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Aggregation of reduced graphene oxide and its nanohybrids with magnetite and elemental silver under environmentally relevant conditions

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Abstract

The aggregation and long-term (25 d) sedimentation behaviors of reduced graphene oxide (RGO) and its three successively self-assembled nanohybrids with magnetite (Fe_3O_4) and zerovalent silver (Ag^0) nanoparticles have been investigated. The aggregation behaviors of the nanomaterials in NaCl and CaCl_2 were found to be in good agreement with the Derjaguin–Landau–Verwey–Overbeek (DLVO)-type interactions and the Schulze-Hardy rule. The colloidal stability decreased with the increasing ratios of the edge-based functional groups (COO^- and C=O) to the total oxygen-containing functional groups decorated on the basal planes (C-O) and edges of RGO, as quantified by X-ray photoelectron spectroscopy analysis. In the presence of natural organic matter (NOM), the aggregation of RGO and its nanohybrids was greatly inhibited as a result of the enhanced electrosteric repulsions arising from the adsorbed NOM macromolecules. The long-term sedimentation kinetics results showed that the RGO nanohybrids were less stable in synthetic groundwater containing higher electrolyte concentrations, which was likely because of the greater charge screening or neutralization effect imparted by higher monovalent and divalent electrolyte concentrations. Our findings have important implications for evaluating the environmental impact and toxicity of the emerging class of multifunctional nanohybrids whose environmental behaviors are currently largely unknown.

Keywords

graphene oxide; magnetite; silver; nanohybrid; aggregation; sedimentation

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1. Introduction

In congruence with an ever-increasing demand for nanomaterials (NMs), carbonaceous NMs (CNMs) such as fullerenes, carbon nanotubes, and graphene oxide (GO), and metallic NMs (MNM) such as silver (Ag), ZnO, and TiO₂ have become the major components of several consumer products in recent years (Vance et al., 2015). CNMs are high-performance materials owing to their light weight, tunable morphology, and multifunctional properties (Gogotsi and Presser, 2013). Over the past few decades, a wide variety of NMs have been utilized for environmental remediation (Dupont et al., 2014; Madadrang et al., 2012; Mahdavi et al., 2013). However, there are several challenges that must be overcome before all of the advantages of NMs can be utilized for many potential applications. For example, in an aquatic environment, bare CNMs tend to form larger aggregates because of inherent hydrophobicity and strong van der Waals attractions (Al-Hamadani et al., 2015). This behavior greatly deteriorates the overall performances of NMs by altering the interfacial surface energy, which lowers the adsorption capacity otherwise desired for water and wastewater treatment. On the other hand, bare MNMs (e.g., ZnO) have very limited applications because of their dissolution, complexation properties, and vulnerability to surface passivation by denitrifying bacteria in soil and groundwater (Ma et al., 2013).

With the recent growing interest in nanotechnology, multifunctional nanohybrids have attracted great attention because they can overcome the above-mentioned disadvantages (Yan et al., 2013) and provide a promising solution to major threats to human health (Zaporotskova et al., 2015). In recent years, GO-MNMs (e.g., GO-Fe₃O₄ and GO-Ag⁰) have generated significant interest because of their high mechanical strength and strong antibacterial activity, with great potentials in (electro)catalysis (Jafri et al., 2010), electrochemical sensing (Guo et al., 2010), and environmental remediation (Liu et al., 2011). Albeit GO is a good supporting material for MNMs, it often requires further treatment such as chemical reduction to produce a graphene-like film with high electrical conductivity and thermal stability. This suggests that GO can be expected to be present in a reduced form (RGO) in the environment (Salas et al., 2010). Recent studies have demonstrated that RGO is more toxic than its unreduced form because it produces more reactive oxygen species (ROS) in comparison. The ROS exert toxic effects by inducing oxidative stress in human cells (Jaworski et al., 2015). The metal/metal oxide nanoparticles (NP) that get detached from the GO-MNMs also exert detrimental effects on living organisms when they are exposed to competing ions in aqueous solutions and proteins in cellular media (Maiorano et al., 2010).

Although considerable research effort has been devoted to the study of the bioavailability and ecotoxicity of GO-MNM hybrids in the environment in recent years, our current understanding of their environmental fate and transport is restricted to the individual (single-component) NPs such as RGO, Fe₃O₄, and Ag NPs (Chowdhury et al., 2015; Huynh and Chen, 2011; Tóth et al., 2012). In this context, critical coagulation concentration (CCC) is a well-known concept that allows a coherent overview of the colloidal stability of NPs. CCC is defined as the electrolyte concentration that electrostatically counterbalances the attractive and repulsive forces between the nanoparticles (Jiang et al., 2017). The CCC values of individual NPs have been reported to be 30–35 mM NaCl for RGO (Chowdhury et al.,

2015), ~1 mM NaCl for uncoated magnetite (Tóth et al., 2012), and 111.5 mM NaCl for polyvinylpyrrolidone (PVP)-stabilized Ag⁰ NPs (Huynh and Chen, 2011). To the best of our knowledge, there is only one published study on the aggregation behavior of RGO-MNMs (i.e., RGO-TiO₂ nanohybrids) (Hua et al., 2016). It was found that the stability of RGO-TiO₂ lay between that of the individual NP components (bare TiO₂ and graphene) and was influenced by environmental conditions such as pH, type of salts, and ionic strength (IS). In general, the colloidal stability of the individual NPs and NPs doped with other elements could be described by the Derjaguin–Landau–Verwey–Overbeek (DLVO)-type interactions and the concentration of surface functional groups (Adeleye et al., 2017). Furthermore, it was found that natural organic matter (NOM) present in the natural and engineered aquatic environments enhanced the stability of the copper-based NPs by electrostatic and steric repulsions (Adeleye et al., 2014). However, a complete understanding of the role of the deposition of metal/metal oxides on RGO in aggregation kinetics is lacking, and the effect of the various influencing factors on the stability of nanohybrid is still inconclusive.

To address these limitations, a detailed study of the stability, aggregation, and long-term sedimentation kinetics of RGO-MNMs was investigated in an aqueous system. To this end, Fe₃O₄ and Ag⁰ NPs were decorated on the bare RGO surface (RGO/Fe₃O₄, RGO/Ag⁰, and RGO/Fe₃O₄/Ag⁰). The fate of the well-dispersed RGO and its nanohybrids was investigated by varying pH, salt type, IS, and NOM conditions. Variations of the CCC values of RGO-based nanohybrids were interpreted by considering the ratios of the edge-based functional groups (carboxyl COO[−] and carbonyl C=O) to the total oxygen-containing functional groups decorated on the basal planes (C–O) and edges of RGO. The morphological change, electrokinetic properties, hydrodynamic size, composition, and surface functional groups of the nanomaterials were also identified in an effort to acquire a comprehensive understanding of the stability of such nanomaterials in the environment. Our findings represent the first attempt at advancing the understanding of aggregation kinetics of aqueous RGO-MNMs under environmentally relevant conditions.

2. Materials and methods

2.1. Material preparation

GO was prepared by the oxidative treatment of natural graphite flakes (Asbury Carbons, 3061 grade material) using the modified Hummers method (Chen et al., 2015). The detailed procedures and reagents used in the synthesis of RGO and its nanohybrids are given in the Supplementary Information (SI). All reagents, including NaCl, CaCl₂, HCl, NaOH, NaNO₃, H₂SO₄, KMnO₄, NaBH₄, FeCl₃·6H₂O, AgNO₃, HNO₃, Cu(NO₃)₂, Cd(NO₃)₂, Co(NO₃)₂, Ni(NO₃)₂, Pb(NO₃)₂, Zn(NO₃)₂, MgCl₂, MgSO₄, KHCO₃, NaHCO₃, CaCO₃, KNO₃, Ca(NO₃)₂, and CaSO₄·2H₂O were of analytical grade (minimum purity of 99%) and purchased from Fischer Scientific (Pittsburgh, PA). Deionized (DI) water purified by a Barnstead Nanopure system (18.2 MΩ cm^{−1} at 25 °C) was used to prepare all the aqueous solutions.

2.2. Characterization of RGO and its nanohybrids with magnetite and elemental silver

The morphology and size of the prepared materials were characterized using a transmission electron microscope (TEM, Titan G2 with ChemiSTEM Cs Probe, FEI Company) and a field emission-scanning electron microscope (FE-SEM, Hitachi SU8220, Japan). Mineralogical compositions were identified using an X-ray diffractometer (XRD) (Rigaku MiniFlex) with Fe K α radiation source ($\lambda = 1.937 \text{ \AA}$) at 30 kV and 15 mA. The surface functional groups (i.e., C–C/C=C, C–O, C=O, COO[−], and O–H) of the materials were characterized via X-ray photoelectron spectroscopy (XPS, ULVAC-PHI Quantera SXM). A Nicolet 6700 FT-IR spectrometer (Thermo Nicolet) was used to record the Fourier transform infrared (FT-IR) spectra of the prepared materials in the wavenumber range of 4000–400 cm^{−1}. Electrokinetic and hydrodynamic properties of RGO and its nanohybrids in suspensions were analyzed by a light-scattering method using the ZetaSizer Nano ZS analyzer (Malvern Instruments) as a function of pH, type of electrolyte (NaCl and CaCl₂), IS, and NOM (Suwannee River humic acid (SRHA) and fulvic acid (SRFA)) at pH 6.3 $\pm$ 0.1. Prior to electrophoretic mobility (EPM) and hydrodynamic diameter (D_h) measurements, the samples were ultrasonicated in a Branson 1510 ultrasonication bath with an output of 70 W at a frequency of 42 kHz for 5 min and then vortexed for 10 s. The measured EPM was converted to the apparent zeta potentials based on the Smoluchowski equation (Fig. S1) (Elimelech et al., 1995; Park et al., 2016).

2.3. Aggregation kinetics experiments

The changes in the hydrodynamic diameters of bare RGO and its nanohybrids were determined by time-resolved dynamic light scattering (TRDLS) method on the ZetaSizer Nano ZS analyzer (described above) (Zheng et al., 2016). For all the light-scattering D_h measurements of the NP aggregates, 1 mL of 20 mg L^{−1} of the ultrasonicated (5 min) and vigorously vortexed (10 s) NP suspensions containing electrolyte or NOM was pipetted into a standard cuvette and then immediately loaded into the ZetaSizer instrument. The acquisition of the first data point began within 30 s after sample loading. Size measurements were performed at 25 °C with the intensity of a scattering angle of 173°. The autocorrelation functions were allowed to accumulate for 15 s over a period of 25 min for each measurement. A total sample volume of 1 mL, including background electrolytes (4–200 mM NaCl and 0.06–2.5 mM CaCl₂) and 5 mg C L^{−1} of SRHA or SRFA in 100 mM NaCl solutions (pH 6.3 $\pm$ 0.1), was placed in 10 mm disposable polystyrene cuvettes (Sarstedt, Germany) to achieve an adequate signal from the light scattering unit. The initial rate of increase (k) of D_h was defined as the initial slope obtained by the linear regression analysis of the early stage kinetics curve up to 1.5 $D_{h, \text{initial}}$ (Chen and Elimelech, 2006; 2007).

$$k \propto \frac{1}{N_0} \left(\frac{dD_h(t)}{dt} \right)_{t \rightarrow 0}$$

where N_0 represents the initial particle concentration in water. The integrity of RGO and its nanohybrids was examined by monitoring the amount of leached iron/silver ions using an inductively coupled plasma-mass spectrometer (ICP-MS, Agilent 7900, Agilent

Technologies). The ion (i.e., Fe and Ag) concentrations after ultrasonic irradiation for 30 min followed by 24 h of storage in deionized water were lower than 3 mg L^{-1} , which indicated the high integrity of the RGO nanohybrids.

2.4. Long-term sedimentation kinetics experiment

The dynamic aggregation processes of RGO and its nanohybrids were examined by monitoring the sedimentation kinetics in synthetic surface and ground waters for 25 days. The artificial surface and ground waters were prepared by following a previously reported protocol (Chowdhury et al., 2015). Details of the preparation and compositions of these water media are provided in the Supporting Information (SI) S5 and Table S3. The suspensions of RGO and its nanohybrids in the synthetic surface and ground waters were placed in borosilicate glass bottles (Fisher Scientific) and ultrasonicated for 30 min. Ultraviolet-visible (UV-Vis) spectra were measured using a Lambda 35 UV-Vis spectrophotometer (Perkin-Elmer) to identify the sedimentation processes at wavelengths of 237.9, 236.9, 244, and 238.1 nm for RGO, RGO/Fe₃O₄, RGO/Ag⁰, and RGO/Fe₃O₄/Ag⁰, respectively. The optical absorbances of the samples (20 mg L^{-1}) at $t = 0$ indicate the point at which UV-Vis absorbance was first acquired after vortexing the ultrasonicated NP suspension for 10 s and then loading into the UV-Vis spectrophotometer within 1 min.

3. Results and discussion

3.1. Characterization of RGO and its nanohybrids with magnetite and elemental silver

The morphologies and sizes of bare RGO and its nanohybrids were investigated by TEM and FE-SEM at different magnifications. Several wrinkles were found on the transparent RGO sheets after the dispersion of Fe₃O₄ and Ag⁰ NPs on RGO, which indicated that the RGO layer was extremely thin (~1 nm thickness after a mild ultrasonication) (Muthoosamy et al., 2015). This is generally observed for the exfoliated GO (Liu et al., 2016). In the TEM images, the RGO nanohybrids exhibited gel networks wrapped in the RGO interlayers, and a fluffy structure could be clearly seen when Ag⁰ NPs were loaded on RGO or RGO/Fe₃O₄ (Fig. 1b, c).

XRD patterns obtained using Fe K α as the radiation source confirmed the internal structures of pristine graphite, RGO, RGO/Fe₃O₄, RGO/Ag⁰, and RGO/Fe₃O₄/Ag⁰ nanohybrids by their characteristic peaks (Fig. S5). A major diffraction peak of RGO was observed at 32.6° (2θ), which was in good agreement with the XRD pattern of pristine graphite (JCPDS No. 01–089-8487). This peak corresponded to the (002) crystalline plane of graphite with a *c*-axis interlayer space of 3.45 Å. For RGO/Fe₃O₄ and RGO/Fe₃O₄/Ag⁰, a diffraction peak at 45.6° (2θ), corresponding to the (311) plane, indicated the formation of the magnetite crystal (Fe₃O₄, JCPDS No. 01–075-0449) with an interlayer space of 2.50 Å, presumably as a result of the complete oxidation of the ferrous iron on the RGO surface (Amemiya et al., 2007). The strong XRD peaks of RGO/Ag⁰ and RGO/Fe₃O₄/Ag⁰ at 48.6° and 56.7° with an interlayer space of 2.35 and 2.03 Å, respectively, were assigned to the (111) and (200) planes of silver-3C, *syn* (JCPDS No. 01–087-0597). The RGO/Fe₃O₄ nanohybrid recovered the graphitic crystal structure with a broad peak of the (002) plane of graphite and a strong peak on the surface centered at 45.5° (2θ), which belonged to the (311) plane of magnetite.

XPS was used to determine the distributions of the various C and O components of RGO and its nanohybrids. Wide XPS survey, C1s, and O1s spectra of RGO, RGO/Fe₃O₄, RGO/Ag⁰, and RGO/Fe₃O₄/Ag⁰ are presented in Fig. 1d, f. The C1s XPS spectra were deconvoluted into different oxygen-containing functional groups and the results are summarized in Table S1 (SI). The characteristic peaks observed in the XPS spectra were those of non-oxygenated (aromatic) carbon (C–C/C=C at 284.1–284.9 eV), epoxy or hydroxyl carbon (C–O at 285.1–286.1 eV), carbon bound to alcohol, phenol, or ethers (C=O at 287.0–287.9 eV), and carboxylate carbon (COO[−] at 288.3–288.8 eV) (Hartono et al., 2009). The deposition of Fe₃O₄ NPs on RGO led to an appreciable decrease in the intensity of the C1s peak of RGO at 284.3 eV and an increase in intensity of the peak at 285.6 eV. Thus, there was a peak shift by 1.3 eV towards a higher binding energy because of the electronic change upon deposition of Fe₃O₄ on RGO, which implied that the non-oxygenated (aromatic) carbon were converted to the epoxy/hydroxyl carbon. The O1s peaks observed at 530.0–530.4, 531.4–531.7, 532.2–532.6, and 533.1–533.9 eV were assigned to quinone-type COO[−]/C=O, C–O/O–H, C=O, and COO[−] functional groups, respectively (Heo et al., 2013). Thus, the XPS spectra indicated that most of the functional groups in RGO were present in the form of carbonyl and carboxyl groups, other oxygenated species such as C–O/O–H, COO[−], and quinone groups.

FT-IR spectra reflect the alterations in the functional groups after hybridization of RGO with Fe₃O₄ or Ag⁰ NPs. The influence of metal deposition on the FT-IR spectra of RGO is presented in Fig. S6. The sharp peaks of RGO at 1402, 1173, and 877 cm^{−1} can be assigned to aliphatic C–H bending, C–O–C stretching, and aromatic C–H bending, respectively. In the presence of Fe₃O₄ and Ag⁰ NPs on the RGO surface, the finger print region shifted from 1600–660 cm^{−1} for RGO to 1800–1100 cm^{−1} for its nanohybrids. This suggests that the RGO nanohybrids were comprised of benzene rings, ketones, and carbonyl groups in conjunction with functional groups that showed the C=O stretching of amide groups (1633–1630 cm^{−1}) and C–N stretching of the amide II band (1540–1537 cm^{−1}), which may be ascribed to a preferred combination of RGO with metal cations in high energy states (Yang et al., 2010). The vibration energy level of C=O increased in the following order: RGO/Ag⁰ > RGO/Fe₃O₄/Ag⁰ > RGO/Fe₃O₄, which is in line with the stabilization of these NPs (described below). The weak O–H (3300 cm^{−1}) and C–H (2926 cm^{−1}) stretching vibrations were also observed for RGO nanohybrids.

3.2. Electrokinetic and hydrodynamic properties of RGO and its nanohybrids with magnetite and elemental silver

EPM measurements showed that RGO and its nanohybrids remained negatively charged over the broad pH range of 3–12 (Fig. 2a). These negative values of EPM can be attributed to the deprotonation of carboxylic and phenolic groups, which play a significant role in the aqueous stability of the nanohybrids (Bhadra et al., 2016). It is worth noting that the variation of EPM as a function of pH and deposition with different metal species was partly a result of an increase in the ionizable carbonyl and carboxylic acid groups, as confirmed by XPS analysis (Fig. 1) (Duch et al., 2011). The isoelectric point (IEP) of RGO/Ag⁰ was found to be less than 3.0, which lies between the IEPs of RGO (pH_{IEP} ~2.5) (Lou et al., 2017) and Ag NPs (pH_{IEP} ~3.1) (Cornelis et al., 2013). Although the previously reported IEP of

magnetite ($pH_{IEP} \sim 6.5$) (Zhao et al., 2008) is higher than that of RGO, a large upward shift of IEP was not observed for RGO/Fe₃O₄ and RGO/Fe₃O₄/Ag⁰ presumably because of the low Fe₃O₄ loading in the nanohybrids (~ 5.4 at% for Fe₂p, Table S2 (SI)). This low IEP is consistent with the previously reported value for the RGO/magnetite nanohybrid ($pH_{IEP} \sim 2.7$) (Hu and Tang, 2013). At pH higher than the IEP of RGO and its nanohybrids, the surface becomes more negatively charged, which increases the stability of the NPs in the aqueous solution. While the absolute values of EPM remained the same from pH 3 to 6, they increased significantly in the pH range from 7 to 12, which could be attributed to an increase in the negative surface charge by the deprotonation of carboxylic groups with increasing pH. This suggests that at low pH, the RGO nanohybrids are more prone to aggregate in the aquatic environment. The response of D_h over a wide range of pH is presented in Fig. 2b. We observed that D_h decreased as the pH increased from 3 to 12, in accordance with the change in EPM.

The EPM and D_h measurements as a function of type of electrolyte salts (NaCl and CaCl₂) and concentrations are presented in Fig. 2c–f. With increasing IS, the EPMs of RGO and its nanohybrids became higher as the surface charge was more screened or neutralized by the monovalent and divalent cations. The influence of CaCl₂ concentration on D_h was much higher than that of NaCl for RGO and its nanohybrids. Interestingly, RGO and RGO/Fe₃O₄ appeared to have a much greater influence on D_h , even though there was no notable change in EPM. The absolute values of EPM for RGO/Ag⁰ were higher than those for RGO, RGO/Fe₃O₄, and RGO/Fe₃O₄/Ag⁰ at all pH values and NaCl/CaCl₂ concentrations, and, correspondingly, lower aggregation of RGO/Ag⁰ was observed.

The effect of SRHA and SRFA on the electrokinetic properties of RGO and its nanohybrids in the presence of 100 mM NaCl is illustrated in Fig. S8 (SI). The total organic carbon (TOC) content for the particulate SRHA and SRFA ($< 0.2 \mu m$) was adjusted to 5 mg C L⁻¹ in the suspension, which represents the typical organic matter levels (0.1 to 10–20 mg C L⁻¹) in surface and ground waters (Thurman, 2012). Previous studies have shown that the hematite NMs exhibited increased negative charges in the presence of NOM (Chen et al., 2006). However, the addition of SRHA and SRFA to the suspensions of RGO and its nanohybrids had a negligible effect on EPM, as observed for pristine and oxidized carbon nanotubes and fullerenes (Chowdhury et al., 2012; Saleh et al., 2010).

3.3. Aggregation kinetics of RGO and its nanohybrids with magnetite and elemental silver

The aggregation behavior of RGO and its nanohybrids was systematically investigated over a wide range of NaCl and CaCl₂ concentrations. The aggregation profiles showed that the colloidal stability was strongly dependent on the electrolyte valence and IS (Fig. S7). An increase in the NaCl and CaCl₂ concentrations reduced the colloidal stability of these materials because of the elevated degree of charge screening (Na⁺ and Ca²⁺) and neutralization (Ca²⁺) (Huynh and Chen, 2011). This was also evident by the increase in attachment efficiency (α) in the reaction-limited regimes (Fig. 3). Among the four samples investigated, the RGO/Ag⁰ nanohybrid showed the highest stability over the entire range of NaCl and CaCl₂, and the attachment efficiency followed the order $\alpha_{RGO/Fe_3O_4} > \alpha_{RGO} > \alpha_{RGO/Fe_3O_4/Ag^0} > \alpha_{RGO/Ag^0}$. The influence of Na⁺ and Ca²⁺ on the colloidal stability

decreased as concentration of the oxygen-containing functional groups at the edges of RGO was reduced after Ag⁰ or Fe₃O₄/Ag⁰ NPs were loaded on RGO (Table S2). This suggests that larger aggregates of RGO are more likely to form by face-to-face interaction in the presence of NaCl and CaCl₂. The attachment efficiencies in the reaction- and diffusion-limited regimes agreed with the DLVO-type of interactions (attractive van der Waals and repulsive electrostatic forces) (El Badawy et al., 2012).

The CCCs of RGO, RGO/Fe₃O₄, RGO/Ag⁰, and RGO/Fe₃O₄/Ag⁰ were determined by using Eq. (2) in the SI and found to be 43.8 ± 0.9 , 41.2 ± 0.5 , 96.3 ± 3.8 , and 83.2 ± 2.6 mM NaCl and 0.71 ± 0.03 , 0.69 ± 0.02 , 2.30 ± 0.04 , and 1.31 ± 0.06 mM CaCl₂, respectively (Table 1).

These CCC values increased with the increasing EPM of the aggregated RGO and its nanohybrids at pH 6.3 ± 0.1 , presumably as a result of the stronger electrostatic repulsive energy making the particles less sensitive to charge screening effects. The CCC of RGO was similar to the value reported for the intermediately-reduced GO (30–35 mM NaCl) (Chowdhury et al., 2015) and N₂H₄-reduced GO (41 mM NaCl) (Qi et al., 2016). The CCCs of the RGO nanohybrids were between the calculated CCC values of bare RGO and those reported for naked magnetite NPs (~1 mM NaCl) (Tóth et al., 2012) at pH ~6.3 and for PVP-Ag NPs (111.5 mM NaCl) (Huynh and Chen, 2011). It is noteworthy that the CCC values in CaCl₂ were significantly lower than those in NaCl, likely because divalent cations are more effective in reducing the interaction energy barrier. This can be explained by the Schulze-Hardy rule, which states that the ratio of the CCC of counter-ions, i.e., CaCl₂/NaCl, is inversely proportional to the sixth power of their valence ($z = 2$) (Elimelech et al., 2013; Hardy, 1899). These ratios were $z^{-5.95}$, $z^{-5.90}$, $z^{-5.39}$, and $z^{-5.99}$ for RGO, RGO/Fe₃O₄, RGO/Ag⁰, and RGO/Fe₃O₄/Ag⁰, respectively, and were in good agreement with the Schulze-Hardy rule (Table 1). In this work, we have correlated the CCC values of RGO and its nanohybrids in NaCl and CaCl₂ with the ratios of edge-based oxygen-containing functional groups such as C=O and COO⁻, which were identified by XPS. As an indication of the various interactions of RGO, the edge ratios of functional groups were negatively correlated to the CCC values, as shown in Fig. 4. Higher ratios of the edge-based functional groups indicated a lesser sensitivity for the edge-edge interactions with the electrolyte cations. This indicated that the aggregation of RGO nanosheets was more likely to occur by basal plane stacking, which decreased the aggregate-resistance in water. Counterintuitively, the degree of this reduction, as indicated by the atomic C/O ratio, poorly correlated with the aggregation tendency of these materials. This behavior has been previously demonstrated for the GO and RGOs in artificial ground water (Qi et al., 2016).

The major approximations in the DLVO theory of interparticle interactions (sphere-sphere interaction), which combines the attractive van der Waals forces (V_{vdW}) and the repulsive electrostatic forces (V_{ed}) are the use of the Poisson–Boltzmann equation (Russel et al., 1989) to describe the electrical potential distribution around the particles and the volume integration approach (Hamaker's technique) (Hamaker, 1937) for van der Waals interactions. In this study, the DLVO theory was not used to quantitatively assess the Hamaker constants because RGO and its nanohybrids show non-spherical and irregular geometries. However, the higher stability of RGO nanohybrids suggests that the Hamaker constants for the RGO nanohybrids should fall within the ranges previously reported for bare GO (6.3×10^{-21} J)

(Hua et al., 2016) and Fe_3O_4 (2.1×10^{-19} J) (Xu et al., 2008) or Ag (3.9×10^{-19} J) (Butt et al., 2005) NPs and can be controlled by the compositions of NPs loaded on RGO.

The presence of SRHA and SRFA ($\text{TOC} \approx 5 \text{ mg C L}^{-1}$) significantly inhibited the aggregation of all RGO nanohybrids in 100 mM NaCl at which the nanohybrids typically displayed diffusion-limited aggregation (Fig. 5). It should be noted that the presence of SRHA and SRFA had no significant effect on the surface charge of RGO and its nanohybrids, which indicates that the stabilization effect was primarily due to non-DLVO forces such as steric repulsion, which originated from the adsorbed macromolecules on RGO (Chowdhury et al., 2013). SRHA had a more compelling effect, particularly on the RGO/ Fe_3O_4 nanohybrid, while there was a considerable influence of SRFA on RGO, RGO/Ag⁰, and RGO/ Fe_3O_4 /Ag⁰. Interestingly, SRHA was unable to hinder the aggregation of bare RGO in aqueous solution. This can be attributed to the weak adsorption of SRHA on RGO, which had lesser epoxy or hydroxyl groups available on its surface, as confirmed by XPS (Table S2, SI). These functional groups can act as Lewis base sites and are responsible for chemical-bond formation with several functional groups of SRHA such as $-\text{COOH}$, $-\text{OH}$, and $-\text{NH}_2$ and strong hydrogen-bonding interactions (Hartono et al., 2009). A similar trend was observed for the fully reduced GO in the presence of SRHA (Chowdhury et al., 2015). These observations suggest that the distribution of surface functional groups of RGO is also important in controlling the stability of RGO nanohybrids.

3.4. Long-term stability in synthetic surface and ground waters

In order to further examine the stability of RGO and its nanohybrids in more complex aquatic environments, sedimentation experiments were conducted in synthetic surface water and groundwater for a prolonged period of 25 d (Fig. 6). For quantitative determination of the NP concentrations using UV-Vis spectrophotometry, Beer-Lambert law (as described below) was used because the unique absorbance of a material is directly proportional to the concentration associated with the surface area of the absorbing material.

$$A = \epsilon cl$$

where A is the absorbance, ϵ is the molar extinction coefficient of the material ($\text{L mol}^{-1} \text{cm}^{-1}$), c is the concentration of the unknown suspension (mol L^{-1}), and l is the length of the path that light travels through the material (cm). In a highly concentrated suspension, the NPs may be ionized and change the pH or precipitate to make the suspension more turbid. This may cause non-linear deviations from the Beer-Lambert Law by altering the absorbance at a certain wavelength. The samples having a concentration of 20 mg L^{-1} and absorbance of 2.9–3.1 a.u. (Fig. S11, SI) used in the sedimentation experiments were diluted to 2 mg L^{-1} to have an absorbance between 0.33 and 0.34 a.u. for best results. While RGO and its nanohybrids settled down faster in an initial 10-day period, there was no noticeable sedimentation over an extended time period (after 10 d). In synthetic groundwater containing higher concentrations of monovalent and divalent cations than synthetic surface water (Table S3, 1SI), an enhanced aggregation was observed as a result of the increased charge screening or neutralization effects (Huynh and Chen, 2011). The relative concentration (C/C_0)

suggested that the RGO/Ag⁰ nanohybrid remained highly dispersed in both synthetic surface and ground waters. This lack of aggregation is understandable as RGO/Ag⁰ was greatly stable at high concentrations of a single electrolyte salt (NaCl or CaCl₂) because of the greater interaction energy barrier between RGO/Ag⁰ in the DLVO theory. In comparison, RGO/Fe₃O₄ underwent faster settling as flocculated aggregates. A substantial attractive energy of RGO/Fe₃O₄ in synthetic waters could reach the deep primary energy minimum in which irreversible particle deposition takes place. RGO showed a more pronounced tendency to aggregate than RGO/Ag⁰ and RGO/Fe₃O₄/Ag⁰ in synthetic surface and ground waters since the lesser number of oxygenated groups on RGO allowed for the NPs to aggregate at high cation concentrations. This behavior has also been observed for L-ascorbic acid-reduced RGO containing small functional groups (Qi et al., 2016).

4. Conclusions

With the concurrent rapid development and utilization of multifunctional RGO-MNMs, it is inevitable that these RGO nanohybrids would be released into the environment. However, our current understanding on the environmental processes is largely restricted to the individual NMs, which hinders our ability to predict the aggregation and long-term sedimentation behaviors of NHs in the environment. In this study, three differently self-assembled Fe₃O₄ and Ag⁰ NPs anchored on the RGO nanosheets were investigated and compared to assess the fate and transport of NHs. There were notable changes in the electrokinetic and hydrodynamic properties of these materials under aquatic environmental conditions (pH 5 to 9) (Chowdhury et al., 2013). Under these conditions, RGO/Fe₃O₄ showed a greater tendency to aggregate and lesser stability owing to insignificant changes in the EPM. Over a wide range of IS, we observed that the degree of aggregation followed the order RGO/Fe₃O₄ > RGO > RGO/Fe₃O₄/Ag⁰ > RGO/Ag⁰, which is consistent with the trend of ratios of the oxygen-containing functional groups at the edges of RGO. A high correlation between the CCC values and the ratios of edge-based functional groups indicates that the edge-edge interactions play a major role in the aggregation of nanohybrids in the aquatic environment. The presence of SRHA and SRFA significantly improved their stability in natural water systems, primarily because of the electrosteric repulsion. In surface and ground waters, RGO and its nanohybrids were somewhat less stable and mobile as a result of charge screening and bridging effects. Consequently, the aggregation and sedimentation behaviors of these materials in complex matrices were subject to change as a function of multiple parameters, including the physicochemical properties of the NPs and the different environmental conditions. This result provides important implications on environmental impacts and toxicity of the emerging class of multi-element NMs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Agency policy and approved for publication. However, the research results do not necessarily reflect the views or policies, and no official endorsement should be inferred. The authors declare that they have no conflict of interest.

References

- Adeleye AS, Conway JR, Perez T, Rutten P, Keller AA (2014) Influence of extracellular polymeric substances on the long-term fate, dissolution, and speciation of copper-based nanoparticles. *Environ. Sci. Technol.* 48(21):12561–12568. [PubMed: 25295836]
- Adeleye AS, Pokhrel S, Mädler L, Keller AA (2017) Influence of nanoparticle doping on the colloidal stability and toxicity of copper oxide nanoparticles in synthetic and natural waters. *Water Res.*
- Al-Hamadani YA, Chu KH, Son A, Heo J, Her N, Jang M, Park CM, Yoon Y (2015) Stabilization and dispersion of carbon nanomaterials in aqueous solutions: A review. *Sep. Purif. Technol.* 156:861–874.
- Amemiya Y, Arakaki A, Staniland SS, Tanaka T, Matsunaga T (2007) Controlled formation of magnetite crystal by partial oxidation of ferrous hydroxide in the presence of recombinant magnetotactic bacterial protein Mms6. *Biomaterials* 28(35):5381–5389. [PubMed: 17720242]
- Bhadra BN, Seo PW, Jhung SH (2016) Adsorption of diclofenac sodium from water using oxidized activated carbon. *Chem. Eng. J.* 301:27–34.
- Butt H-J, Cappella B, Kappl M (2005) Force measurements with the atomic force microscope: Technique, interpretation and applications. *Surf. Sci. Rep.* 59(1):1–152.
- Chen J, Li Y, Huang L, Li C, Shi G (2015) High-yield preparation of graphene oxide from small graphite flakes via an improved Hummers method with a simple purification process. *Carbon* 81:826–834.
- Chen KL, Elimelech M (2006) Aggregation and deposition kinetics of fullerene (C60) nanoparticles. *Langmuir* 22(26):10994–11001. [PubMed: 17154576]
- Chen KL, Elimelech M (2007) Influence of humic acid on the aggregation kinetics of fullerene (C60) nanoparticles in monovalent and divalent electrolyte solutions. *J. Colloid Interface Sci.* 309(1):126–134. [PubMed: 17331529]
- Chen KL, Mylon SE, Elimelech M (2006) Aggregation kinetics of alginate-coated hematite nanoparticles in monovalent and divalent electrolytes. *Environ. Sci. Technol.* 40(5):1516–1523. [PubMed: 16568765]
- Chowdhury I, Duch MC, Gits CC, Hersam MC, Walker SL (2012) Impact of synthesis methods on the transport of single walled carbon nanotubes in the aquatic environment. *Environ. Sci. Technol.* 46(21):11752–11760. [PubMed: 23016910]
- Chowdhury I, Duch MC, Mansukhani ND, Hersam MC, Bouchard D (2013) Colloidal properties and stability of graphene oxide nanomaterials in the aquatic environment. *Environ. Sci. Technol.* 47(12):6288–6296. [PubMed: 23668881]
- Chowdhury I, Mansukhani ND, Guiney LM, Hersam MC, Bouchard D (2015) Aggregation and stability of reduced graphene oxide: complex roles of divalent cations, pH, and natural organic matter. *Environ. Sci. Technol.* 49(18):10886–10893. [PubMed: 26280799]
- Cornelis G, Pang L, Doolette C, Kirby JK, McLaughlin MJ (2013) Transport of silver nanoparticles in saturated columns of natural soils. *Sci. Total Environ.* 463:120–130. [PubMed: 23792254]
- Duch MC, Budinger GS, Liang YT, Soberanes S, Urich D, Chiarella SE, Campochiaro LA, Gonzalez A, Chandel NS, Hersam MC (2011) Minimizing oxidation and stable nanoscale dispersion improves the biocompatibility of graphene in the lung. *Nano Lett.* 11(12):5201. [PubMed: 22023654]
- Dupont D, Brullot W, Bloemen M, Verbiest T, Binnemans K (2014) Selective uptake of rare earths from aqueous solutions by EDTA-functionalized magnetic and nonmagnetic nanoparticles. *ACS Appl. Mater. Interfaces* 6(7):4980–4988. [PubMed: 24641094]
- El Badawy AM, Scheckel KG, Suidan M, Tolaymat T (2012) The impact of stabilization mechanism on the aggregation kinetics of silver nanoparticles. *Sci. Total Environ.* 429:325–331. [PubMed: 22578844]
- Elimelech M, Gregory J, Jia G, Williams R (1995) *Surface interaction potentials*, Butterworth-Heinmann.

- Elimelech M, Gregory J, Jia X (2013) Particle deposition and aggregation: measurement, modelling and simulation, Butterworth-Heinemann.
- Gogotsi Y, Presser V (2013) Carbon nanomaterials, CRC Press.
- Guo S, Wen D, Zhai Y, Dong S, Wang E (2010) Platinum nanoparticle ensemble-on-graphene hybrid nanosheet: one-pot, rapid synthesis, and used as new electrode material for electrochemical sensing. *ACS Nano* 4(7):3959–3968. [PubMed: 20568706]
- Hamaker H (1937) The London—van der Waals attraction between spherical particles. *physica* 4(10): 1058–1072.
- Hardy W (1899) A preliminary investigation of the conditions which determine the stability of irreversible hydrosols. *Proc. R. Soc. London* 66(424–433):110–125.
- Hartono T, Wang S, Ma Q, Zhu Z (2009) Layer structured graphite oxide as a novel adsorbent for humic acid removal from aqueous solution. *J. Colloid Interface Sci.* 333(1):114–119. [PubMed: 19233379]
- Heo Y, Im H, Kim J (2013) The effect of sulfonated graphene oxide on sulfonated poly (ether ether ketone) membrane for direct methanol fuel cells. *J. Membr. Sci.* 425:11–22.
- Hu G, Tang B (2013) Photocatalytic mechanism of graphene/titanate nanotubes photocatalyst under visible-light irradiation. *Mater. Chem. Phys.* 138(2):608–614.
- Hua Z, Zhang J, Bai X, Ye Z, Tang Z, Liang L, Liu Y (2016) Aggregation of TiO₂–graphene nanocomposites in aqueous environment: Influence of environmental factors and UV irradiation. *Sci. Total Environ.* 539:196–205. [PubMed: 26360460]
- Huynh KA, Chen KL (2011) Aggregation kinetics of citrate and polyvinylpyrrolidone coated silver nanoparticles in monovalent and divalent electrolyte solutions. *Environ. Sci. Technol.* 45(13): 5564–5571. [PubMed: 21630686]
- Jafri RI, Arockiadoss T, Rajalakshmi N, Ramaprabhu S (2010) Nanostructured Pt dispersed on graphene-multiwalled carbon nanotube hybrid nanomaterials as electrocatalyst for PEMFC. *J. Electrochem. Soc.* 157(6):B874–B879.
- Jaworski S, Sawosz E, Kutwin M, Wierzbicki M, Hinzmann M, Grodzik M, Winnicka A, Lipińska L, Włodzka K, Chwalibog A (2015) In vitro and in vivo effects of graphene oxide and reduced graphene oxide on glioblastoma. *Int. J. Nanomed.* 10:1585.
- Jiang Y, Raliya R, Liao P, Biswas P, Fortner JD (2017) Graphene oxides in water: assessing stability as a function of material and natural organic matter properties. *Environ. Sci. Nano* 4(7):1484–1493.
- Liu W, Ma J, Shen C, Wen Y, Liu W (2016) A pH-responsive and magnetically separable dynamic system for efficient removal of highly dilute antibiotics in water. *Water Res.* 90:24–33. [PubMed: 26724436]
- Liu X, Pan L, Lv T, Zhu G, Lu T, Sun Z, Sun C (2011) Microwave-assisted synthesis of TiO₂-reduced graphene oxide composites for the photocatalytic reduction of Cr(VI). *RSC Adv.* 1(7):1245–1249.
- Lou Z, Cao Z, Xu J, Zhou X, Zhu J, Liu X, Baig SA, Zhou J, Xu X (2017) Enhanced removal of As(III)/(V) from water by simultaneously supported and stabilized Fe-Mn binary oxide nanohybrids. *Chem. Eng. J.*
- Ma R, Levard C, Michel FM, Brown GE Jr, Lowry GV (2013) Sulfidation mechanism for zinc oxide nanoparticles and the effect of sulfidation on their solubility. *Environ. Sci. Technol.* 47(6):2527–2534. [PubMed: 23425191]
- Madadrang CJ, Kim HY, Gao G, Wang N, Zhu J, Feng H, Gorrington M, Kasner ML, Hou S (2012) Adsorption behavior of EDTA-graphene oxide for Pb(II) removal. *ACS Appl. Mater. Interfaces* 4(3):1186–1193. [PubMed: 22304446]
- Mahdavi S, Jalali M, Afkhami A (2013) Heavy metals removal from aqueous solutions using TiO₂, MgO, and Al₂O₃ nanoparticles. *Chem. Eng. Commun.* 200(3):448–470.
- Maiorano G, Sabella S, Sorce B, Brunetti V, Malvindi MA, Cingolani R, Pompa PP (2010) Effects of cell culture media on the dynamic formation of protein–nanoparticle complexes and influence on the cellular response. *ACS Nano* 4(12):7481–7491. [PubMed: 21082814]
- Muthoosamy K, Bai RG, Abubakar IB, Sudheer SM, Lim HN, Loh H-S, Huang NM, Chia CH, Manickam S (2015) Exceedingly biocompatible and thin-layered reduced graphene oxide nanosheets using an eco-friendly mushroom extract strategy. *Int. J. Nanomed.* 10:1505.

- Park CM, Heo J, Her N, Chu KH, Jang M, Yoon Y (2016) Modeling the effects of surfactant, hardness, and natural organic matter on deposition and mobility of silver nanoparticles in saturated porous media. *Water Res.* 103:38–47. [PubMed: 27429353]
- Qi Y, Xia T, Li Y, Duan L, Chen W (2016) Colloidal stability of reduced graphene oxide materials prepared using different reducing agents. *Environ. Sci. Nano* 3(5):1062–1071.
- Russel WB, Saville DA, Schowalter WR (1989) *Colloidal dispersions*, Cambridge university press.
- Salas EC, Sun Z, Lüttge A, Tour JM (2010) Reduction of graphene oxide via bacterial respiration. *ACS Nano* 4(8):4852–4856. [PubMed: 20731460]
- Saleh NB, Pfefferle LD, Elimelech M (2010) Influence of biomacromolecules and humic acid on the aggregation kinetics of single-walled carbon nanotubes. *Environ. Sci. Technol.* 44(7):2412–2418. [PubMed: 20184360]
- Tóth IY, Illés Eb, Bauer RA, Nesztör D, Szekeres Mr, Zupkó I, Tombácz E (2012) Designed polyelectrolyte shell on magnetite nanocore for dilution-resistant biocompatible magnetic fluids. *Langmuir* 28(48):16638–16646. [PubMed: 23140279]
- Thurman E (2012) *Organic geochemistry of natural waters*, Springer Science & Business Media, Denver, Colorado.
- Vance ME, Kuiken T, Vejerano EP, McGinnis SP, Hochella MF Jr, Rejeski D, Hull MS (2015) Nanotechnology in the real world: Redeveloping the nanomaterial consumer products inventory. *Beilstein J. Nanotechnol.* 6(1):1769–1780. [PubMed: 26425429]
- Xu C, Ohno K, Ladmira V, Composto RJ (2008) Dispersion of polymer-grafted magnetic nanoparticles in homopolymers and block copolymers. *Polymer* 49(16):3568–3577.
- Yan W, Lien H-L, Koel BE, Zhang W-x (2013) Iron nanoparticles for environmental clean-up: recent developments and future outlook. *Environ. Sci. Processes Impacts* 15(1):63–77.
- Yang S-T, Chang Y, Wang H, Liu G, Chen S, Wang Y, Liu Y, Cao A (2010) Folding/aggregation of graphene oxide and its application in Cu²⁺ removal. *J. Colloid Interface Sci.* 351(1):122–127. [PubMed: 20705296]
- Zaporotskova IA, Kozhitov LV, Anikeev NA, Davletova OA, Popkova AV, Muratov DG, Yakushko EV (2015) Metal–carbon nanocomposites based on pyrolysed polyacrylonitrile. *Mod. Electron. Mater.* 1(2):43–49.
- Zhao X, Shi Y, Wang T, Cai Y, Jiang G (2008) Preparation of silica-magnetite nanoparticle mixed hemimicelle sorbents for extraction of several typical phenolic compounds from environmental water samples. *J. Chromatogr. A* 1188(2):140–147. [PubMed: 18329033]
- Zheng T, Bott S, Huo Q (2016) Techniques for accurate sizing of gold nanoparticles using dynamic light scattering with particular application to chemical and biological sensing based on aggregate formation. *ACS Appl. Mater. Interfaces* 8(33):21585–21594. [PubMed: 27472008]

Fig. 1.

(a–c) Representative TEM images of aggregated RGO/Fe₃O₄, RGO/Ag⁰, and RGO/Fe₃O₄/Ag⁰ nanohybrids. Insets in (a–c) are TEM images at low magnification. The scale bars in (a–c) are 50, 100, and 20 nm, respectively. (d) Wide survey XPS, (e) C1s, and (f) O1s spectra of RGO, RGO/Fe₃O₄, RGO/Ag⁰, and RGO/Fe₃O₄/Ag⁰. Measurements were performed at 25 °C.

Fig. 2.

Electrophoretic mobility (EPM) and hydrodynamic diameter of RGO and its nanohybrids in water as a function of (a, b) pH, and (c, d) NaCl and (e, f) CaCl₂ concentrations at pH 6.3 ± 0.1. Measurements were performed at 25 °C. Error bars indicate the standard deviations of at least three sample measurements.

Fig. 3. Attachment efficiency (α) of RGO, RGO/Fe₃O₄, RGO/Ag⁰, and RGO/Fe₃O₄/Ag⁰ nanohybrids as a function of (a) NaCl and (b) CaCl₂ concentrations at pH 6.3 ± 0.1. The dashed lines are a guide to the eye to show the CCCs in NaCl and CaCl₂.

Fig. 4. Linear relationship between the CCCs of RGO and its nanohybrids and the edge-based surface functional groups identified by XPS in (a) NaCl and (b) CaCl_2 .

Fig. 5. Aggregation profiles of RGO/Fe₃O₄, RGO/Ag⁰, and RGO/Fe₃O₄/Ag⁰ nanohybrids in the presence of (a) SRHA and (b) SRFA at 100 mM NaCl and pH 6.3 ± 0.1. (c) Aggregation rate plots of RGO and its nanohybrids in the presence of SRHA and SRFA at pH 6.3 ± 0.1. Measurements were performed at 25 °C. [SRHA]₀ = [SRFA]₀ = 5 mg C L⁻¹.

Fig. 6. Long-term stability of RGO, RGO/Fe₃O₄, RGO/Ag⁰, and RGO/Fe₃O₄/Ag⁰ nanohybrids in synthetic (a) surface water and (b) groundwater at pH 6.3 ± 0.1. Error bars indicate the standard deviations of at least three sample measurements.

Table 1.

Critical Coagulation Concentration (CCC) values of RGO and its nanohybrid samples and fitting of Schulze-Hardy rule ($z = 2$).

Nanohybrids	CCC _{NaCl} (mM)	CCC _{CaCl₂} (mM)	ratio of CCC _{CaCl₂} /CCC _{NaCl}
RGO	43.8 ± 0.9	0.71 ± 0.03	$z^{-5.95}$
RGO/Fe ₃ O ₄	41.2 ± 0.5	0.69 ± 0.02	$z^{-5.90}$
RGO/Ag ⁰	96.3 ± 3.8	2.30 ± 0.04	$z^{-5.39}$
RGO/Fe ₃ O ₄ /Ag ⁰	83.2 ± 2.6	1.31 ± 0.06	$z^{-5.99}$