



Attachment of bacteriophages MS2 and Φ X174 onto kaolinite and montmorillonite: Extended-DLVO interactions

Constantinos V. Chrysikopoulos*, Vasiliki I. Syngouna

Environmental Engineering Laboratory, Department of Civil Engineering, University of Patras, Patras 26500, Greece

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ABSTRACT

This study aims to gain insights into the interaction of virus particles with clay colloids. Bacteriophages MS2 and Φ X174 were used as model viruses and kaolinite (KGa-1b) and montmorillonite (STx-1b) as model colloids. The experimental data obtained from batch experiments of MS2 and Φ X174 attachment onto KGa-1b and STx-1b suggested that virus attachment is adequately described by the Freundlich isotherm equation. Both MS2 and Φ X174 were attached in greater amounts onto KGa-1b than STx-1b with MS2 having greater affinity than Φ X174 for both clays. Furthermore, extended-DLVO interaction energy calculations explained that the attachment of viruses onto model clay colloids was primarily caused by hydrophobic interaction. The theoretical and experimental results of this study were found to be in good agreement with previous findings.

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1. Introduction

Pathogenic viruses present in groundwater are likely to originate from accidental (landfills, broken sewer pipelines, leaking septic tanks, graveyards, urban runoff, irrigation) and intentional (direct injection wells, recharge basins) pollution sources [1–3]. Clay minerals are the finest inorganic components in soils and sediments and occur naturally in groundwater. Clay minerals have a very high surface area to volume ratio and great affinity for contaminants and biocolloids (viruses and bacteria) [4–8]. Therefore, suspended clay minerals are instrumental in providing contaminants and biocolloids with the opportunity to migrate while sorbed or attached onto their surfaces [9,10]. Numerous studies have provided evidence that contaminants as well as biocolloids sorb or attach onto suspended colloids and can migrate substantially farther than in the absence of mobile colloids [11–24].

The transport of viruses in porous and fractured subsurface environments is governed by numerous factors including virus inactivation, temperature, and water chemistry [25–29], and has been investigated extensively theoretically [30–35] as well as experimentally at the laboratory [36–43] and at the field scale [2,44–48]. Although the attachment of viruses onto clay minerals

has been examined by a number of investigators [7,49–51], more work is needed in order to fully understand and precisely define the fundamental mechanisms governing virus attachment onto the various clay minerals commonly found in groundwater.

Colloid stability is often predicted by the Derjaguin–Landau–Verwey–Overbeek (DLVO) theory [52,53], which was developed for smooth, homogeneous particles with ideal geometries. However, the majority of the colloids found in the subsurface are of irregular geometries with rough surfaces and heterogeneous composition. Despite the success of DLVO theory, numerous investigators have modified the DLVO theory to include factors not accounted for in the DLVO model. The extended-DLVO (XDLVO) theory includes the magnitude of the Lewis acid–base interaction [54,55]. The XDLVO theory is currently the subject of much research and discussion [40,56].

In the present study, batch experiments were conducted to characterize the attachment of viruses onto clay colloids by extending the research efforts by Syngouna and Chrysikopoulos [7], who studied the effect of temperature and agitation on the interactions of MS2 and Φ X174 with untreated, “readily available” kaolinite and bentonite (90% montmorillonite) under quite different experimental and analytical procedures than those employed here. The extended-DLVO approach was used to evaluate the attachment of bacteriophages MS2 and Φ X174 onto well-crystallized kaolinite (KGa-1b) and montmorillonite (STx-1b) (colloidal fraction $<2\ \mu\text{m}$). Furthermore, the surface properties of viruses and clays were

* Corresponding author. Tel.: +30 6945373208; fax: +30 2610996573.

E-mail address: gios@upatras.gr (C.V. Chrysikopoulos).

Nomenclature

A	absorbance (–)
A_{ii}	Hamaker constant of individual component, $M \cdot L^2/t^2$
A_{ij}	combined Hamaker constant, $M \cdot L^2/t^2$
C_i	aqueous phase concentration of species i , M/L
C_{i_0}	initial aqueous phase concentration of species i , M/L
C^*	concentration of viruses attached onto clay colloids, $(M \text{ viruses})/(M \text{ clay})$
e	elementary charge (C)
h	separation distance between two approaching surfaces, L
h_0	minimum separation distance between two approaching surfaces, L
i	subscript indicating the various materials used in this study
I_s	ionic strength (mol/L)
k_B	Boltzman's constant, $M \cdot L^2/(t^2 \cdot K)$
K_f	Freundlich constant, $(L^3/M)^m$
K_{123}	hydrophobic force constant (J), $M \cdot L^2/t^2$
m	Freundlich exponent (–)
N_A	Avogadro's number (1/mol)
r_p	average colloidal particle radius, L
T	Temperature (K), T
β_i	contact angle of material i (°)
ε	dielectric constant of the suspending liquid ($C^2/(J \cdot m)$)
ε_0	permittivity of free space ($C^2/(J \cdot m)$)
ε_r	relative dielectric constant of the suspending liquid (–)
θ	XRD scanning angle increment (°)
κ	Debye–Huckel parameter, $1/L$
λ	characteristic wavelength of interaction between two approaching surfaces, L
λ_{AB}	decay (Debye) length of water, L
σ_{Born}	Born collision parameter, L
Φ_{AB}	Lewis acid–base potential energy (J), $M \cdot L^2/t^2$
$\Phi_{AB(h_0)}$	Lewis acid–base free energy of interaction at $h = h_0$ (J/m^2), M/t^2
Φ_{Born}	Born potential energy (J), $M \cdot L^2/t^2$
Φ_{dl}	double layer potential energy (J), $M \cdot L^2/t^2$
Φ_{max1}	primary maximum of Φ_{tot} (J), $M \cdot L^2/t^2$
Φ_{min1}	primary minimum of Φ_{tot} (J), $M \cdot L^2/t^2$
Φ_{min2}	secondary minimum of Φ_{tot} (J), $M \cdot L^2/t^2$
Φ_{vdW}	van der Waals potential energy (J), $M \cdot L^2/t^2$
Ψ_p	surface potential of the colloidal particle (V)
Ψ_s	surface potential of the collector surface (V)

evaluated by electrophoretic mobility measurements and were used for the construction of DLVO and XDLVO potential energy profiles.

2. Materials and methods

2.1. Bacteriophages and assay

The bacteriophages MS2 (an F-specific single-stranded RNA phage with effective particle diameter ranging from 24 to 26 nm) and Φ X174 (a somatic single-stranded DNA phage with effective particle diameter ranging from 25 to 27 nm), were used in this study as surrogates for human viruses. MS2 has a hydrophobic protein coat, and Φ X174 has a hydrophilic protein coat [57]. Both bacteriophages infect *E. coli*, and were assayed by the double-layer overlay method [58], as outlined by Syngouna and Chrysikopoulos [43].

For the separation of viruses adsorbed onto clay colloids from suspended viruses in the liquid phase, in 2 mL of the liquid sample was added 0.3 mL of the density gradient separation reagent Histodenz (60% by weight, AXIS-SHIELD PoC AS Company, Norway) [5,6,10]. The mixture was centrifuged at $2000 \times g$ for 30 min so that the supernatant was free of clay colloids. The optimum separation conditions were determined experimentally. The suspension of unattached viruses in the supernatant was pipetted out and the suspended viruses were determined by the double-layer overlay method [58]. The absence of clay colloids in the supernatant was verified by a UV–vis spectrophotometer (UV-1100, Hitachi) at a wavelength of 280 nm. The concentration of attached viruses was determined by subtracting the mass of viruses that remained in suspension from the initial virus concentration in each sample. In addition, preliminary control experiments were conducted, as outlined by Vasiliadou and Chrysikopoulos [10], to verify that Histodenz did not interfere with the virus concentration measurements.

2.2. Clays

The clays used in this study were kaolinite (KGa-1b, is a well-crystallized kaolin from Washington County, Georgia [59]) and montmorillonite (STx-1b, a Ca-rich montmorillonite, white, from Gonzales County, Texas), purchased from the Clay Minerals Society, Columbia, USA. KGa-1b has a specific surface area of $10.1 \text{ m}^2/\text{g}$, as evaluated by the Brunauer–Emmet–Teller (BET) method, and a cation exchange capacity of $2.0 \text{ meq}/100 \text{ g}$ [60]. STx-1b has a specific surface area of $82.9 \text{ m}^2/\text{g}$ [61], and assuming that the characteristics of STx-1b are comparable to those of STx-1, which is the previous batch of montmorillonite from the same area, its cation exchange capacity is $84.4 \text{ meq}/100 \text{ g}$ [60].

Fifty grams of each clay mineral were mixed with 100 mL distilled deionized water (ddH₂O) in a 2 L beaker. Sufficient hydrogen peroxide (30%, solution) was added to oxidize all organic matter. The mineral suspension was adjusted to pH 10 with 0.1 M NaOH solutions and dispersed by ultrasonication for 20 min. The suspension was diluted to 2 L and the $<2 \mu\text{m}$ colloidal fraction was separated by sedimentation. The separated colloid suspension was flocculated by adding 0.5 M CaCl₂ solution. The colloidal particles were washed with ddH₂O and ethanol to remove the Cl[–] ions and subsequently dried at 60 °C [6]. The optical density of the clay colloids was analyzed at a wavelength of 280 nm by a UV–vis spectrophotometer, and the corresponding clay concentrations were calibrated with the standard curves of clay optical densities presented in Fig. 1, which are based on dry weights. Using the calibration curves (Fig. 1), each measured KGa-1b absorbance, $A_{(KGa-1b)}$ [–], was converted to KGa-1b concentration, $C_{(KGa-1b)}$ [g/L], using the relationship:

$$C_{(KGa-1b)} = 0.314 A_{(KGa-1b)} + 0.026 \quad (1)$$

Similarly, each measured STx-1b absorbance, $A_{(STx-1b)}$ [–], was converted to STx-1b concentration, $C_{(STx-1b)}$ [g/L], using the relationship:

$$C_{(STx-1b)} = 0.326 A_{(STx-1b)} - 0.007 \quad (2)$$

Note that the absorbance was assumed to be a non-dimensional number as recorded by the spectrophotometer.

Transmission electron microscopy (TEM) by a JEOL (JEM-2100 system, operated at 200 kV) was performed by diluting each clay colloid fraction $<2 \mu\text{m}$ in ddH₂O, placed in an ultrasonic bath for 10 min, and air-dried onto a carbon-coated copper grid (200 mesh). Micrograph images were recorded by a Erlangshen CCD Camera (Model 782 ES500W). Two representative images are shown in Fig. 2. The TEM analyses suggested that KGa-1b was dominated by kaolinite hexagonal platy particles with size in the range of

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