nC₆₀ deposition kinetics: the complex contribution of humic acid, ion concentration, and valence

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Abstract

The demonstrated toxicity coupled with inevitable environmental release of nC₆₀ raise serious concerns about its environmental fate and transport, therefore it is crucial to understand how nC₆₀ will interact with subsurface materials including attached phase soil and sediment organic matter (AP-SOM). This study investigated the attachment of nC₆₀ onto a Harpeth humic acid (HHA) coated silica surface under various solution conditions using a quartz crystal microbalance with dissipation monitoring. The HHA coating greatly enhanced nC₆₀ attachment at low ion concentrations while hindering attachment at high ion concentrations in the presence of both mono and divalent cations. At low ion concentrations, the HHA greatly reduced the surface potential of the silica, enhancing nC₆₀ deposition through reduction in the electrostatic repulsion. At high ion concentrations however, the reduced surface potential became less important due to the near zero energy barrier to deposition and therefore non-DLVO forces dominated, induced by compaction of the HHA layer, and leading to hindered attachment. In this manner, ob-

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served contributions from the HHA layer were more complex than previously reported and by monitoring surface charge and calculated DLVO interaction energy alongside attachment experiments, this study advances the mechanistic understanding of the variable attachment contributions from the humic acid layer.

Keywords: nC₆₀, humic acid, NOM, QCM-D, DLVO

1. Introduction

The popularity of engineered nanomaterials (ENMs) has grown rapidly over the last decade, with the ENM market expected to reach a total of \$64.2 billion over the next five years (McWilliams, 2014). This increase in production will certainly lead to the eventual release of ENMs into the environment through production and manufacturing of the products or use and disposal from the consumer (Gottschalk and Nowack, 2011). One particular ENM of note is buckminsterfullerene (C₆₀), which has applications throughout many fields, including biomedical technology, electronics, and optics (Nel et al., 2006; Mauter and Elimelech, 2008). Since the solubility of fullerene in water is very low (Jafvert and Kulkarni, 2008), the nano-sized colloidal particle it forms in water (nC₆₀) is the most environmentally relevant form to study. The antimicrobial properties, aquatic toxicity, and potential human toxicity of nC₆₀ (Nel et al., 2006; Nakajima et al., 1996; Kamat et al., 2000; Fang et al., 2007) combined with the inevitable release to the environment raise serious concerns about the environmental fate and transport of nC₆₀.

As with other colloidal particles, the transport of nC₆₀ throughout the environment will be determined by its aggregation and deposition processes.

19 These processes are controlled by a complex system of variables including
20 ionic strength, the presence of organic matter, and pH (Lin et al., 2010).
21 The aggregation behavior of nC_{60} in clean systems (absent of organic mat-
22 ter) is usually described by the classic Derjaguin-Landau-Verwey-Overbeek
23 (DLVO) theory (Derjaguin, 1941; Verwey, 1947), which describes the stabil-
24 ity of aqueous colloids by combining the effects of an electrical double layer
25 repulsion (EDL) and a van der Waals (vdW) attraction. An increase in ionic
26 strength screens the EDL repulsion and lowers the energy barrier to aggre-
27 gation. Furthermore, the Schulze-Hardy rule (Elimelech et al., 1998) states
28 that the valence (z) of counterions play a major role as well, predicting the
29 critical coagulation concentration (CCC) to be proportional to z^{-6} . The ag-
30 gregation behavior of nC_{60} has been shown to be in agreement with DLVO
31 theory both in terms of sensitivity to ionic strength and proportionality to z
32 (Chen and Elimelech, 2006, 2007).

33 The deposition of nC_{60} occurs when nC_{60} particles collide with and sorb
34 to an immobile surface in the complex subsurface environment. Relative
35 to the nC_{60} particles, these mineral and soil surfaces can be considered in-
36 finitely large particles and therefore deposition can be studied as a form of
37 heteroaggregation (Elimelech et al., 1998). Several studies have monitored
38 the deposition of nC_{60} using both quartz crystal microbalance with dissipa-
39 tion monitoring (QCM-D) (Chen and Elimelech, 2006, 2007, 2008; Qu et al.,
40 2012) and packed column experiments (Qu et al., 2012; Brant et al., 2005; Es-
41 pinasse et al., 2007; Wang et al., 2008a,b; Li et al., 2008), however most were
42 conducted in a simple electrolyte system with bare mineral surfaces. As ionic
43 strength increased in the aforementioned QCM-D studies, the attachment ef-

44 efficiency of nC_{60} onto silica surfaces increased until reaching a maximum of 1
45 at the CCC, suggesting qualitative agreement with DLVO theory. Addition-
46 ally, two of these studies (Chen and Elimelech, 2006, 2008) reported a much
47 larger charge screening effect when a divalent cation (Ca^{2+}) was introduced,
48 suggesting qualitative agreement with the Schulze-Hardy rule. DLVO theory
49 describes the deposition of nC_{60} well in these clean systems, however the
50 subsurface often contains additional variables for consideration, namely the
51 presence of organic matter.

52 Soil organic matter (SOM) is ubiquitous in the subsurface environment,
53 either dissolved or immobilized (attached) onto the surface of mineral and
54 soil surfaces and humic substances typically represent the dominant organic
55 components in soils and sediments (Stevenson, 1994). Dissolved organic mat-
56 ter has been shown to adsorb onto the surface of nC_{60} particles and increase
57 their stability to aggregation and deposition through steric repulsion (Chen
58 and Elimelech, 2007, 2008) and, in turn, increase their mobility. The effect of
59 attached phase soil organic matter (AP-SOM) on the deposition behavior of
60 nC_{60} , however, appears to be much more complex. The presence of AP-SOM
61 has been shown to enhance, hinder, or have no effect on the attachment of
62 nC_{60} , depending on the type of AP-SOM and the ionic strength of solution
63 (Chen and Elimelech, 2007, 2008; Qu et al., 2012). One study reported that
64 an alginate layer enhanced nC_{60} attachment in the presence of 10 mM NaCl,
65 while hindering it in the presence of 30 mM NaCl (Chen and Elimelech, 2008).
66 The variable behavior was attributed to its compaction at higher NaCl con-
67 centrations, resulting in a smoother, more rigid layer, with less room for nC_{60}
68 particle entanglement. Furthermore, in our previous work, we reported that

69 increasing the temperature of a humic acid layer while holding all other vari-
70 ables constant, raised the attachment efficiency of nC_{60} from low attachment
71 ($\alpha = 0.3$) to complete attachment ($\alpha = 1$) (McNew and LeBoeuf, 2015). We
72 attributed this to the temperature-dependent hydration of the layer, opening
73 up previously unavailable sorption sites. Since the interaction between nC_{60}
74 and environmentally relevant surfaces will likely play a very important role in
75 the fate and transport of nC_{60} throughout the environment (Lecoanet et al.,
76 2004), it is clear that more study is needed in order to understand what role
77 AP-SOM will play.

78 The purpose of this study is to further the understanding of nC_{60} depo-
79 sition behavior in the presence of AP-SOM. By varying the ionic strength of
80 the system, through both cation concentration and valence, while holding all
81 other variables constant, the deposition rate of nC_{60} is directly monitored us-
82 ing a QCM-D. Deposition rate and attachment efficiency curves are then con-
83 structed over a range of ion concentrations and valences. By comparing these
84 curves between surfaces of bare silica and humic acid coated silica, the role of
85 ionic strength in nC_{60} deposition onto AP-SOM is elucidated, discussed, and
86 compared to DLVO theory. Combined with our previous work regarding the
87 role of AP-SOM physicochemical properties (McNew and LeBoeuf, 2015),
88 this study illustrates the importance of AP-SOM in the transport of nC_{60}
89 and the need for a better quantitative understanding of its role. A better
90 understanding of these interactions would lead to more precise predictions on
91 the potential pathways of exposure and their eventual impact on ecosystems
92 and human health.

2. Materials and Methods

2.1. Materials

Sublimed C₆₀ powder (>99.9%) was purchased from MER Corporation (Tucson, AZ). Dry Harpeth humic acid (HHA) was isolated as described in our previous publication (DeLapp et al., 2005). Poly-L-lysine hydrobromide (PLL, P1274) and 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were purchased from Sigma-Aldrich (St. Louis, MO). The concentration of PLL and HEPES was held constant at 0.1 g/L and 10 mM, respectively. Reagent grade NaCl (S671) and CaCl₂ (C79) were purchased from Fisher Scientific (Pittsburgh, PA). Silica-coated QCM-D sensors (Q5X-303) were purchased from Q-Sense (Biolin Scientific, Stockholm, Sweden). 900 nm silica microspheres were purchased from Polysciences Inc (Warrington, PA) as a surrogate for estimating the electrophoretic mobility of the silica sensor surface. All solutions were prepared using ultra pure water purified by the Milli-Q Water Purification System from Millipore (Billerica, MA).

2.2. Preparation of Stock Suspensions and Solutions

The nC₆₀ stock suspension was prepared using the same method as described in our previous publication (McNew and LeBoeuf, 2015), similar to that described by Andrievsky et al. (1995) (details of the preparation method are provided in the Appendix). The resulting nC₆₀ stock suspension was determined to have a total carbon content (Shimadzu TOC-V CPH/CPN, Columbia, MD) of 33.6 mg L⁻¹ and a pH of 5.6. The nC₆₀ stock suspension was stored in the dark at 4 °C for the entirety of the study and all experiments took place within 4 months of preparation, during which time the nC₆₀ stock

suspension was stable as confirmed by periodic Dynamic Light Scattering (DLS) measurements (Malvern Zetasizer Nano ZS, Worcestershire, UK).

The method for preparing the HHA stock solution is provided in the Appendix. The HHA stock solution was kept in the dark for the entirety of the study, during which time the solution was stable as confirmed by periodic DLS measurements. The HHA used in this study has been extensively characterized by DeLapp et al. (2005) and shown to undergo thermal transitions at elevated temperatures. Due to the constant temperature of 25 °C employed throughout this study, however, no such transition behavior is expected. All prepared solutions were filtered through 0.22 μm pore size membrane filter paper (Fisher, GSWP02500) and pH adjusted to 5.6, so that all experiments could take place at the unadjusted pH of the nC_{60} stock suspension.

2.3. nC_{60} Aggregation Kinetics

Since particle size can control the rate of diffusion, it is important to characterize the aggregation of nC_{60} under all experimental conditions. By mixing measured amounts of concentrated electrolyte and stock nC_{60} immediately before introduction into the DLS, the nC_{60} particle size at each experimental condition can be monitored over time. The nC_{60} concentration and pH were held constant at 6.1 mg L^{-1} and 5.6, respectively, for all experiments throughout this study for consistency with other relevant studies (Chen and Elimelech, 2006, 2008; Qu et al., 2012) and to maintain environmentally relevant conditions. The particle size was monitored once per minute, with each point representing 10 independent measurements. In order to calculate the aggregation attachment efficiency factor (α_A) (Eq 1), the aggregation rate for each experimental condition was normalized by the aggregation rate un-

142 der diffusion limited conditions. The aggregation rate under diffusion limited
 143 conditions was taken to be the aggregation rate at which an increase in NaCl
 144 or CaCl₂ concentration did not result in an increase in aggregation rate.

$$\alpha_A = \frac{da_p/dt}{(da_p/dt)_{diff\ limited}} \quad (1)$$

145 2.4. Electrophoretic Mobility Measurements

146 In order to measure the electrophoretic mobility (EPM) of nC₆₀ and silica
 147 under all experimental conditions, the DLS was used in combination with a
 148 universal dip cell (Malvern ZEN1002, Worcestershire, UK). Each reported
 149 EPM value was the result of 10 independent measurements. 900 nm silica
 150 microspheres were used as a surrogate for the silica QCM-D sensor surface.
 151 EPM values were measured at each experimental condition for uncoated,
 152 PLL-coated, and HHA-coated silica particles. In order to coat the negatively
 153 charged silica particles with HHA, they were first coated with positively
 154 charged PLL, rinsed with DI water, and then coated with HHA. The sil-
 155 ica particles were introduced into a 100 mg L⁻¹ solution of PLL, stirred for
 156 20 minutes, and then drained through a 0.45 μm pore size membrane fil-
 157 ter (Fisher, GSWP02500) while rinsing in DI water, allowing the unbound
 158 PLL to pass through, leaving the coated silica particles behind. The process
 159 was then repeated for an HHA coating. The uncoated silica particles were
 160 also taken through an identical procedure without the addition of PLL or
 161 HHA, as a control. The silica concentration was 10 mg L⁻¹ for all EPM
 162 measurements. Zeta potentials were estimated using the Smoluchowski ap-
 163 proximation (Hunter, 2013).

164 2.5. QCM-D Experiments

165 The deposition rate of nC₆₀ under varying experimental conditions was
 166 monitored using a QCM-D (Q-Sense E4 unit, Biolin Scientific, Stockholm,
 167 Sweden). Flowrates were 0.1 mL min⁻¹, ensuring laminar flow within the
 168 module, as confirmed by a Re of 0.26 (details can be found in the Appendix).
 169 Temperature was held constant at 25 ± 0.02 °C by a Peltier element. The
 170 nC₆₀ concentration and pH were held constant at 6.1 mg L⁻¹ and 5.6, respec-
 171 tively, for all experiments throughout this study in order to remain consistent
 172 with other relevant studies (Chen and Elimelech, 2006, 2008; Qu et al., 2012)
 173 and environmentally relevant conditions. Before each experiment, the silica
 174 coated quartz sensors were soaked in a 2% Hellmanex II solution (Mullheim,
 175 Germany) overnight, rinsed with copious amounts of DI water, dried with
 176 ultrapure N₂ gas and then cleaned in a UV-Ozone cleaner (Jelight 42, Irvine,
 177 CA) for 20 min. The QCM-D experimental protocol is explained in detail in
 178 the Appendix and a representative experimental procedure is illustrated in
 179 Fig. 1.

180 2.6. nC₆₀ Deposition Kinetics

181 A continuous increase in mass during deposition results in a continuous
 182 decrease in frequency, as described by the Sauerbrey Relation (Sauerbrey,
 183 1959), which can be seen in Eq. 2, where m is the mass of material deposited,
 184 C is the crystal constant (17.7 ng cm⁻²Hz⁻¹), and f_n is the frequency at
 185 overtone n .

$$\Delta m = -C \frac{1}{n} \Delta f_n \quad (2)$$

Figure 1: Representative nC_{60} deposition experiment. The initial baseline was collected in HEPES solution (A) before PLL was attached to the silica surface and rinsed in HEPES solution (B). The PLL layer was then rinsed in 1 mM NaCl (C) before HHA was attached to the PLL surface and rinsed in 1 mM NaCl (D). Finally, the HHA layer was rinsed in the electrolyte concentration at which the deposition experiment would take place (100 mM NaCl in this case) (E). Once a stable baseline was observed the nC_{60} was mixed with a premeasured volume of electrolyte to form the desired concentration (100 mM NaCl in this case) and immediately introduced onto the sensor surface (F).

186 In order to compare deposition rates under different experimental con-
 187 ditions, independent of nC₆₀ aggregation effects, the deposition attachment
 188 efficiency factor was calculated (α_D) as seen in Eq. 3. The initial change
 189 of the third overtone of frequency with respect to time ($\frac{df_3}{dt}$) was calculated
 190 from the first 120 seconds of nC₆₀ deposition. Since the nC₆₀ particles are
 191 negatively charged at all experimental conditions examined (Fig. D.7), any
 192 particles that flow near the positively charged PLL-coated surface will at-
 193 tach, meaning the deposition in this case is limited only by diffusive mass
 194 transport. Therefore, the diffusion limited deposition rate ($(\frac{df_3}{dt})_{diff\ limited}$)
 195 was taken to be the nC₆₀ deposition rate onto the PLL-coated surface at each
 196 corresponding experimental condition.

$$\alpha_D = \frac{df_3/dt}{(\frac{df_3}{dt})_{diff\ limited}} \quad (3)$$

197 3. Results and Discussion

198 3.1. nC₆₀ Aggregation Kinetics

199 Since the size of nC₆₀ particles can affect their mass transport rate to-
 200 wards the sensor surface and therefore control their deposition, it is important
 201 to first understand their aggregation behavior. Presented in Fig. 2 are the
 202 aggregation attachment efficiency factors (Eq. 1) for a range of NaCl (a) and
 203 CaCl₂ (b) concentrations. As the NaCl or CaCl₂ concentration is increased,
 204 the EDL repulsion becomes more suppressed and therefore the energy bar-
 205 rier to aggregation is reduced, allowing the nC₆₀ particles to aggregate more
 206 rapidly. When the concentration becomes high enough, the EDL repulsion is
 207 reduced to zero, the energy barrier to aggregation vanishes, and the aggrega-

208 tion rate is limited only by the diffusion of particles towards each other. This
 209 concentration is known as the CCC (Elimelech et al., 1998). Above the CCC,
 210 α_A values level off at 1, as can be seen in Fig. 2. By interpolation of the
 211 data in Fig. 2 to find the point of intersection between the reaction limited
 212 and diffusion limited regimes, the CCC was estimated as 217 mM NaCl (a)
 213 and 3.7 mM CaCl_2 (b), illustrating a much higher sensitivity to aggregation
 214 in the presence of the divalent cation Ca^{2+} . The ratio of CCC values derived
 215 here (3.7/217) is proportional to $z^{-5.9}$, where z is the valence of Ca^{2+} . This
 216 is in very close agreement with the Schulze-Hardy rule which predicts a value
 217 of z^{-6} , (Elimelech et al., 1998) and much closer than the previously reported
 218 value of $z^{-4.64}$ for nC_{60} (Chen and Elimelech, 2006), though it is worth not-
 219 ing that the properties of nC_{60} have been shown to be somewhat dependent
 220 upon preparation method (Espinasse et al., 2007).

221 3.2. nC_{60} Deposition Kinetics

222 The initial deposition rate of nC_{60} onto the sensor surface was accom-
 223 panied by a linear change in frequency (Fig. 1), suggesting continuous de-
 224 position on the clean sensor surface with no ripening or detachment. The
 225 Voigt viscoelastic model (Voinova et al., 1999) is typically employed to es-
 226 timate the mass and thickness of a viscoelastic film (Reviakine et al., 2011)
 227 such as nC_{60} in this study. Because of the limitations of the Voigt model,
 228 for example, it assumes a homogenous, uniform layer, the mass calculated
 229 includes water, and the density of the layer is assumed and held constant,
 230 we chose to quantify deposition rate as the initial slope of the third overtone
 231 of frequency.

232 The nC_{60} deposition rate as a function of NaCl (a) and CaCl_2 (b) concen-

Figure 2: nC_{60} aggregation attachment efficiency factors for a range of NaCl (a) and CaCl_2 (b) concentrations. Each point consists of three measurements and error bars represent standard deviation.

trations onto three different surfaces can be seen in Fig. 3. The calculated Peclet number (Pe) for the experiments in this study ranged from 7.94×10^{-8} to 4.34×10^{-7} (details can be found in the Appendix). Pe describes the rate of convective mass transport to diffusive mass transport, therefore such small values suggest diffusive mass transport dominates for all experimental conditions investigated. Since nC_{60} is negatively charged over all experimental conditions investigated (Fig. D.7)), deposition onto the oppositely charged PLL surface is limited only by diffusion of the particles towards the sensor surface and therefore the deposition onto PLL in Fig. 3 represents diffusion limited conditions. As NaCl concentration increases in Fig. 3(a), the nC_{60} particle size increases (Fig. 2(a)) causing a decrease in diffusive mass transport and a corresponding decrease in deposition for the PLL-coated trend, with the highest decrease occurring as the NaCl concentration in Fig. 3(a) approaches the CCC. The PLL-coated trend in the presence of $CaCl_2$ (Fig. 3(b)) shows a small decrease throughout, without the sharp decrease observed in Fig. 3(a), which can be explained by noticing that the highest $CaCl_2$ concentration in Fig. 3(b) is far from reaching the corresponding CCC for $CaCl_2$ in Fig. 2(b).

As NaCl or $CaCl_2$ concentration increases, the EDL repulsion between the bare silica surface and nC_{60} is increasingly suppressed, lowering the energy barrier to deposition and therefore increasing the deposition rate for the bare silica trend in Fig. 3. As NaCl or $CaCl_2$ concentration increases, it eventually reaches a point where the energy barrier is completely suppressed, the deposition is limited by diffusion alone, and the bare silica and PLL-coated trends coincide, known as the critical deposition concentration (CDC).

Figure 3: nC_{60} deposition rates as a function of NaCl (a) and $CaCl_2$ (b) concentrations onto three different surfaces. Each point consists of three measurements and error bars represent standard deviation.

258 In this manner, the bare silica trend in Fig. 3 represents the summation of
 259 the energy barrier reduction and particle aggregation effects. At low NaCl
 260 and CaCl_2 concentrations, an increase in concentration results in a large
 261 reduction in the energy barrier, but only a small increase in nC_{60} particle
 262 size, so the bare silica deposition trend increases rapidly. Conversely, at high
 263 concentrations of NaCl and CaCl_2 an increase in concentration results in a
 264 large increase in nC_{60} particle size but no reduction in the energy barrier,
 265 since it is already reduced to zero, so the bare silica deposition trend decreases
 266 (Fig. 3 (a)) or remains the same (Fig. 3 (b)). As explained previously, the
 267 lack of a decrease in the bare silica trend in Fig. 3 (b) is likely due to the
 268 CDC occurring at a CaCl_2 concentration far from the CCC. At intermediate
 269 concentrations of NaCl and CaCl_2 , the two effects offset and result in smaller
 270 changes to the bare silica deposition trend.

271 3.3. *Effect of Attached Phase Humic Acid*

272 In order to elucidate the effect of the HHA coating, the deposition at-
 273 tachment efficiency was calculated at every experimental condition (Eq. 3)
 274 and presented in Fig. 4. For both cations investigated, the HHA coating en-
 275 hanced nC_{60} attachment at low concentrations and hindered attachment at
 276 high concentrations. Though this variable effect has been previously reported
 277 for Suwannee River humic acid (SRHA) in the presence of CaCl_2 (Chen and
 278 Elimelech, 2008), it has never before been reported for humic acid in the
 279 presence of NaCl. The HHA coating raised the attachment efficiency an or-
 280 der of magnitude for both of the lowest NaCl concentrations (Fig. 4 (a)) and
 281 the lowest CaCl_2 concentration (Fig. 4 (b)). As the concentration increased
 282 however, the HHA coating hindered attachment and raised the CDC to near

283 100 mM NaCl (Fig. 4 (a)) or, in the case of CaCl_2 , failed to reach the CDC
 284 within the concentration range investigated (Fig. 4 (b)). The presence of the
 285 HHA coating hindered the attachment for NaCl concentrations of 30 and 55
 286 mM and CaCl_2 concentrations of 0.95 and 1.2 mM. Quite clearly, this behav-
 287 ior deviates from the typical steric stabilization often seen in the presence of
 288 DOM (Chen and Elimelech, 2007, 2008; Terashima and Nagao, 2007; Hyung
 289 et al., 2007; Lin and Xing, 2008) and further validates the variable nature
 290 observed in other deposition studies of NOM coatings (Chen and Elimelech,
 291 2007, 2008; Qu et al., 2012; McNew and LeBoeuf, 2015). In order to further
 292 explain the observed behavior of the HHA-coating, the electrophoretic mo-
 293 bility (EPM) of silica with and without coatings of HHA was measured and
 294 presented in Fig. 5.

295 The bare silica remains negatively charged throughout the concentration
 296 range investigated (Fig. 5), as expected from reported zeta potential values
 297 of SiO_2 thin films (Bousse et al., 1991) and silica sands (Qu et al., 2012).
 298 Interestingly, however, the HHA coating reduced the surface charge (closer
 299 to neutral) of the silica throughout all concentrations investigated, except for
 300 the case of 100 mM NaCl, at which point the two values were statistically
 301 identical. A less negatively charged (closer to neutral) surface would certainly
 302 provide less resistance to nC_{60} deposition through a reduction in electrostatic
 303 repulsion, which is evident by the enhanced nC_{60} attachment at low NaCl
 304 and CaCl_2 concentrations (Fig. 4). If this were the only contributing effect,
 305 however, enhanced attachment would continue throughout the entire range of
 306 concentrations investigated, instead of hindering nC_{60} attachment at higher
 307 concentrations, as observed in Fig. 4. In order to further understand the

Figure 4: nC_{60} deposition attachment efficiency factors as a function of NaCl (a) and $CaCl_2$ (b) concentrations onto bare and HHA-coated silica. Each point consists of three measurements and error bars represent standard deviation. Figure insets provide a more detailed view of experimental values at high electrolyte concentrations, presented on an arithmetic scale.

Figure 5: Electrophoretic mobility measurements of 900 nm silica microspheres with and without coatings of HHA, as a function of NaCl and CaCl_2 concentration. Each value was the result of 10 independent measurements and error bars represent standard deviation.

Figure 6: DLVO interaction energy profile of nC_{60} approaching a silica surface with and without a HHA coating in the presence of 7 (a) and 55 (b) mM NaCl.

complex interactions taking place in this system, a DLVO interaction energy profile was calculated for a 100 nm nC_{60} particle approaching a silica surface with and without a coating of HHA, with properties matching the nC_{60} (Fig. D.7 and Table E.1), silica (Fig. 5), and HHA coating (Fig. 5) measured in this study (details can be found in the Appendix).

In the presence of 7 mM NaCl, the DLVO interaction energy curve predicts a much lower energy barrier to nC_{60} deposition when the silica surface is coated with HHA (Fig. 6 (a)) as observed in Fig. 4 (a). At a concentration of 55 mM NaCl (Fig. 6 (b)) DLVO predicts a much larger EDL screening effect and therefore the energy barrier to nC_{60} deposition is much lower for both cases, bare silica and HHA-coated silica. In the presence of 55 mM NaCl, the energy barrier to nC_{60} deposition is still slightly smaller onto the HHA-coated silica, due to a reduction in surface charge. The significance of this difference, however, is much lower since the energy barrier has nearly disappeared at the increased NaCl concentration. Similar results were ob-

served in the presence of CaCl_2 and are presented in the Appendix (Fig. E.8). Since DLVO does not correctly predict the attachment hindering behavior of the HHA coating at increased NaCl and CaCl_2 concentrations, a non-DLVO force is likely contributing.

The structure of immobilized macromolecules has been shown to depend on ionic strength (Yuan et al., 2008; Hong and Elimelech, 1997) and, in particular, NOM layers have been shown to condense with increased ionic strength (Chen and Elimelech, 2008; Chen et al., 2006). At increased NaCl and CaCl_2 concentrations, the HHA layer compacts and has a more condensed, less extended (smoother) surface with reduced potential for nC_{60} particle entanglement, effectively lowering attachment efficiency (Fig. 4). Furthermore, the compaction of the HHA layer may reduce the amount of available hydrophobic regions for nC_{60} attachment, contributing to the reduction in attachment efficiency. This finding is in agreement with two studies which have reported a reduction in nC_{60} attachment due to the compaction of alginate (Chen and Elimelech, 2008) and humic acid (McNew and LeBoeuf, 2015) layers.

At low electrolyte concentrations, the HHA coating enhances nC_{60} attachment for both mono and divalent cations. By lowering the surface potential of the silica surface, the HHA coating significantly reduces the energy barrier to nC_{60} deposition at low electrolyte concentrations. As electrolyte concentration rises, the HHA coating eventually results in a hindering of attachment for both mono and divalent cations. Though still lowering the surface potential of silica, the HHA coating does not significantly lower the energy barrier to deposition at these elevated electrolyte concentrations, due to the signif-

348 ificant suppression of EDL repulsion resulting in a near zero energy barrier
 349 to nC₆₀ deposition. Furthermore, the structure of the HHA coating likely
 350 condenses at elevated electrolyte concentration, leading to a more compact
 351 and smoother surface with less potential for nC₆₀ particle entanglement, con-
 352 tributing to the observed reduction in attachment efficiency. Because of the
 353 aforementioned reasons, the observed trend in nC₆₀ attachment onto HHA-
 354 coated silica is attributed to the summation of two contributing forces: (1)
 355 the reduction in electrostatic repulsion due to the HHA-coating and (2) HHA
 356 layer compaction at increased NaCl and CaCl₂ concentrations.

357 **4. Conclusions**

358 In this study, we have shown that the presence of a HHA layer can both
 359 hinder and enhance nC₆₀ attachment onto a silica surface, depending on so-
 360 lution chemistry. At low NaCl and CaCl₂ concentrations, the presence of
 361 HHA enhances nC₆₀ attachment due to a reduction in electrostatic repulsion
 362 and the extended confirmation of the HHA layer. At high NaCl and CaCl₂
 363 concentrations, the HHA layer condenses, hindering nC₆₀ attachment. The
 364 findings presented within this study suggest that NOM coatings affect nC₆₀
 365 deposition in a more complex way than previously reported and may play a
 366 major role in determining the environmental fate and transport of ENMs. In
 367 order to further clarify the complex contributions of NOM coatings, different
 368 types of NOM and ENMs should be evaluated while remaining in environ-
 369 mentally relevant solution conditions. Fully understanding the contributions
 370 from NOM coatings may prove crucial to predicting potential pathways of
 371 exposure and their impact on ecosystems and human health.

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377 6. References

378 Grigoriy V Andrievsky, Marina V Kosevich, M Vovk, Vadim S Shelkovsky,
379 and Lyudmila A Vashchenko. On the production of an aqueous colloidal
380 solution of fullerenes. *Journal of the Chemical Society-Chemical Commu-*
381 *nications*, (12):1281–1282, 1995.

382 Luc Bousse, Shahriar Mostarshed, Bart Van Der Shoot, NF De Rooij, Peter
383 Gimmel, and Wolfgang Göpel. Zeta potential measurements of ta_2o_5 and
384 sio_2 thin films. *Journal of colloid and interface science*, 147(1):22–32, 1991.

385 Jonathan Brant, Helene Lecoanet, and Mark R Wiesner. Aggregation and
386 deposition characteristics of fullerene nanoparticles in aqueous systems.
387 *Journal of Nanoparticle Research*, 7(4-5):545–553, 2005.

388 Kai Loon Chen and Menachem Elimelech. Aggregation and deposition ki-
389 netics of fullerene (c-60) nanoparticles. *Langmuir*, 22(26):10994–11001,
390 2006.

391 Kai Loon Chen and Menachem Elimelech. Influence of humic acid on the
392 aggregation kinetics of fullerene (c_60) nanoparticles in monovalent and di-

- 393 valent electrolyte solutions. *Journal of Colloid and Interface Science*, 309
394 (1):126–134, 2007.
- 395 Kai Loon Chen and Menachem Elimelech. Interaction of fullerene (c-60)
396 nanoparticles with humic acid and alginate coated silica surfaces: Mea-
397 surements, mechanisms, and environmental implications. *Environmental*
398 *Science & Technology*, 42(20):7607–7614, 2008.
- 399 Kai Loon Chen, Steven E Mylon, and Menachem Elimelech. Aggregation
400 kinetics of alginate-coated hematite nanoparticles in monovalent and di-
401 valent electrolytes. *Environmental science & technology*, 40(5):1516–1523,
402 2006.
- 403 Rossane C DeLapp, Eugene J LeBoeuf, Jie Chen, and Baohua Gu. Advanced
404 thermal characterization of fractionated natural organic matter. *Journal*
405 *of Environmental Quality*, 34(3):842–853, 2005.
- 406 BV Derjaguin. Theory of the stability of strongly charged lyophobic sols and
407 the adhesion of strongly charged particles in solutions of electrolytes. *Acta*
408 *Physicochim. USSR*, 14:633–662, 1941.
- 409 Menachem Elimelech, Xiadong Jia, John Gregory, and Richard Williams.
410 *Particle deposition & aggregation: measurement, modelling and simula-*
411 *tion*. Butterworth-Heinemann, 1998.
- 412 Benjamin Espinasse, Ernest M Hotze, and Mark R Wiesner. Transport
413 and retention of colloidal aggregates of cmedia: Effects of organic macro-
414 molecules, ionic composition, and preparation method. *Environmental*
415 *science & technology*, 41(21):7396–7402, 2007.

- 416 Jiasong Fang, Delina Y Lyon, Mark R Wiesner, Jinping Dong, and Pedro JJ
417 Alvarez. Effect of a fullerene water suspension on bacterial phospholipids
418 and membrane phase behavior. *Environmental science & technology*, 41
419 (7):2636–2642, 2007.
- 420 Fadri Gottschalk and Bernd Nowack. The release of engineered nanomaterials
421 to the environment. *Journal of Environmental Monitoring*, 13(5):1145–
422 1155, 2011.
- 423 R. Hogg, T.W. Healy, and D.W. Fuerstenau. Mutual coagulation of colloidal
424 dispersions. *Transactions of the Faraday Society*, 62:1638–1651, 1966.
- 425 Seungkwan Hong and Menachem Elimelech. Chemical and physical aspects of
426 natural organic matter (nom) fouling of nanofiltration membranes. *Journal*
427 *of membrane science*, 132(2):159–181, 1997.
- 428 Robert J Hunter. *Zeta potential in colloid science: principles and applica-*
429 *tions*. Academic press, 2013.
- 430 Hoon Hyung, John D Fortner, Joseph B Hughes, and Jae-Hong Kim. Natural
431 organic matter stabilizes carbon nanotubes in the aqueous phase. *Envi-*
432 *ronmental Science & Technology*, 41(1):179–184, 2007.
- 433 Chad T Jafvert and Pradnya P Kulkarni. Buckminsterfullerene’s (c60)
434 octanol- water partition coefficient (kow) and aqueous solubility. *Envi-*
435 *ronmental science & technology*, 42(16):5945–5950, 2008.
- 436 X. Jiang, M. Tong, H. Li, and K. Yang. Deposition kinetics of zinc oxide
437 nanoparticles on natural organic matter coated silica surfaces. *Journal of*
438 *colloid and interface science*, 350(2):427–434, 2010.

- 439 J.P. Kamat, T.P.A. Devasagayam, K.I. Priyadarsini, and H. Mohan. Re-
 440 active oxygen species mediated membrane damage induced by fullerene
 441 derivatives and its possible biological implications. *Toxicology*, 155(1):55–
 442 61, 2000.
- 443 Hélène F Lecoanet, Jean-Yves Bottero, and Mark R Wiesner. Laboratory as-
 444 sessment of the mobility of nanomaterials in porous media. *Environmental*
 445 *science & technology*, 38(19):5164–5169, 2004.
- 446 Yusong Li, Yonggang Wang, Kurt D Pennell, and Linda M Abriola. Inves-
 447 tigation of the transport and deposition of fullerene (c60) nanoparticles
 448 in quartz sands under varying flow conditions. *Environmental science &*
 449 *technology*, 42(19):7174–7180, 2008.
- 450 Daohui Lin and Baoshan Xing. Tannic acid adsorption and its role for stabi-
 451 lizing carbon nanotube suspensions. *Environmental science & technology*,
 452 42(16):5917–5923, 2008.
- 453 Daohui Lin, Xiaoli Tian, Fengchang Wu, and Baoshan Xing. Fate and trans-
 454 port of engineered nanomaterials in the environment. *Journal of environ-*
 455 *mental quality*, 39(6):1896–1908, 2010.
- 456 Meagan S Mauter and Menachem Elimelech. Environmental applications
 457 of carbon-based nanomaterials. *Environmental Science & Technology*, 42
 458 (16):5843–5859, 2008.
- 459 Coy P McNew and Eugene J LeBoeuf. The role of attached phase soil and
 460 sediment organic matter physicochemical properties on fullerene (nc60) at-
 461 tachment. *Chemosphere*, 2015.

- 462 Andrew McWilliams. Nanotechnology: A realis-
463 tic market assessment, 2014. URL [http://www.](http://www.bccresearch.com/market-research/nanotechnology/nanotechnology-market-assessment-report-nan031f.html)
464 [bccresearch.com/market-research/nanotechnology/](http://www.bccresearch.com/market-research/nanotechnology/nanotechnology-market-assessment-report-nan031f.html)
465 [nanotechnology-market-assessment-report-nan031f.html](http://www.bccresearch.com/market-research/nanotechnology/nanotechnology-market-assessment-report-nan031f.html).
- 466 N Nakajima, C Nishi, FM Li, and Y Ikada. Photo-induced cytotoxicity of
467 water-soluble fullerene. *Fullerene Science and Technology*, 4(1):1–19, 1996.
- 468 Andre Nel, Tian Xia, Lutz Mädler, and Ning Li. Toxic potential of materials
469 at the nanolevel. *Science*, 311(5761):622–627, 2006.
- 470 Xiaolei Qu, Pedro JJ Alvarez, and Qilin Li. Impact of sunlight and humic
471 acid on the deposition kinetics of aqueous fullerene nanoparticles (nc60).
472 *Environmental Science & Technology*, 46(24):13455–13462, 2012.
- 473 Ilya Reviakine, Diethelm Johannsmann, and Ralf P Richter. Hearing what
474 you cannot see and visualizing what you hear: interpreting quartz crystal
475 microbalance data from solvated interfaces. *Analytical chemistry*, 83(23):
476 8838–8848, 2011.
- 477 Günter Sauerbrey. Verwendung von schwingquarzen zur wagung dünner
478 schichten und zur mikrowagung. *Z. Phys*, 155:206–222, 1959.
- 479 Frank J Stevenson. *Humus chemistry: genesis, composition, reactions*. Wiley,
480 New York, 2nd edition, 1994.
- 481 Motoki Terashima and Seiya Nagao. Solubilization of [60] fullerene in water
482 by aquatic humic substances. *Chemistry Letters*, 36(2):302–303, 2007.

- 483 E.J.W. Verwey. Theory of the stability of lyophobic colloids. *The Journal of*
484 *Physical Chemistry*, 51(3):631–636, 1947.
- 485 Marina V Voinova, M Rodahl, M Jonson, and B Kasemo. Viscoelastic acous-
486 tic response of layered polymer films at fluid-solid interfaces: continuum
487 mechanics approach. *Physica Scripta*, 59(5):391, 1999.
- 488 Yonggang Wang, Yusong Li, John D Fortner, Joseph B Hughes, Linda M
489 Abriola, and Kurt D Pennell. Transport and retention of nanoscale c60
490 aggregates in water-saturated porous media. *Environmental science &*
491 *technology*, 42(10):3588–3594, 2008a.
- 492 Yonggang Wang, Yusong Li, and Kurt D Pennell. Influence of electrolyte
493 species and concentration on the aggregation and transport of fullerene
494 nanoparticles in quartz sands. *Environmental toxicology and chemistry*, 27
495 (9):1860–1867, 2008b.
- 496 Baoling Yuan, Mai Pham, and Thanh H Nguyen. Deposition kinetics of
497 bacteriophage ms2 on a silica surface coated with natural organic matter
498 in a radial stagnation point flow cell. *Environmental science & technology*,
499 42(20):7628–7633, 2008.

500 **Appendix A. nC₆₀ and HHA Stock Preparation Details**

501 The method used to produce the stock nC₆₀ suspension in this study is
502 similar to that provided by Andrievsky et al. (1995) and identical to that
503 used in our previous publication (McNew and LeBoeuf, 2015). First, ap-
504 proximately 35 mg of C₆₀ powder was dissolved in 30 mL toluene, forming a

505 clear, dark purple solution. Then, 5 mL of this mixture was introduced into a
506 beaker containing 50 mL deionized water, forming two distinct phases. This
507 solution was then sonicated (Fisher Sonic Dismembrator 300, Pittsburgh,
508 PA) for more than 3 hours in a fume hood to allow for evaporation of the
509 toluene. The beaker was topped off with deionized water every 20 minutes to
510 account for the evaporated water. The resulting transparent, brown-yellow
511 liquid was filtered through 0.22 μm pore size membrane filter paper (Fisher,
512 GSWP02500), resulting in a clear, bright yellow liquid. This method was
513 repeated a total of 6 times to produce approximately 330 mL of nC_{60} stock
514 suspension.

515 50 mg of dry HHA was introduced into 400 mL deionized water and stirred
516 for more than 3 hours. During this time, the pH was adjusted drop wise with
517 0.1 N NaOH to a pH of 10.0 to allow the HHA to completely dissolve. After
518 3 hours and complete dissolution of the HHA, the pH was adjusted drop wise
519 with 0.1 N HCl down to a pH of 5.6 and filtered through 0.22 μm pore size
520 membrane filter paper. The stock solution was then diluted to 500 mL total
521 volume to make for a concentration of 0.1 g/L HHA.

522 **Appendix B. Detailed QCM-D Experimental Protocol**

523 For nC_{60} deposition experiments taking place on bare silica, the desired
524 electrolyte type and concentration was first flowed across the sensor surface
525 until a stable baseline was achieved. The signal was considered stable when
526 the third overtone of frequency (f_3) failed to drift more than 0.3 Hz over
527 the course of 10 minutes. The nC_{60} stock was then diluted to 6.05 mg/L
528 with a measured amount of electrolyte to form the desired concentration and

529 immediately introduced into the measurement chamber. As the nC₆₀ flowed
 530 across the sensor surface, the first 120 seconds of the drop in frequency were
 531 used to calculate the initial deposition rates for each set of experimental
 532 conditions.

533 For nC₆₀ deposition experiments taking place on PLL or HHA surfaces,
 534 the surfaces were first constructed using the following method. The initial
 535 baseline was collected in a buffer solution consisting of 10 mM HEPES and
 536 100 mM NaCl. Then, 2 mL of a 0.1 g/L PLL solution was introduced,
 537 containing the same HEPES and NaCl concentrations. During this time,
 538 oppositely charged PLL attached rapidly to the surface, forming a positively
 539 charged layer. The PLL layer was then rinsed with 2 mL of the HEPES
 540 solution. If PLL was the terminal layer, then the nC₆₀ deposition experiment
 541 proceeded from here, as described previously. If not, the PLL layer was then
 542 rinsed with 2 mL of 1 mM NaCl, followed by 2 mL of 0.1 g/L HHA. During
 543 this period, the negatively charged HHA attached rapidly to the oppositely
 544 charged PLL layer, forming a second layer. After the HHA layer was rinsed
 545 with 2 mL of 1 mM NaCl, the nC₆₀ deposition experiment proceeded as
 546 described previously.

547 **Appendix C. Reynolds and Peclet Number Calculations**

548 In order to calculate Re and Pe of the QCM-D system, a parallel-plate
 549 channel configuration was assumed (Elimelech et al., 1998).

$$Re = \frac{\rho v L}{\mu} \quad (C.1)$$

550 Eq. C.1 was used to calculate the Re of the QCM-D system where ρ is the

551 density of water, v is the flow velocity within the chamber ($2*10^{-4}$ ms $^{-1}$), L
552 is the half depth of the channel ($3.2*10^{-4}$ m), and μ is the viscosity of water.

$$Pe = \frac{3va^3}{2L^2D} \quad (C.2)$$

$$D = \frac{kT}{3\pi\mu d} \quad (C.3)$$

553 Eq. C.2 was used to calculate the Pe of the QCM-D system where a
554 is the nC₆₀ particle radius and D is the diffusion coefficient. The diffusion
555 coefficient was estimated using the Stokes-Einstein equation (Eq. C.3) where
556 k is the Boltzmann constant ($1.38*10^{-23}$ JK $^{-1}$), T is the temperature (298
557 K), and d is the nC₆₀ particle diameter.

558 Appendix D. nC₆₀ Characterization Details

Figure D.7: The intensity weighted average hydrodynamic particle diameter and electrophoretic mobility of nC₆₀ at each NaCl and CaCl₂ concentration investigated. Error bars represent standard deviation.

559 Appendix E. DLVO Calculations

560 By assuming a sphere-plate interaction, Eq. E.1 was used to calculate the
 561 van der Waals attraction energy between nC₆₀ and the coated or uncoated
 562 silica surface (Hogg et al., 1966), where A is the combined Hamaker constant
 563 for the nC₆₀ water silica system (4.71×10^{-21} J) (Qu et al., 2012), a is the
 564 radius of nC₆₀ particles at a given electrolyte concentration (Fig. D.7), h is
 565 the separation distance between the nC₆₀ particle and silica sensor surface,

566 and λ is the characteristic wavelength of interaction (usually assumed to be
 567 100 nm) (Jiang et al., 2010).

$$V_A = -\frac{Aa}{6h} \left(1 + \frac{14h}{\lambda}\right)^{-1} \quad (\text{E.1})$$

568 Eq. E.2 was used to calculate the electrical double layer repulsion energy
 569 between nC₆₀ and the coated or uncoated silica surface (Hogg et al., 1966),
 570 where ϵ_0 is the dielectric permittivity in a vacuum ($8.85 \times 10^{-12} \text{ C}^2 \text{ J}^{-1} \text{ m}^{-1}$),
 571 ϵ_r is the relative dielectric permittivity of water (78.3), ζ_p is the zeta potential
 572 of the nC₆₀ particles at a given electrolyte concentration (Table E.1), ζ_c is the
 573 zeta potential of the coated or uncoated silica surface at a given electrolyte
 574 concentration (Fig. 5), and κ is the inverse Debye length.

$$V_R = \pi\epsilon_0\epsilon_r a \left\{ 2\zeta_p\zeta_c \ln \left[\frac{1 + \exp(-\kappa h)}{1 - \exp(-\kappa h)} \right] + (\zeta_p^2 + \zeta_c^2) \ln [1 - \exp(-2\kappa h)] \right\} \quad (\text{E.2})$$

575 Eq. E.3 was used to calculate the inverse Debye length for the electrolyte
 576 system, where e is the elementary charge, n_j is the number concentration of
 577 ions in the bulk solution, z_j is ion valence, k is the Boltzmann constant, and
 578 T is the absolute temperature.

$$\kappa = \sqrt{\frac{e^2 \sum n_j z_j^2}{\epsilon_0 \epsilon_r k T}} \quad (\text{E.3})$$

Figure E.8: DLVO interaction energy profile of nC_{60} approaching a silica surface with and without a HHA coating in the presence of 0.55 (a) and 1.2 (b) mM CaCl_2 .

Table E.1: nC_{60} zeta potential values for use in DLVO calculations. Estimated from the electrophoretic mobility values using the Smoluchowski approximation (Hunter, 2013).

Electrolyte Concentration	nC_{60} Electrophoretic Mobility ($10^{-4}\text{cm}^2\text{V}^{-1}\text{s}^{-1}$)	Zeta Potential (mV)
7 mM NaCl	-3.43	-44.1
16 mM NaCl	-3.36	-43.1
30 mM NaCl	-2.70	-34.7
55 mM NaCl	-2.80	-36.9
100 mM NaCl	-2.37	-30.5
0.30 mM CaCl_2	-1.30	-16.7
0.55 mM CaCl_2	-1.55	-19.9
0.95 mM CaCl_2	-1.52	-19.5
1.2 mM CaCl_2	-1.35	-17.3