

<sup>1</sup> Environmental Microbiology Unit, Department of Public Health, University of Patras, Patras, Greece (avanta@upatras.gr),

<sup>2</sup> Department of Civil Engineering, Environmental Engineering Laboratory, University of Patras, Patras, Greece

<sup>3</sup> Environmental Engineering Laboratory (TUceel), School of Environmental Engineering, Technical University of Crete, Chania, Greece

Abstract

Clays are used to establish low permeability liners in landfills, sewage lagoons, water retention ponds, golf course ponds, and hazardous waste sites. Human adenoviruses (HAdVs) are waterborne viruses which have been used as viral indicators of fecal pollution. The objective of this study was to investigate the survival of HAdV in static and dynamic clay systems. The clays used as a model were crystalline aluminosilicates: Kaolinite and Bentonite. The adsorption and survival of HAdVs onto these clays were characterized at two different controlled temperatures (4 and 25° C) under static and dynamic batch conditions. Control tubes, in the absence of clay, were used to monitor virus inactivation due to factors other than adsorption to clays (e.g. inactivation or sorption onto the tubes walls). For both static and dynamic batch experiments, samples were collected for a maximum period of seven days. This seven day time – period was determined to be sufficient for the virus-clay systems to reach equilibrium. To infer the presence of infectious HAdV particles, all samples were treated with Dnase and the extraction of viral nucleid acid was performed using a commercial viral RNA kit. All samples were analyzed by Real – Time PCR which was used to quantify viral particles in clays. Samples were also tested for virus infectivity by A549 cell cultures. Exposure time intervals in the range of seven days (0.50-144 hours) resulted in a load reduction of 0.74 to 2.96 logs for Kaolinite and a reduction of 0.89 to 2.92 for Bentonite. Furthermore, virus survival was higher onto Bentonite than Kaolinite (p<0.005). The experimental results of this work indicate that viruses were systematically more persistent at 4° C than at 25° C onto both clays (p<0.005). The adsorption of HAdV onto both clays increase with increasing time and it is higher in dynamic experiments (P<0.005). The increased reduction of waterborne viruses by their contact with clays systems could play an important role in the prevention of viral waterborne diseases.

Materials & Methods

## Virus stock preparation

- Human Adenovirus serotype 35 (HAdV35) as model virus
- Cultivation in human lung carcinoma cell line A549
- The initial concentration of adenoviral stock: 10<sup>8</sup>-10<sup>9</sup> genome copies/ml
- Quantification by Real-Time Polymerase Chain Reaction (qPCR)

## Real – Time PCR (qPCR) virus detection

- Hexon gene was used as the target area
- All samples were tested in duplicate (two neat and two diluted)
- Use of TaqMan Universal PCR Master Mix (Applied Biosystems) and a carry-over contamination prevention system, uracil N-glycosylase (Hemroth et al., 2002)

## Electrokinetic Measurements

The Zeta potentials of the HAdV35 and clay particles (Kaolinite, Bentonite) were measured at pH 7.6 in the DNase I reaction buffer solution in both I<sub>s</sub> ≈ 1.4 mM and I<sub>s</sub> ≈ 14 mM by a zetasizer (see Table 1)

## Clay minerals

- Kaolinite (03584 Kaolinite, Fluka, chemical composition: Al<sub>2</sub>O<sub>3</sub> ~37.6%, SiO<sub>2</sub> ~47.3%, Fe<sub>2</sub>O<sub>3</sub> ~0.5%, TiO<sub>2</sub> ~0.4%, K<sub>2</sub>O ~1.8%, Na<sub>2</sub>O ~0.1%, loss on calcination ~12%)
- Bentonite (18609 Bentonite, Riedel de Haen, > 90% montmorillonite, chemical composition: SiO<sub>2</sub> 59.2%, Al<sub>2</sub>O<sub>3</sub> 18.5%, Fe<sub>2</sub>O<sub>3</sub> 5.6%, CaO 2.0%, MgO 4.0%, Na<sub>2</sub>O 0.2%, K<sub>2</sub>O 0.9%, weight loss on ignition 8.7%).

Table 1. Zeta potential measurements

Particles	Zeta Potential (mV) ±SD	
	I <sub>s</sub> = 1.4 mM	I <sub>s</sub> = 14 mM
Adenovirus (HAdV35)	-21.78±1.39	-9.18±1.35
Kaolinite	-19.95±0.50	-16.73±0.48
Bentonite	-29.76±0.78	-14.80±1.34

Materials & Methods

## Batch experiments

### Kinetic Adsorption Experiments

- Two different controlled temperatures (4 and 25° C)
- DNase I reaction buffer solution at pH 7.6
- Two different ionic strengths
- I<sub>s</sub> ≈ 1.4 mM
- I<sub>s</sub> ≈ 14 mM
- Static and dynamic batch conditions
- Control tubes (virus in the absence of clay)
- Reactor tubes (virus in the presence of clay: 10 mg/ml)
- Virus concentration 10<sup>6</sup> copies/mL
- Dynamic batch experiments: reactor and control tubes attached to a rotator
- Samples were collected every 24 h for a period of 7 days and centrifuged at 2000g (6000 rpm) for 10 min

### Isotherm Adsorption Experiments

- Five concentrations of HAdV35: 10<sup>4</sup> to 10<sup>9</sup> copies/ml
- Dynamic Conditions, Temperature of 4° C
- DNase I reaction buffer (I<sub>s</sub> ≈ 1.4 mM, pH 7.6)
- The mass balance equation for computing virus sorbed onto clay particles is described by (Syngouna and Chrysikopoulos, 2010):

$$C_v^*(t) = \frac{C_{v0}(t) - C_v(t)}{M}$$

where

- C<sub>v0</sub>, virus concentration at time t in the controls (copies/ml)
- C<sub>v</sub>, virus concentration at time t in the reactors (copies/ml)
- C<sub>v</sub><sup>\*</sup> concentration of viruses sorbed onto a clay (copies /mg clay)
- M (mg clay/ml) mass of clay added per volume of virus suspension

## DLVO interaction energy calculations

(Loveland et al., 1996)

$$\Phi_{DLVO}(h) = \Phi_{vdW}(h) + \Phi_{dl}(h) + \Phi_{Born}(h)$$

where

- Φ<sub>vdW</sub> [J], the van der Waals
- Φ<sub>dl</sub> [J] the electrostatic double layer
- Φ<sub>Born</sub> [J] the Born, interaction energies
- h [m] the separation distance between the approaching surfaces

Table 2. Estimated values of Φ<sub>min1</sub>, Φ<sub>min2</sub> and Φ<sub>max1</sub> for the HAdV35 virus-clay interactions

DLVO Interactions	Φ <sub>min1</sub> (K <sub>B</sub> T)	Φ <sub>max1</sub> (K <sub>B</sub> T)	Φ <sub>min2</sub> (K <sub>B</sub> T)
I <sub>s</sub> = 1.4 mM			
Kaolinite	-8.692	14.39	-0.0089
Bentonite	-1.063	22.25	-0.0082
I <sub>s</sub> = 14 mM			
Kaolinite	-24.47	0.4937	-0.2181
Bentonite	-24.56	0.2119	-0.2350

## Virus-clay interactions

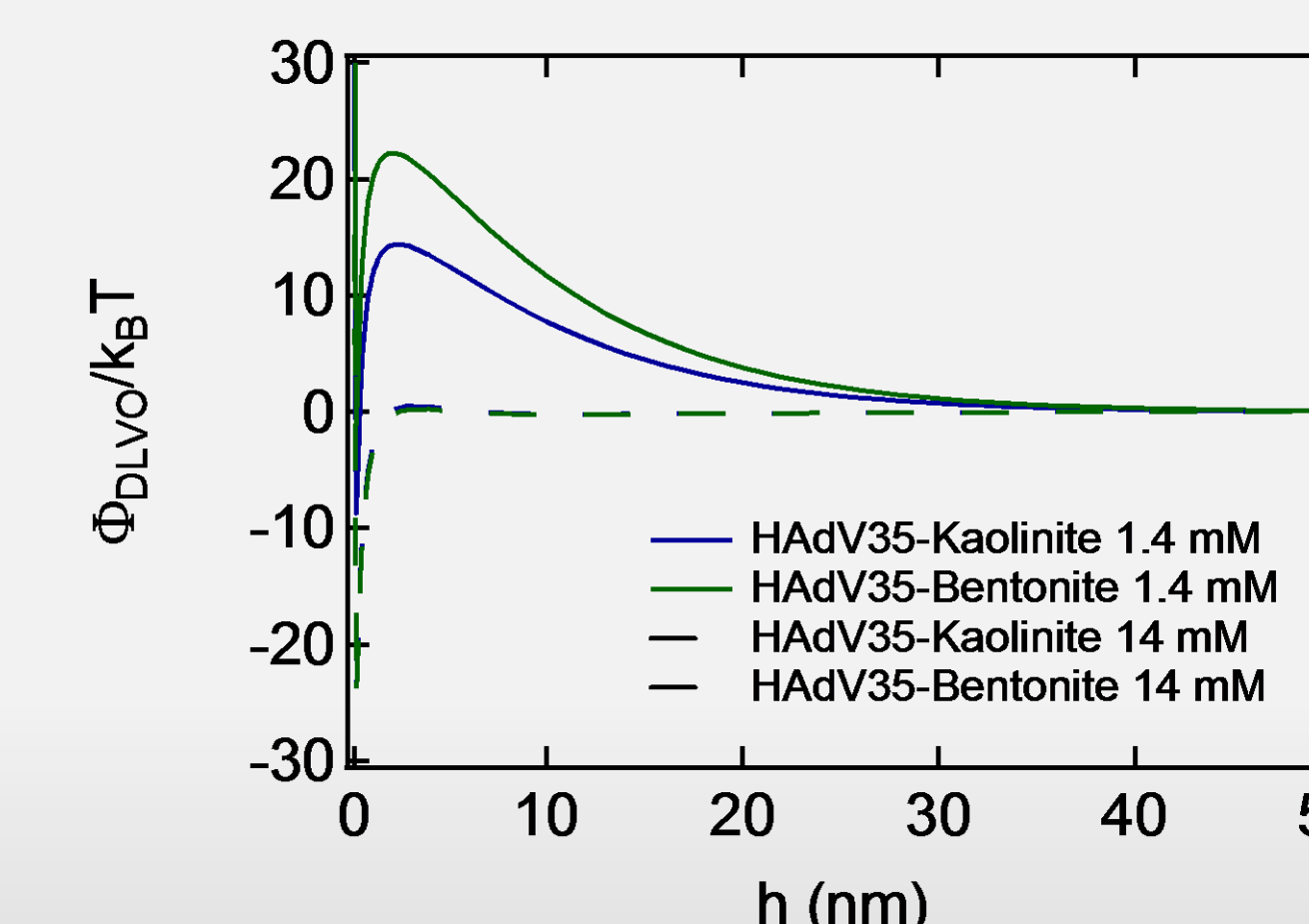


Figure 1. Predicted DLVO interaction energy profiles for HAdV35 with clay minerals (Kaolinite, Bentonite) as a function of separation distance for the experimental conditions of I<sub>s</sub>=1.4mM and I<sub>s</sub>=14mM at pH 7.6, using sphere-plate approximation.

## Particle Aggregations

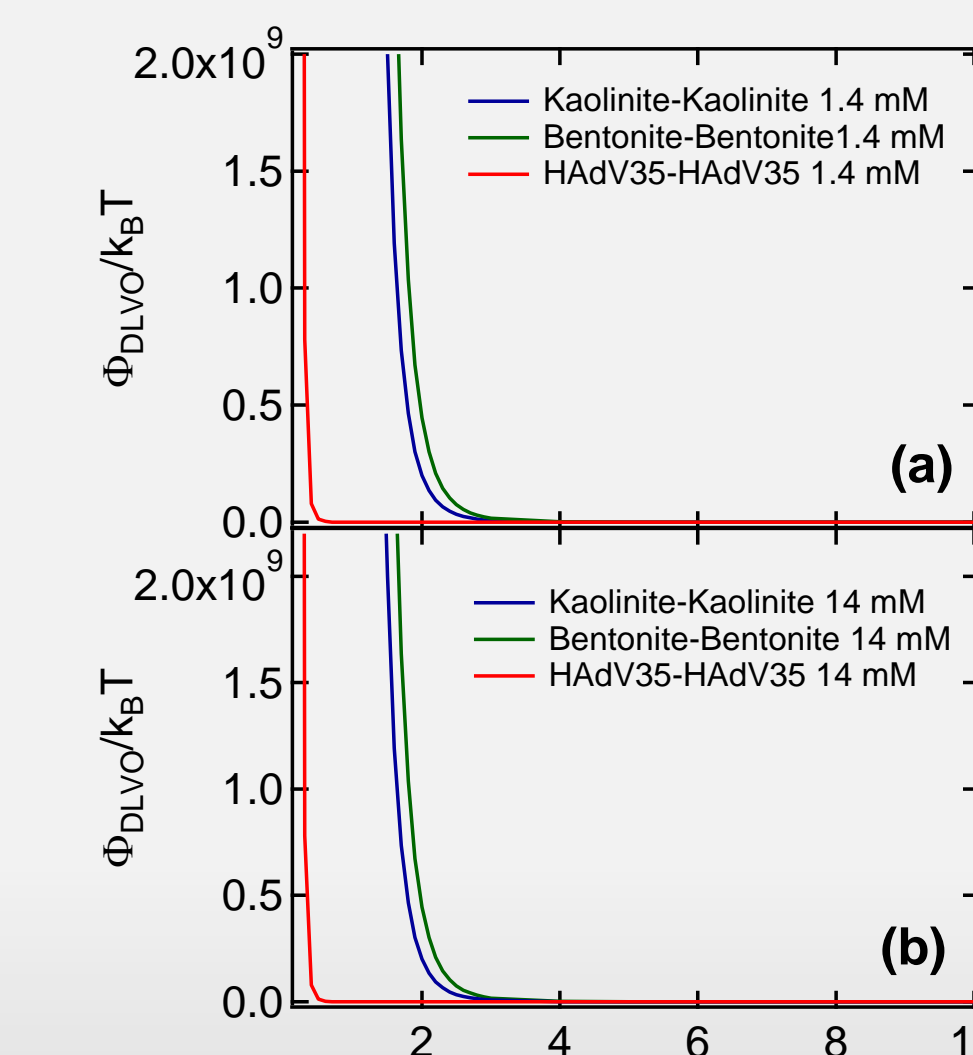


Figure 2. Predicted sphere-sphere Φ<sub>DLVO</sub> interaction energy profiles for Kaolinite-Kaolinite, Bentonite-Bentonite, and HAdV35-HAdV35 as a function of separation distance, for the experimental conditions of (a) I<sub>s</sub>=1.4mM and (b) I<sub>s</sub>=14mM.

Results

## Batch Kinetic Experiments

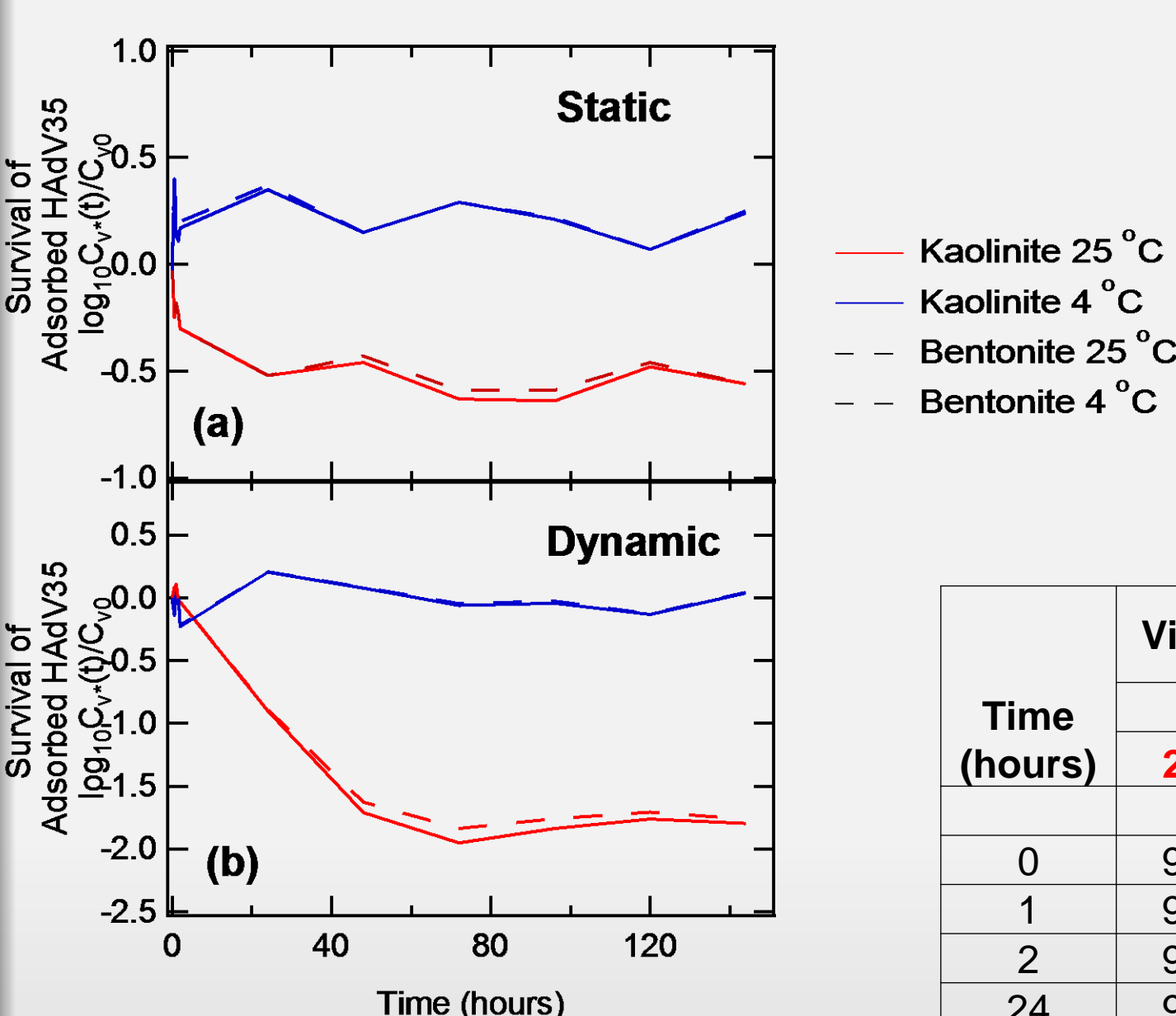


Figure 3: Survival of Adsorbed HAdV35 expressed as log<sub>10</sub> C<sub>v</sub><sup>\*</sup>(t)/C<sub>v0</sub> at two different temperature conditions, 4° C and 25° C at pH 7.6 and I<sub>s</sub>=1.4 mM: a) Static experiments and b) Dynamic experiments.

Table 3. Kinetics of HAdV35 adsorption to clays under static conditions, at 4° C and 25° C, pH 7.6 and two ionic strength values (I<sub>s</sub>=1.4 mM and I<sub>s</sub>=14 mM)

Time (hours)	Virus adsorbed (% of control at time t)			
	Kaolinite		Bentonite	
	25° C	4° C	25° C	4° C
I <sub>s</sub> =1.4mM				
0	99.80	92.51	99.61	95.16
1	98.52	88.34	99.52	96.95
2	99.01	90.69	99.59	98.47
24	98.68	94.01	99.42	98.69
48	92.86	99.18	98.80	98.87
I <sub>s</sub> =14mM				
0	98.34	99.97	95.64	99.17
1	60.36	99.50	75.53	97.83
2	99.95	52.05	93.14	29.78
24	99.67	99.99	97.72	99.87
48	98.59	99.94	79.77	99.73

## Batch Isotherm Experiments

The experimental data from the equilibrium attachment experiments of HAdV35 onto Kaolinite and Bentonite was fitted to the logarithmic form of the Freundlich equation:

$$\log C_{veq}^* = \log K_f + m \log C_{veq}$$

where

- C<sub>veq</sub><sup>\*</sup> [(M viruses)/(M clay)] is the virus concentration attached onto clay colloids (copies/μg clay)
- C<sub>veq</sub> [M/L<sup>3</sup>] is the aqueous phase virus concentration (copies/ml)
- K<sub>f</sub> [(L<sup>3</sup>/M)<sup>m</sup>] is the Freundlich constant {(ml)<sup>m</sup>/[(μg clay)(copies)<sup>m-1</sup>}
- m [-] is the Freundlich exponent

Table 4. Freundlich isotherm parameter values

Interacting materials	K <sub>f</sub> (mL/μg) <sup>m</sup>	m
HAdV35 & Kaolinite	0.42×10 <sup>2</sup>	0.773
HAdV35 & Bentonite	3.25×10 <sup>-2</sup>	1.494

Theoretical Calculations

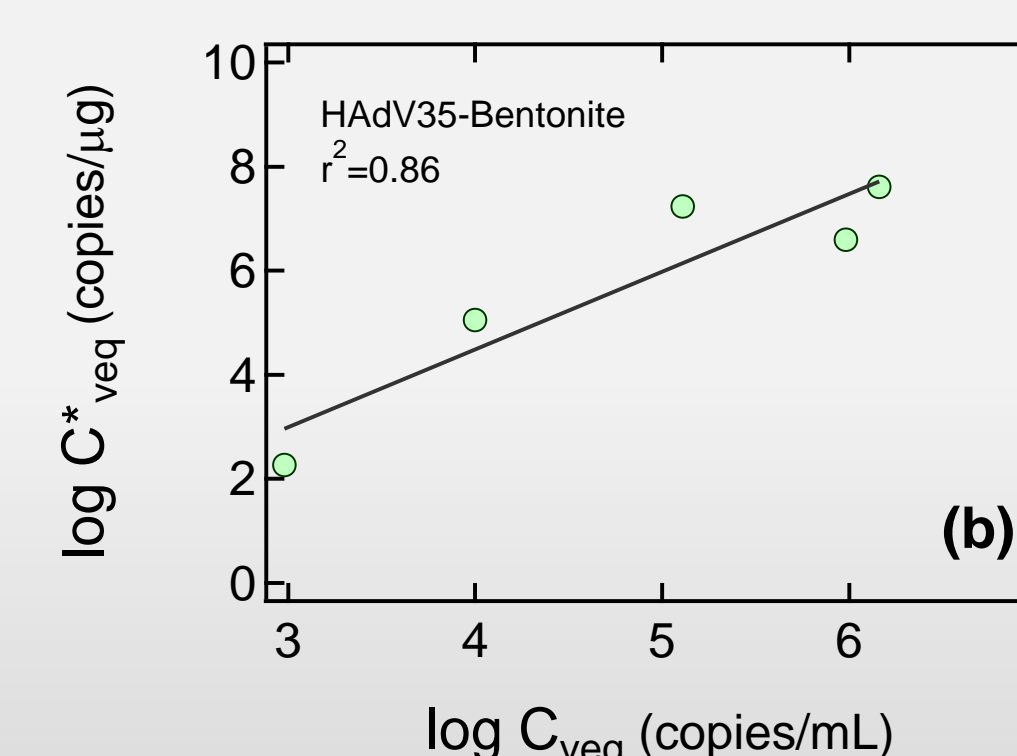
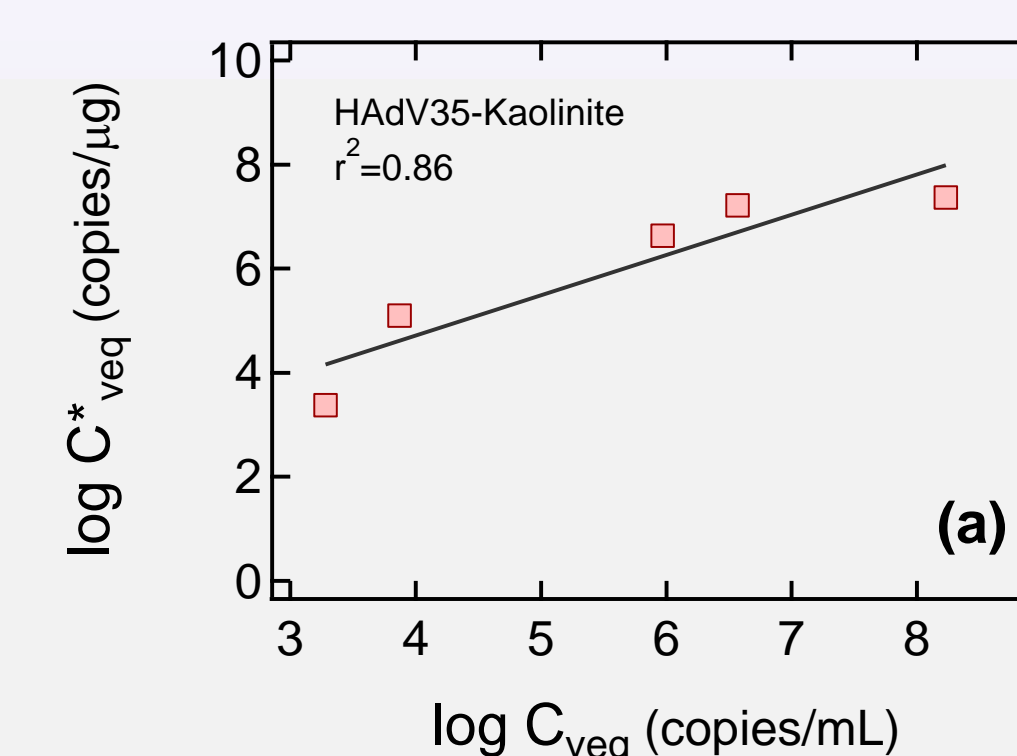


Figure 4. Freundlich isotherms for the attachment of (a) HAdV35 onto Kaolinite, (b) HAdV35 onto Bentonite, under dynamic conditions at pH 7.6 and 4° C. The corresponding Freundlich parameters K<sub>f</sub> and m are listed in Table 4.

Conclusions

Higher adsorption onto both Kaolinite and Bentonite was observed for dynamic than static conditions under both temperatures.

For the most of the cases examined under static conditions the adsorption of HAdV35 increased with increasing temperature at I<sub>s</sub>=1.4mM while for the case of I<sub>s</sub>=14mM no clear trend was observed.

The experimental data from the equilibrium attachment experiments of HAdV35 onto Kaolinite and Bentonite (dynamic conditions, I<sub>s</sub>=1.4 mM, pH 7.6, and 4° C) suggested that the affinity of HAdV35 is greater for Kaolinite than Bentonite.

The DLVO theory suggests that, for all cases considered, no coagulation between like particles is expected to occur under the experimental conditions (I<sub>s</sub>=1.4 mM, I<sub>s</sub>=14 mM, pH=7.6).

Φ<sub>max1</sub> energy barrier values are slightly higher for Bentonite than Kaolinite interactions with HAdV35. For the case of I<sub>s</sub>=14 mM the short energy barrier Φ<sub>max1</sub> makes the system completely unstable.

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