to 977.6 ± 229.5 nm when pH decreased or NaCl concentration increased (Figure 6-2 (C)), resulting in CNT to sand collector size ($d_{50}$) ratio $d_c/d_{50}$ of 0.002 to 0.004; straining is typically expected at a $d_c/d_{50}$ value of 0.003 or higher.$^{142}$

Furthermore, the very large aspect ratio of CNT and its bundled structure favor straining as the longitudinal dimension of the CNTs can be much larger than the column pore size. Similar observations were reported in a previous study on singled walled CNTs$^{133}$. As shown in Figure 6-2 (C), the 1-hr mixing significantly increased particle size of CNT in 1 mM NaCl at pH 3 and 100 mM NaCl at pH 9, indicating that the CNT homoaggregation in these conditions were mass transfer-limited.

It is also noticed the CNT breakthrough curves with low mass recovery peaked at pore volume less than 1 except for in 10 mM NaCl at pH 9, suggesting size exclusion. Given the unusual bundled structure and large aspect ratio, CNT aggregates may be blocked from narrow pores, which leads to a reduced effective pore volume and hence a shorter breakthrough time.
6.3.3 Transport of kaolinite.

Compared with the CNT, kaolinite was much more mobile in the saturated quartz sand column (Figure 6-3 (A) and (B)), with up to 94% of the input mass exiting the column under the conditions tested. This is consistent with the higher negative EPM of kaolinite (Figure 6-1), and the greater repulsive energy barrier between kaolinite and the quartz sand (Figure 6-S3 (D)) under most solution conditions. One exception was its transport in 1 mM NaCl at pH 3; no kaolinite was detected in the effluent under this condition though the energy barrier between

Figure 6-2 Transport of CNT control. (A) Breakthrough curves of CNT control; (B) mass recovery of CNT under various solution conditions. Sample names as seen in the legend; (C) intensity mean diameter of CNTs in control suspensions before and after 1-hr mixing; (D) calculated DLVO interaction energy as a function of separation distance under various solution conditions between CNT and quartz sand.
kaolinite and quartz sand was the highest (Figure 6-S3 (D)). This is attributed to the very large kaolinite homoaggregate size (1551.6 ± 167.6 nm), and the straining of these large aggregates by the quartz sand. For the other solution conditions tested, kaolinite retention increased with increasing ionic strength and decreasing pH, consistent with the calculated DLVO interaction. Notable retardation effect (retardation coefficient of 1.29) was observed in kaolinite transport in 100 mM NaCl at pH 9.

It is noted that most breakthrough curves of CNT and kaolinite exhibited significant asymmetry with a tailing effect. This suggests that deposition of CNT and kaolinite on quartz sand may be reversible and detachment may have occurred\textsuperscript{143,144}. 
Figure 6-3 Transport of kaolinite control. (A) Breakthrough curves of kaolinite control and (B) mass recovery of kaolinite in kaolinite control column experiments under various solution conditions; (C) intensity mean diameter of kaolinite control suspensions before and after 1-hr mixing.
6.3.4 Co-transport of CNT and kaolinite.

When both CNTs and kaolinite were present in the influent, heteroaggregation led to CNT attachment to kaolinite, especially at high NaCl concentration of 100 mM or pH 3, where 63 and 86 %, respectively, of the total CNT mass was associated with kaolinite (Figure 6-4 (A)).

Consistent with the DLVO calculation, the average particle size of the mixture increased with increasing NaCl concentration and decreasing pH (Figure 6-4 (B)). Due to the high kaolinite-to-CNT concentration ratio used, the light scattering by free CNTs can be considered negligible. Therefore, comparison between the particle size distribution of the CNT-kaolinite mixture and the kaolinite control provides insights on the formation of CNT-kaolinite heteroaggregates. For example, the average particle size of the CNT-kaolinite mixture in 1 mM NaCl at pH 3 (2526 ± 18.24 nm) was much larger than that of the kaolinite control (1551.6 ± 167.6 nm) under the same solution condition, while that in 100 mM NaCl at pH 9 (875.4 ± 54.43 nm) was similar to the kaolinite control (801.8 ± 26.05 nm) although a large percentage of CNTs were associated with kaolinite in both cases. The very large particle size at pH 3 suggests the formation of secondary heteroaggregates (i.e., aggregates formed by multiple primary heteroaggregates). This is attributed to the charge heterogeneity of kaolinite at pH 3. At pH 3, kaolinite has positive edges (PZC~5-8), which can interact with the negatively charged COOH-MWCNT and kaolinite faces, forming very large edge-CNT-edge structures, i.e., secondary heteroaggregates. Similar secondary heteroaggregation resulting from surface heterogeneity of clays was also reported for kaolinite and TiO$_2$ nanoparticles$^{38}$. In 100 mM NaCl at pH 9, however, heteroaggregation seems to form primarily through CNT attaching to individual kaolinite particles or kaolinite homoaggregates, i.e., primary heteroaggregation. Because of the large kaolinite to CNT concentration ratio, the number of primary heteroaggregates formed was small, and hence had
only small impact on the average particle size. In all conditions, there was no significant
difference in particle size before and after mixing (p > 0.05), suggesting that heteroaggregation
between CNT and kaolinite occurred quickly and was surface reaction-limited.

Figure 6-4 Compositions and particle sizes in CNT-kaolinite mixture suspensions. (A)
Proportions of free CNT and attached CNT in total CNT in the mixture after 1-hr mixing. (B)
Intensity mean diameter of CNT-kaolinite mixture before and after 1-hr mixing.

Interactions between kaolinite and CNT had great impact on their transport behaviors. In 1 mM
NaCl at pH 3, the presence of kaolinite reduced the mobility of total CNT, with mass recovery
decreasing from 10.8% to 0. On the other hand, CNT transport was enhanced in the presence of
kaolinite in 100 mM NaCl at pH 9, mass recovery increasing from 2.7% to 26% (Figure 6-5 (B)). In both cases, the mass recovery of the attached CNTs was very similar to that of kaolinite (Figure 6-5 (B)): there was no detectable CNT or kaolinite in the column effluent in 1 mM NaCl at pH 3, while 36.3% attached CNT in the influent was recovered in the effluent, similar to the 36.4% mass recovery of kaolinite control in 100 mM NaCl at pH 9. This strongly suggests that the attached CNTs co-transport with kaolinite in the form of CNT-kaolinite heteroaggregates, and the presence of the attached CNTs in these heteroaggregates does not affect kaolinite transport.

Figure 6-5 Co-transport of CNT and kaolinite at 100 mM NaCl pH 9 and 1 mM NaCl pH 3. (A) Breakthrough curves of total CNT in the mixture compared with CNT control and (B) mass
recovery of compositions in mixture. The mass of free CNT and attached CNT was normalized by the corresponding influent compositions and total influent CNT, respectively.

Free CNTs exhibited interesting transport behavior in the presence of kaolinite. Under the three solution conditions where heteroaggregation was minimal, i.e., CNTs were present mostly as free CNTs in the influent in 1 mM NaCl at pH 5 or 9 and 10 mM NaCl at pH 9 (Figure 6(A)), the presence of kaolinite greatly enhanced transport of free CNTs compared with the CNT control experiment (Figure 6-B). This could be attributed to competition for attachment sites and site blocking by kaolinite due to its much higher concentration. Kaolinite occupied a much larger portion of the quartz sand surface (0.27 - 0.50%, see Table 6-S3) compared with CNT (0.012 - 0.018%). The decreased number of available surface attachment sites led to enhanced mobility of the free CNTs through the column.
Figure 6-6 Co-transport of CNT and kaolinite at 1 mM NaCl pH 5, 1 mM NaCl pH 9 and 10 mM NaCl pH 9. (A) Breakthrough curves of total CNT in mixture compared with CNT control and (B) mass recovery of compositions in mixture. The mass of free CNT and attached CNT was normalized by the corresponding influent compositions and total influent CNT, respectively.

Due to the very high kaolinite-to-CNT concentration ratio, kaolinite transport was not affected by the presence of CNT. In 100 mM NaCl at pH 9, when primary heteroaggregation occurred, the CNT surface coverage on the kaolinite was probably very low and the number of heteroaggregates was small due to the low CNT concentration. Under the conditions when heteroaggregation was negligible, the competition for attachment sites by the small number of
CNTs was not sufficient to cause notable changes in kaolinite transport. Therefore, transport of kaolinite in the presence of CNT was similar to that of the kaolinite control.

6.4 Environmental implications

Colloidal materials such as natural clay particles are ubiquitous. ENMs that enter the aquatic environment will inevitably encounter naturally occurring colloids. As shown in this study, the presence of a natural clay particle, kaolinite, has important impact on the transport of carboxylated multi-walled carbon nanotubes through the formation of heteroaggregates even though the bulk physicochemical properties of the COOH-MWCNTs and kaolinite suggest strong repulsive interactions, as well as competition for and blockage of attachment sites on mineral surfaces. Furthermore, the formation and structure of CNT-kaolinite heteroaggregates depend strongly on the solution pH and ionic strength; heteroaggregates of different structures (e.g., primary vs. secondary heteroaggregates) exhibit distinct transport behavior. At large kaolinite-to-CNT concentration ratios, which is expected in realistic environmental conditions, the transport of CNT-kaolinite primary heteroaggregates is dominated by the transport properties of kaolinite. This suggests significantly enhanced mobility of the CNTs in natural aquatic environment. Secondary heteroaggregates, on the other hand, have limited mobility in the porous media due to their very large size. These findings underline the importance of ENM-NOC heteroaggregates in the transport of ENMs in the natural environmental, and point to the need for characterizing EMN-NOC heteroaggregates in order to better predict ENM transport behaviors. Even under conditions where heteraggregation is insignificant, kaolinite could greatly enhance CNT mobility by competing for and blocking surface attachment sites. This further underscores the importance of NOC in the transport of ENMs. Future investigation also needs to consider the complex interactions with other important environmental factors including natural organic matter
and divalent cations, which may mediate ENM-NOC heteroaggregation through specific interactions.

### 6.5 Supporting information

![Column setup diagram](image)

**Figure 6-S7 Schematic of column setup**

**Control experiments to validate the protocol of separating free CNT from attached CNT and kaolinite**

To validate that this protocol can effectively remove free CNTs from a mixture sample, the same centrifugation protocol was applied to 90 mg/L CNT control, 18,000 mg/L kaolinite control, and a mixture sample containing 90 mg/L CNT and 18,000 mg/L kaolinite. After 1-hr mixing, the samples were diluted 20 times, and light absorbance at 800 nm was measured using a UV-Vis spectrophotometer (Shimadzu, UV 2550). The diluted samples were then transferred to 1.5-mL microtubes and sonicated in a cup horn for 1 min at 100 W followed by centrifugation at 5,000
rpm for 2 to 4 min in order to obtain an optimal separation conditions. The supernatant was collected and the light absorbance was measured at 800 nm. For the CNT-kaolinite mixture, both the free CNT and the attached CNT, which is now detached from kaolinite, contribute to the light absorbance. Therefore, comparison between the measured light absorbance with that of a 4.5 mg/L (after 20-time dilution) CNT suspension is used to measure the effectiveness of sonication in detaching the CNTs from kaolinite.

Results in Table 6-S3 show that 92 - 99% of free CNT is recoverable from the supernatant after the centrifugation protocol, but only 0.2 - 0.7% of kaolinite is in the supernatant (Table 6-S1). The relative error of the total CNT measured were 1% - 7%. Thus, the washing method was reasonable.

Table 6-S3 A series of light absorbance (Abs) for kaolinite (kao.) and CNT control samples before and after the separation test. Percent absorbances are shown for CNT and kaolinite after centrifugation compared to their respective controls and for CNT/kaolinite mixture after treatment compared to the CNT control.

<table>
<thead>
<tr>
<th>Solution condition</th>
<th>Optimal condition</th>
<th>Absorbance / Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CNT control</td>
</tr>
<tr>
<td>1 mM NaCl pH 3</td>
<td>5k rpm 3 min</td>
<td>0.072 ± 0.005</td>
</tr>
<tr>
<td>1 mM NaCl pH 5</td>
<td>5k rpm 3.5 min</td>
<td>0.078 ± 0.003</td>
</tr>
</tbody>
</table>
Table 6-S4 Aggregation rate derived from particle size of CNT, kaolinite and the mixture after the mixing.

<table>
<thead>
<tr>
<th>Solution condition</th>
<th>Aggregation rate (d(dp)/dt, nm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CNT control</td>
</tr>
<tr>
<td>1 mM NaCl, pH 9</td>
<td>0.079 ± 0.003</td>
</tr>
<tr>
<td>10 mM NaCl, pH 9</td>
<td>0.078 ± 0.002</td>
</tr>
<tr>
<td>100 mM NaCl, pH 9</td>
<td>0.075 ± 0.003</td>
</tr>
</tbody>
</table>

DLVO calculation

For CNT-CNT, kaolinite-kaolinite and CNT-kaolinite interaction energy, equations applied are shown below for sphere-sphere geometry\textsuperscript{145,146}:

\[ V_T = V_{EDL} + V_{VDW} \]  \hspace{1cm} (6.S1)
$$V_{EDL} = 64\pi\varepsilon_0\varepsilon_r \frac{a_1 a_2}{a_1 + a_2} \left( \frac{k_B T}{ze} \right)^2 \Gamma_1 \Gamma_2 \exp(-\kappa h)$$  \hspace{1cm} (6.52)$$

$$\kappa = \sqrt{\frac{1000e^2 N_A \sum n_j z_j^2}{\varepsilon_0 \varepsilon_r kT}}$$  \hspace{1cm} (6.53)$$

$$V_{VDW} = -A_{132} \frac{a_1 a_2}{6h(a_1 + a_2)}$$  \hspace{1cm} (6.54)$$

Here, $V_{EDL}$ is electric double layer repulsion energy; $V_{VDW}$ is van der Waals attraction energy; $\varepsilon_0$ and $\varepsilon_r$ are dielectric constant of vacuum and water, respectively; $a_1/a_2$ is the particle radius; $k_B$ is the Boltzmann constant, $1.3805\times10^{-23}$ J/K; $T$ is absolute temperature; $\Gamma_1/\Gamma_2$ is the dimensionless surface potential ($\Gamma_i = \tanh \left( \frac{ze\psi_i}{4k_B T} \right)$, where $z$ is the counterion valence, $e$ is the electron charge ($1.602\times10^{-19}$ C), $\psi_i$ is the surface potential); $\kappa$ is the inverse Debye length; $N_A$ is Avogadro’s number, $6.02\times10^{23}$ mol$^{-1}$; $n_j$ is the molar concentration of ion $j$ in the bulk solution; $z_j$ is the valence of ion $j$; and $A_{132}$ is the Hamaker constant for materials 1 and 2 in medium 3.

For CNT-quartz sand and kaolinite-quartz sand interaction energy, $V_{EDL}$ and $V_{VDW}$ were calculated using the equations for sphere-plate system$^{147}$:

$$V_{EDL} = 64\pi a e \varepsilon_0 \left[ \frac{kT}{ze} \right]^2 \Gamma_1 \Gamma_2 \exp(-\kappa h)$$  \hspace{1cm} (6.55)$$

$$V_{VDW} = -A_{132} \frac{a}{6} \left[ \frac{a}{h} + \frac{a}{h + 2a} + \ln \left( \frac{h}{h + 2a} \right) \right]$$  \hspace{1cm} (6.56)$$

where $a$ is the particle radius of CNT or kaolinite, and $h$ is the separation distance.
In calculations for CNT-CNT, kaolinite-kaolinite and CNT-kaolinite interaction energy, particle radii \((a)\) of CNT and kaolinite used were 146.5 and 200 nm, respectively, obtained from the DLS measurement of the stock suspensions immediately after 30-min ultra-sonication at 100 W. For CNT-sand and kaolinite-sand interaction, \(a\) values of particles were obtained from the DLS measurement of CNT and kaolinite control samples after 1-hr mixing. Surface potential \((\psi_i)\) was approximated using the zeta potential value (Figure 6-S2). The Hamaker constants used for CNT, kaolinite, water and sand are \(6.0 \times 10^{-20}\), \(4.7 \times 10^{-20}\), \(3.7 \times 10^{-20}\), and \(7.93 \times 10^{-20}\) J, respectively.

![Graph A](image)

**Figure 6-S8** Zeta potential of CNT and kaolinite as a function of (A) pH (in 1 mM NaCl solution) and (B) ionic strength (pH 9).
Figure 6-S9 Calculated DLVO interaction energy as a function of separation distance under various solution conditions between (A) CNT and CNT, (B) kaolinite and kaolinite, (C) CNT and quartz sand, (D) kaolinite and quartz sand and (E) CNT and kaolinite.
Calculation of attachment efficiency.

Table 6-S5 lists the calculated attachment rate constant ($k_{att}$) and attachment efficiency ($\alpha$) for different column experiments. Calculation was not done for kaolinite control in 1 mM NaCl at pH 3 because no kaolinite was detected in the effluent under this solution condition. Calculation for attached CNT was shown for 100 mM NaCl at pH 9 only because the amount of attached CNT in the effluent was negligible under other conditions.

Table 6-S5 Calculated deposition rate constant ($k_{att}, s^{-1}$) and attachment efficiency ($\alpha$) of CNT control, kaolinite control and attached CNT in mixture onto quartz sand.
Percentages of sand surface area for retained CNT and kaolinite

Surface area on quartz sand occupied by kaolinite and CNT in their corresponding control column experiments was calculated. Kaolinite and CNT particles were assumed spherical and the particle radius after the 1 hr mixing was used in the calculation. The mean particle size of the quartz sand is 0.274 mm in radius. Density of kaolinite, CNT and quartz sand is 2.65, 2.1 and 2.56 g/cm$^3$, respectively.

Table 6-S6 Percentage of quartz sand surface area occupied by kaolinite and CNT in the respective control experiments.

<table>
<thead>
<tr>
<th>Solution condition</th>
<th>Percentage of sand surface area occupied</th>
<th></th>
</tr>
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<tbody>
<tr>
<td></td>
<td>kaolinite control</td>
<td>CNT control</td>
</tr>
<tr>
<td>1 mM NaCl, pH 5</td>
<td>0.27%</td>
<td>0.018%</td>
</tr>
<tr>
<td>1 mM NaCl, pH 9</td>
<td>0.42%</td>
<td>0.012%</td>
</tr>
<tr>
<td>10 mM NaCl, pH 9</td>
<td>0.50%</td>
<td>0.018%</td>
</tr>
</tbody>
</table>
Chapter 7

Conclusions

The wide application and increasing production\(^1\) of carbon nanotubes (CNT) result in their inevitable release into the natural aquatic systems. At the same time, the toxicities of carbon nanotubes to living organisms have been reported\(^8\). When released into the water systems, the universal existence of naturally occurring colloids (NOC) and dissolved natural organic matter (NOM) will greatly alter the size and surface properties of carbon nanotubes depending on the solution chemistry, resulting in changes on the toxicity and bioavailability of the nanomaterials. Therefore, investigating the fate and transport of CNT in natural water conditions in the presence of NOC and NOM is crucial for the environmental risk assessment of this carbon-based nanomaterial. There are three primary objectives in this study: 1) To explore the impact of kaolinite and various environmental compositions (solution pH, ionic strength, Ca\(^{2+}\) and NOM) on the stability of CNTs in aqueous phase. 2) To investigate the effect of kaolinite, solution ionic strength and pH on the subsurface transport of CNT in saturated porous media. 3) To study the morphology of various compositions in CNT-kaolinite binary systems under various CNT-kaolinite concentration ratios and solution conditions and investigate how morphological information will help explain the mechanisms that cause the differences in the stability and mobility between CNT-only and CNT-kaolinite systems. The following points highlighted the main conclusions of this study.
• Though negatively charged in most natural pH ranges, COOH-MWCNT can aggregate with kaolinite and form both primary and secondary heteroaggregates with kaolinite depending on the solution pH and CNT: kaolinite ratio. DLVO forces and Louis acid-base interaction were proposed to be the mechanisms of the heteroaggregation.

• Calcium ions were more effective in inducing heteroaggregation and destabilized the system than monovalent sodium ions due to the charge screening and bridging effects.

• In low to no calcium ions, NOM hindered heteroaggregation through steric effect and stabilized the CNT. When in the high calcium ion concentration range, despite the NOM concentration, CNT formed extremely large heteroaggregates and was totally destabilized due to the dominating impact of calcium ions.

• Flow cytometer can detect from extremely diluted mixture samples the presence and extent of AFCNT homoaggregation and its heteroaggregation with kaolinite. It is limited in figuring out the configurations of aggregates especially for the non-spherical and polydispersed CNT and kaolinite suspensions.

• Large CNT-clay aggregates can form in acidic water conditions and retain in subsurface porous media. Facilitated transport of CNT by natural colloids is likely to occur in high ionic strength and high pH conditions.

This research is the first study that systematically investigates the impact of naturally occurring colloids on the structure, fate and transport of CNT in natural water systems. It is also the first study regarding the applicability of flow cytometry on NOC-ENM systems. This study contributes to the understanding of the role of naturally occurring colloid and dissolved natural
organic matter in the aggregation and deposition behaviors of CNT particles. It also contributes to the knowledge of analytical techniques for particle aggregations by introducing flow cytometry as an efficient tool in analyzing aggregation behaviors in NOC-ENM suspensions with ENM concentration ranges close to that in the environment. Findings in this study provide fundamental understanding of stability and mobility of CNT in natural aquatic systems and a novel and efficient analytical tool in analyzing CNT-NOC heteroaggregations. These findings enable better environmental risk assessment and management of CNT. Specifically, results from this study enables the prediction of aggregation/dispersion status of CNT in aqueous phase, which directly affect the bioavailability and toxicity: free CNT can be more bioavailable and toxic than attached CNT. In acidic water conditions such as thermal springs, large CNT-clay secondary aggregates can retain in subsurface porous media, which may result in a potential source of CNT pollution as particles accumulate. In low to medium ionic strength and normal pH conditions, CNT might be very mobile due to the reversible depositions of natural clays and thus enlarge the polluted areas.

To improve the prediction of fate and transport of ENMs in aquatic systems, recommendations for future research are: 1) quantitative analysis via co-transport modelling and investigations on multiple environmentally related impacting factors such as ion types, nano-suspension solvents, porous media characteristics and influent concentration. 2) Using flow cytometry to determine the configurations of ENM-NOC heteroaggregates. By selecting a spherical, mono-sized nanoparticle as the model ENM, the fluorescence vs. size dot plot will probably show distinguishable gates representing different structures of the aggregates.


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