



Contents lists available at ScienceDirect

## Environmental Pollution

journal homepage: [www.elsevier.com/locate/envpol](http://www.elsevier.com/locate/envpol)

# Alginate affects agglomeration state and uptake of $^{14}\text{C}$ -labeled few-layer graphene by freshwater snails: Implications for the environmental fate of graphene in aquatic systems<sup>☆</sup>

Yu Su<sup>a</sup>, Chi Huang<sup>a</sup>, Fenxiao Lu<sup>a</sup>, Xin Tong<sup>a</sup>, Junfeng Niu<sup>b</sup>, Liang Mao<sup>a,\*</sup>

<sup>a</sup> State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing 210093, China

<sup>b</sup> Beijing Normal University, School of Environment, State Key Lab Water Environmental Simulation, Beijing 100875, China

## ARTICLE INFO

## Article history:

Received 5 June 2017

Received in revised form

24 November 2017

Accepted 27 November 2017

## Keywords:

Graphene

Sodium alginate

Agglomeration

Biological uptake

Freshwater snail

## ABSTRACT

Understanding of the interaction of graphene with natural polysaccharides (e.g., alginate) is crucial to elucidate its environmental fate. We investigated the impact of alginate on the agglomeration and stability of  $^{14}\text{C}$ -labeled few-layer graphene (FLG) in varying concentrations of monovalent (NaCl) and divalent ( $\text{CaCl}_2$ ) electrolytes. Enhanced agglomeration occurred at high  $\text{CaCl}_2$  concentrations ( $\geq 5$  mM) due to the alginate gel networks formation in the presence of  $\text{Ca}^{2+}$ . FLG enmeshed within extended alginate gel networks was observed under transmission electron microscope and atomic force microscope. However, background  $\text{Na}^+$  competition for binding sites with  $\text{Ca}^{2+}$  at the alginate surfaces shielded the gelation of alginate. FLG was readily dispersed by alginate under environmentally relevant ionic strength conditions (i.e.,  $<200$  mM  $\text{Na}^+$  and  $<5$  mM  $\text{Ca}^{2+}$ ). In comparison with the bare FLG, the slow sedimentation of the alginate-stabilized FLG (158  $\mu\text{g/L}$ ) caused continuous exposure of this nanomaterial to freshwater snails, which ingested 1.9 times more FLG through filter-feeding within 72 h. Moreover, surface modification of FLG by alginate significantly increased the whole-body and intestinal levels of FLG, but reduced the internalization of FLG to the intestinal epithelial cells. These findings indicate that alginate will act as a stabilizing agent controlling the transport of FLG in aqueous systems. This study also provides the first evidence that interaction of graphene with natural polysaccharides affected the uptake of FLG in the snails, which may alter the fate of FLG in aquatic environments.

© 2017 Elsevier Ltd. All rights reserved.

## 1. Introduction

Graphene, a two-dimensional sheet of  $\text{sp}^2$ -hybridized carbon atoms packed into a honeycomb lattice, has attracted substantial scientific interests because of its excellent mechanical, optical, and electronic properties (Mao et al., 2013; Novoselov et al., 2004). The commercialization of this new emerging nanomaterial has raised concerns about its possible release into the environment (Segal, 2009; Zhao et al., 2014). Given the limited number of ecotoxicological studies (Guo et al., 2013; Hu and Zhou, 2013; Mao et al., 2016b), the extent to which graphene will pose threat to ecological receptor is uncertain. In particular, understanding and connection between the physicochemical properties of graphene and its interaction with biological systems are still limited.

In aquatic environments, the hydrophobic graphene will inevitably interact with ubiquitous natural organic matter (NOM) (Hyung et al., 2007), which mainly consists of humic substances and non-humic substances (Nebbioso and Piccolo, 2013). The effects of humic substances on the stability of graphene have been reported previously (Chowdhury et al., 2013, 2015). Because of the heterogeneity and diversity of NOM, graphene will interact with non-humic substances such as polysaccharides and hence exists in different dispersion and agglomeration states. Alginate secreted by brown algae in the form of extracellular polymeric substance has been widely used as a model polysaccharide due to its large-scale application in the food and biomedical industries (Dettmar et al., 2011; Liu et al., 2008) and wastewater treatment (Devrimci et al., 2012; Kivilcimdan Moral et al., 2016). A number of studies have shown that the presence of alginate has a strong impact on the agglomeration and stability of nanoparticles (e.g., hematite,  $\text{TiO}_2$ , and carbon nanotubes (CNTs)) (Chen et al., 2006, 2007; Loosli et al., 2013, 2015; Saleh et al., 2010). For example, the “egg-box” shaped

<sup>☆</sup> This paper has been recommended for acceptance by Baoshan Xing.

\* Corresponding author.

E-mail address: [lmiao@nju.edu.cn](mailto:lmiao@nju.edu.cn) (L. Mao).

alginate gel networks that form as a result of inter- and intra-molecular interactions with divalent cation (e.g.,  $\text{Ca}^{2+}$ ) can destabilize nanoparticles via adsorption and bridging effects (Grant et al., 1973; Gregor et al., 1996). It is very likely that changes in conformation of the alginate polymers with ionic composition of the surrounding solution will affect the surface chemistry and agglomeration state of graphene. To date, the influence of alginate on colloidal stability of graphene has not been systematically studied.

Several studies have demonstrated that interaction with NOM has a notable impact on the bioaccumulation and toxicity of graphene. For instance, the presence of humic acid facilitated the elimination of FLG by *D. magna* (Guo et al., 2013) and mitigated the adverse effects of graphene oxide (GO) on wheat seed germination and zebrafish embryogenesis (Chen et al., 2015; Hu et al., 2014), but enhanced the absorption of small FLG in the gut epithelium of zebrafish (Lu et al., 2017). Binding of biological secreted proteins to FLG or GO led to greater accumulation in earthworms (Mao et al., 2016b) and higher toxicity to zebrafish embryos (Mu et al., 2016) when compared to the bare nanoparticles. It is reported that alginate can reduce the bioavailability of  $\text{TiO}_2$  nanoparticles to *Artemia franciscana* and *Phaeodactylum tricornutum* (Callegaro et al., 2015). We hypothesized that association with alginate may affect the interaction of graphene with biological species.

The objectives of this study were to investigate how the presence of alginate affects the agglomeration and colloidal stability of  $^{14}\text{C}$ -labeled FLG (Guo et al., 2013) under different solution chemistry conditions (i.e., cation species and concentrations) and how this in turn influences the uptake, distribution, and bioavailability of FLG by a filter feeder freshwater snail *Cipangopaludina cathayensis*. The snails are important members of freshwater ecosystem (prefer living in shallow water) and have been used in eco-toxicity testing of nanoparticles (Croteau et al., 2011; Gonçalves et al., 2017). The results of our work have the potential to provide valuable insights on the transport and fate of graphene in aquatic environments as affected by surface modification and biological uptake.

## 2. Material and methods

### 2.1. Chemicals

Commercial sodium alginate (Sigma Aldrich) extracted from brown algae *Macrocystis pyrifera* was used. This unbranched block copolymer consists of mannuronic acid (M) and guluronic acid (G) residues (Schürks et al., 2002). The ratio of M-block to G-block is 1.7 and the frequency of homopolymeric G-block ( $F_{\text{GG}}$ ) is 0.23 (Davis et al., 2003). The alginate was dissolved in deionized (DI) water and filtered through 0.22  $\mu\text{m}$  cellulose acetate membranes to prepare a stock solution, which was then stored at 4 °C before use. The total organic carbon (TOC) content of the alginate was determined to be  $48.2 \pm 0.03\%$  of its mass ( $n = 3$ ; uncertainties always indicate standard deviation values) through high-temperature oxidation (TOC-L, Shimadzu). The apparent molecular weight (Fig. S1 of the Supporting Information, SI) of the alginate ranged from 10 kDa to 70 kDa via high performance size-exclusion chromatogram using a procedure described by Wang et al. (2011). The concentration of alginate in the following agglomeration, sedimentation, and uptake experiments was varied from 1 to 5 mg/L TOC.

### 2.2. Graphene synthesis and characterization

Synthesis and characterization of the  $^{14}\text{C}$ -labeled four-layer graphene have been described elsewhere (Guo et al., 2013). The specific radioactivity of FLG was determined to be  $16 \pm 0.59$  mCi/g

( $n = 3$ ) (Feng et al., 2015). Using atomic force microscopy (AFM), the lateral size and thickness of FLG were 60–590 nm and 1.05–4.05 nm ( $n = 214$ ), respectively (Lu et al., 2015). FLG surfaces consisted of 89% C, 6% O, 1.4% H, and 3.6% N via X-ray photoelectron spectroscopy (Guo et al., 2013). The detected oxygen-containing groups (e.g., C=O and C-OH) (Feng et al., 2015) was introduced by the addition of  $^{14}\text{C}$ -phenol during FLG synthesis. A stock suspension of 10 mg/L FLG was prepared through probe tip sonication as described elsewhere (Guo et al., 2013; Lu et al., 2015). The concentration of FLG stock was determined by mixing 1 mL of this suspension with a 3 mL of scintillation cocktail (Goldstar, Meridian) in triplicate and measuring radioactivity via liquid scintillation counting (LSC) (LS 6500, Beckman Coulter).

### 2.3. Electrophoretic mobility measurements and agglomeration/sedimentation kinetics

Electrophoretic mobility (EPM) and hydrodynamic diameter ( $D_h$ ) of FLG in varying concentrations of electrolytes (i.e., NaCl and  $\text{CaCl}_2$ ) and alginate were measured by dynamic light scattering (DLS) (Nano ZS, Malvern) at 25 °C. The FLG samples (2.5 mg/L) for the EPM and  $D_h$  measurements were diluted with background electrolytes by a factor of 4 from the original stock. The FLG concentrations in all measurements were quantified by radioactivity measurements as described above. For each solution chemistry condition, ten EPM measurements were conducted for each of three samples. For each agglomeration experiment, the scattered light intensity was detected by a photodetector at a scattering angle of 173°; the  $D_h$  was recorded every 10 s by autocorrelation function until 360 data points had been acquired (Chowdhury et al., 2013; Smith et al., 2008). All agglomeration experiments were conducted in triplicate at pH 7.0. The initial agglomeration period was defined as the time period from experiment initiation ( $t_0$ ) to the time when measured  $D_h$  values exceeded  $1.5D_{h, \text{initial}}$  (Bouchard et al., 2012; Chowdhury et al., 2013). The initial agglomeration rate constants ( $k_a$ ) for FLG are proportional to the initial rate of increase in  $D_h$  with time (Eq. (1)) (Chen and Elimelech, 2006; Holthoff et al., 1996).

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

where  $N_0$  is the initial particle concentration. The initial increase in the  $D_h$  was usually linear with respect to time. Therefore, the initial rate of increase in  $D_h$  was obtained by determining the slope of the agglomeration profile within the initial agglomeration period. Detailed equations and parameters for calculation attachment efficiency ( $\alpha_a$ ) and critical coagulation concentration (CCC) values are provided in the SI.

To simulate the effects of alginate on the agglomeration and settling of FLG in relatively complex aqueous systems, agglomeration kinetics of FLG (2.5 mg/L) in artificial freshwater (AF water) (KCl, 1.2 mg/L;  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 58.8 mg/L;  $\text{MgSO}_4 \cdot 2\text{H}_2\text{O}$ , 24.7 mg/L;  $\text{NaHCO}_3$ , 13.0 mg/L) (Guo et al., 2013; Petersen et al., 2009) and artificial seawater (AS water) (KCl, 0.36 g/L; NaCl, 11.31 g/L;  $\text{CaCl}_2$ , 0.54 g/L;  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ , 1.97 g/L;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 2.39 g/L;  $\text{NaHCO}_3$ , 0.17 g/L) (Cooper et al., 2014) with or without addition of alginate (5 mg/L) were monitored for 1 h via DLS using the same method as described above; sedimentation kinetics was performed by mixing the FLG stock with 30 mL of AF water (or AS water) in the absence and presence of alginate (5 mg/L) in a glass Petri dish (inner diameter = 90 mm) to yield an initial concentration of  $97.5 \pm 0.8$   $\mu\text{g/L}$  ( $n = 3$ ). The capped dishes were left undisturbed for up to 48 h. The water samples (200  $\mu\text{L}$ ) were collected at each time point and the radioactivity was measured via LSC as described above. All

Download English Version:

<https://daneshyari.com/en/article/8857270>

Download Persian Version:

<https://daneshyari.com/article/8857270>

[Daneshyari.com](https://daneshyari.com)