Heteroaggregation of an enveloped bacteriophage with colloidal sediments and effect on virus viability

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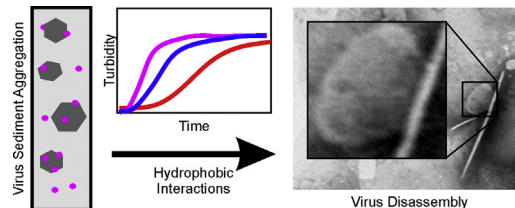[d] Department of Physics, Fairfield University, Fairfield, CT 06824, United States

HIGHLIGHTS

- Sediments interact with enveloped viruses but effects on viability are unknown.
- Light scattering measured aggregation rates of virus with four mineral types.
- Heteroaggregation was hydrophobic and faster with negatively-charged sediments.
- The greatest reduction in infectivity occurred in virus-montmorillonite aggregates.
- Aggregation with sediment caused virus disassembly and infectivity loss.

GRAPHICAL ABSTRACT

ARTICLE INFO

ABSTRACT

Four sediments in the colloidal size range: goethite, montmorillonite, illite, and kaolinite, were suspended with the bacteriophage φ6, a model enveloped virus, to determine relative rates of heteroaggregation and the effect of aggregation on virus viability. Turbidity was measured on combinations of virus and each sediment type at low concentration to determine aggregation rates. Aggregation of sediment with virus occurred regardless of mineral type, and larger fraction of virus is expected to aggregate with increasing sediment concentration leading to higher deposition rates. The negatively charged sediments, aggregated with φ6 (also negatively charged at neutral pH) at a faster rate than the positively charged sediments, yielding turbidity slopes of 4.94×10^{-3} s^{-1} and 7.50×10^{-4} s^{-1} for φ6-montmorillonite and φ6-illite aggregates, respectively, and 2.98×10^{-5} s^{-1} and 2.84×10^{-5} s^{-1}, for φ6-goethite and φ6-kaolinite, respectively. This indicates that the interaction between sediments and virus is hydrophobic, rather than electrostatic. Large numbers of virions remained viable post-aggregation, despite the fragility of the viral envelope, indicating that small-sized aggregates, which may travel more readily through porous media, may pose an infection risk. The fraction of φ6 that remained viable varied with sediment type, with montmorillonite-φ6 aggregates experiencing the greatest reduction in infectivity at 35%. TEM analyses reveal that in all sediment-φ6 combinations, infectivity loss was likely due to disassembly of the viral envelope as a result of aggregation.

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1. Introduction

The mechanisms that influence the transport of viruses are important for management of natural systems and freshwater resources.

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These may include human pathogens such as those introduced by fecal matter, but, more importantly, bacteriophage which serve to regulate bacterial populations (O'Brien et al., 2017). Bacteriophage alter biogeochemical cycles through lysis of bacterial cells (Díaz-Muñoz and Koskella, 2014; Koskella and Brockhurst, 2014) and impact eukaryotes that depend on bacterial populations, thus having a significant impact on the greater ecosystem. The distribution and residence time of viruses is affected by the chemistry of the water, e.g., dissolved organic matter, metals, and contaminants, but also the suspended materials, such as particulate organic matter (POM) and suspended sediment, primarily clays, which serve as substrate and habitat for biofilm-producing bacteria (Alimova et al., 2006; Alimova et al., 2009). In fact, sediment-bearing biofilms have been shown to sequester virus particles in wetlands where they are subsequently concentrated and subject to re-release (Flood and Ashbolt, 2000). Furthermore, bacteriophages are utilized as subsurface tracers and indicators for a variety of environmental applications (Ghanem et al., 2018; Keswick et al., 1982; Redman et al., 1997) and, therefore, their interaction with sediments may affect their quantification in hydrological experiments.

An estimated 10^{31} virus particles world-wide are prevalent in both soils and aquatic systems (Breitbart and Rohwer, 2005; Weinbauer and Rassoulzadegan, 2004). In aquatic systems, the length of time in which a virus can interact with potential hosts is largely controlled by sinking rates (Fuhrman, 1999). These rates are strongly influenced by aggregation with suspended particles. Sediments are known to influence virus transport and survival in porous media by controlling the potential for viruses to contaminate groundwater (Jin and Flury, 2002; Chu et al., 2000) and to promote lysis of bacteria in soils (Kimura et al., 2008). The mineralogy and size of suspended sediments affects the extent to which viruses will aggregate and sorb to sediments (Chu et al., 2000; Jin and Flury, 2002; McGechan and Lewis, 2002; Syngouna and Chrysikopoulos, 2015). With a few recent exceptions, enveloped viruses (Block et al., 2016; Block et al., 2014), have been largely omitted from studies of virus-sediment interaction, aggregation, and viability. Enveloped viruses possess different structural properties from non-enveloped viruses and include important plant and animal pathogens such as herpesvirus, coronavirus and Influenza A and B, all of which are found in natural and wastewaters (Batik et al., 1980; Gundy et al., 2008; Rosenberg et al., 1980; Sharp et al., 1975; Stallknecht et al., 1990a; Stallknecht et al., 1990b).

Virus particles typically range from 20 nm to 500 nm in diameter, and are therefore considered colloidal particles. For non-enveloped viruses, the surface charge is determined by the protonation/deprotonation of amino acids in the capsid (Elimelech et al., 1995). However, for enveloped virions, surface charge is determined by envelope proteins and lipids. The colloidal interaction between virus particles and the small fraction of suspended sediments can be described by DLVO theory (Derjaguin and Landau, 1941; Verwey and Overbeek, 1948), in which the potential between colloidal particles is the sum of the attractive van der Waals potential and the Coulomb potential (repulsive for like-charged particles and attractive for oppositely-charged particles). DLVO theory has been extended to include hydrophobic forces (van Oss, 1993; van Oss et al., 1990).

Previous research has shown that for non-enveloped viruses, sorption is largely influenced by hydrophobicity. Chattopadhyay and Puls (1999), (2000) studied the attachment of three, non-enveloped, bacteriophages, T2, MS2 and φX174 to several clay fractions and calculated the electrostatic and hydrophobic contributions to the free energy. Their calculations showed that surface hydrophobicity dictates sorption of viruses to clays. Chrysikopoulos and Syngouna (2012) looked at attachment of the bacteriophages MS2 and φX174 to kaolinite or montmorillonite, and using extended DLVO energy calculations, concluded that the virus-clay attachment was primarily through hydrophobic interaction.

A study of heteroaggregation of the non-enveloped cowpea mosaic virus with colloidal hematite revealed that at pH 6, at which hematite carries a positive surface charge and the virus a negative charge, the aggregates accumulated four times as many viruses as hematite particles Vilker et al. (1994). However, at pH 3, in which both particle types are positively charged, the aggregates contained three times as many hematite particles as virus. From these results, they concluded that attraction between the virus and hematite is mostly governed by electrostatic interactions.

The above studies all investigated non-enveloped viruses which are structurally different to viruses with lipid envelopes (e.g. influenza, paramyxovirus). In this work, we employ turbidity measurements to investigate the heteroaggregation of a model envelope virus, the bacteriophage φ6, with colloidal goethite and three clay minerals: illite, kaolinite and montmorillonite. φ6 is a member of the cystoviridae family of bacteriophage. The cystoviridae are the only phage family with an outer bi-lipid envelope. φ6 is a dsRNA virus whose host cell is *Pseudomonas phaseolicola*, a common plant pathogen. φ6 consists of an icosahedral nucleocapsid surrounded by a bi-lipid envelope and is therefore employed as a model for virus emergence in evolutionary studies and for human pathogens such as coronavirus (Dennehy, 2017; Mindich, 2004). The diameter of φ6 is ~80 nm. The φ6 virus carries negative charge at neutral pH (Block et al., 2014).

Montmorillonite and illite platelets both have positive charged edges and negative charged faces with an overall negative charge at neutral pH (González Sánchez et al., 2008; Van Olphen, 1962). Kaolinite platelets have negatively charged faces, and at neutral pH, negatively charged edges (Gupta et al., 2011; Schroth and Sposito, 1997). Goethite is positively charged at neutral pH (Gaboriaud and Ehrhardt, 2003; Zeltner and Anderson, 1988).

The effect of the interaction of colloidal sediments and enveloped viruses on virus viability, the nature of that interaction is unknown. We will present aggregation rates as determined by turbidity experiments, the effect of the interaction between φ6 and sediments on φ6 infectivity, and discuss the environmental implications of our findings.

2. Turbidity and colloidal aggregation

2.1. Early stage turbidity and colloidal aggregation

In the early stage of colloidal aggregation, suspensions consist mostly of primary particles and aggregation is a bimolecular process in which the kinetics are dominated by the merging of individual primary particles to form doublets: $N + N \rightarrow N_2$ (García-García et al., 2006; García-García et al., 2007). The rate equation for the loss of primary particles into doublets can be written as:

$$\frac{dN(t)}{dt} = -KN^2(t) \tag{1}$$

where $N(t)$ is the number density of particles and K is the aggregation rate constant for doublet formation. The corresponding rate equation for doublet, N_2, formation is:

$$\frac{dN_2(t)}{dt} = \frac{K}{2}N^2(t) \tag{2}$$

Integration of Eq. (1) gives:

$$\frac{1}{N(t)} = \frac{1}{N_0} + Kt \tag{3}$$

where N_0 is the number density of primary particles at $t = 0$. The average number of primary particles in an aggregate is $\eta = N_0/N(t)$ and thus in the early stage of aggregation:

$$\eta(t) = 1 + KN_0t. \tag{4}$$