



Phage adsorption to bacteria in the light of the electrostatics: A case study using *E. coli*, T2 and flow cytometry

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ABSTRACT

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The addition of sodium chloride to freshwater or diluted minimal salt medium increases the adsorption of T2 phages on *Escherichia coli*. For the first time the adsorption in diluted minimal salt medium was measured by counting unadsorbed phages (i.e. free particles) using flow cytometry, allowing a gentle separation between adsorbed and unadsorbed phages. Flow cytometry was able to detect weakly adsorbed phage that remained undetected using classical centrifugation-based methods and this allowed us to show that increasing ionic strength enhances the phage adsorption to its bacterial host with an extremely low detection limit. A key result was that the adsorption in high ionic strength (i.e. 100 mM) reached $4.5 \pm 0.1 \times 10^{-5}$ mL/min which is 1400 fold higher than previously reported values.

In order to understand the mechanism underpinning such a weak phage adsorption, the zeta potentials and the diffusion coefficient of the particles were measured by dynamic light scattering. The bacterial cells and the phages had zeta potentials between -60 mV and -10 mV and -30 mV and -10 mV, respectively. The diffusion coefficient of the phage was $2.8 \pm 0.4 \times 10^{-12}$ m² s⁻¹ corresponding to a hydrodynamic radius of 104 ± 15 nm. However significant adsorption occurs in conditions where the DLVO theory predicts that minimal encounter, suggesting that forces other than electrostatic repulsion and Van der Waals interaction (e.g. potential impurities, particle shape and other biological characteristics) are likely to interplay.

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1. Introduction

Bacteria play an important role in the carbon cycle by recycling and concentrating dissolved organic matter in aquatic environments, thus making it available for higher trophic levels (Azam, 1983). It has been shown that phages can be a major driver of the carbon cycle by “killing the winner” (Bouvier and del Giorgio, 2007; Fuhrman, 1999), which impacts the nutrient uptake and releases a variety of dissolved organic molecules in the environment (Middelboe et al., 2003). For example, the addition of freshwater viruses to seawater impacts the coastal bacterial communities (Auguet et al., 2009). The role of phages has been reviewed recently (Jacquet et al., 2010; Middelboe et al., 2008) but the mechanisms driving the initial adsorption are not fully understood (Moldovan et al., 2007).

Interestingly the adsorption in situ may be hindered by an energetic barrier because most phages and bacteria are negatively charged in environmental conditions (Michen and Graule, 2010). For example, the PWH3a-P1 phage has a zeta potential at the slipping plane of -20 mV in 0.5 M NaNO₃ at pH 8, which is close to the ionic strength of seawater (Daughney et al., 2004). However, higher ionic strength of the medium is decreasing this energetic barrier, which is particularly interesting in the context of bodies of freshwater arriving to the sea and being confronted with a brutal increase in the concentration of cations. Therefore, phage adsorption can be favored by the increase of salinity occurring when the freshwater mixes with seawater. Early studies on *Escherichia coli* have shown that the T2 or the T4 phage does not adsorb to *E. coli* in the absence of salt while they adsorb when 100 mM of sodium chloride or potassium chloride is present (Puck et al., 1951). Further studies showed that the bacteriophage nt-1 needs a minimum concentration of 0.3 M of NaCl to replicate, which corresponds to the ionic strength of a brackish environment (Zachary, 1976).

Understanding the general mechanism of phage adsorption and being able to predict its dependence to salinity would be useful to understand the dynamics of bacterial populations in

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estuaries and therefore estimate the carbon fluxes in these ecosystems. Interestingly, the weak interactions between charged particles in an aqueous solution of moderate ionic strength are described by the DLVO theory, named after Derjaguin, Landau, Verwey and Overbeek. The DLVO theory was designed to predict stability between charged lyophilic colloids such as metal oxide nanoparticles and polyelectrolyte complexes but fails to predict emulsion and microemulsion stability (Evans and Wennerstrom, 1999). It can be used to make quantitative comparisons between the energy of the Brownian motion and the electrostatic repulsion. It was used in the phage–host context to illustrate that the adsorption was less likely to occur if the energetic barrier is higher than $10k_B T$ (Beumer et al., 1957). However, the dependence of the adsorption to salinity was not part of that study. Later, more refined versions of this theory were successfully applied to the interaction between phages and clay (Chattopadhyay and Puls, 2000) or between phages and iron oxide (Murray et al., 1978; Murray, 1980). In order to understand if the Brownian motion and the screening effect of the cations can explain the adsorption behavior of the phages, the adsorption rates need to be determined in a range of ionic strength on living bacteria.

Experimental methods include determination of equilibrium concentrations (Murray et al., 1978), column filtration (Knappett et al., 2008; Sadeghi et al., 2011) or immobilized cells in a microplate (Tortorello et al., 1991). However the method of choice for living material such as bacteria is the method used by Delbrück (1940). These authors mixed living bacteria and phages in controlled conditions and sampled an aliquot of the solution that was then centrifuged to pellet the bacteria together with the phages potentially adsorbed to them. The unadsorbed phages were counted from the supernatant. The rate of adsorption can then be simply calculated from the decrease of the unadsorbed phages over time. Despite the uncertainty that some adsorbed phages may be released during the centrifugation ($16,000 \times g$, 1 min, 4°C), this method has still been applied 67 years later with minor modifications (Moldovan et al., 2007).

In the present study a new method to measure the adsorption of a model phage (i.e. T2) on its bacterial host (*E. coli*) is proposed. This method avoids the centrifugation step, allowing a sensitive measurement of the adsorption, even in the case of weak adsorption forces. This flow cytometry-based method was proved to be more sensitive than the standard method to measure the adsorption of the T2 phage on living *E. coli* cells. The experimental adsorption results were compared to those calculated using the DLVO theory, which quantitatively estimates the Brownian motion of the virus particles with the electrostatic repulsion based on the zeta potentials and the diffusion rates of the phages and the bacteria. The relevance of salinity to adsorption in environmental conditions was shown by the increased viral adsorption both in freshwater and in a minimal salt medium of known ionic composition when the ionic strength was increased.

2. Materials and methods

2.1. Phage strain, bacterial host, freshwater sampling and media preparation

The T2-phage was chosen as a model (order *Caudovirales*, family *Myoviridae*, genus *T4-like viruses*) Plaque forming units of T2 were cut and used for amplification on *E. coli* XL1. The bacterial cells were grown overnight on LB₁₀ at 30°C unless stated otherwise. Phages were concentrated from the supernatant of an infected culture by the following procedure: addition of 0.1% of chloroform to lyse the cells completely, addition of polyethylene glycol (PEG) 8000 (1% final concentration, overnight incubation at 4°C) to precipitate the phage particles by a $16,000 \times g$ centrifugation for

4 h and finally re-suspension in the relevant medium. The freshwater was sampled on September 2009 from the Randwick pond (Sydney, Australia). The water was then filtered through a $0.2 \mu\text{m}$ filter and divided into 2 bottles stored at 4°C . The conductivity was 0.224 mS/cm (i.e. equivalent ionic strength of $3.5 \times 10^{-3} \text{ M}$). One of the bottle received 100 mM of NaCl to test the effect of salt on the adsorption in freshwater. The 10 mM phosphate buffer at pH 7 was prepared by dissolving 2.26 g/L of disodium orthophosphate dodecahydrate and 0.56 g/L of anhydrous potassium dihydrogen orthophosphate. The stock solution was diluted to 0.1 mM before each experiment and the appropriate amount of sodium chloride was added. The medium was then heated to fully dissolve the sodium chloride and left overnight at room temperature. The T2 phages were resistant to the media used in this study over the time course of the experiments (not shown). All experiments were done at 19°C unless specified otherwise.

2.2. Adsorption experiment based on flow cytometry

A FACSCalibur (Becton Dickinson) flow cytometer hosted at INRA Thonon (France) was used to quantify the adsorption rate of the T2 phage on its bacterial host. This benchtop device is equipped with a laser providing 15 mW at 488 nm able to count accurately free bacterial cells and viral particles (Thomas et al., 2011). The phages were diluted in 10^{-6} M phosphate buffer containing reference beads ($1 \mu\text{m}$, Molecular Probes) to which sodium chloride was added to the appropriate ionic strength for the following experiment and incubated with SYBR Green I (at a final 10^{-4} dilution of the commercial stock solution; Molecular Probes) to label the nucleic acids of the dsDNA phage T2. The ionic strength did not affect the staining of the viral particles. It should be noted that this equilibration step also prevented the possible adsorption of T2 phages on reference beads (if any) to interfere with the measurement of the adsorption rate on bacterial cells. The phage concentration was chosen to avoid co-occurrence, which was tested by diluting the sample (not shown). Therefore, one event with “low fluorescence” corresponds to one phage. Controls were achieved before each experiment to ensure that no event was detected in the phage window cytogram when no phage was added to the medium. Controls without addition of bacteria showed that stained viral particles were stable over the course of the experiments.

The bacterial cells were washed three times in the appropriate saline phosphate buffer by centrifugation for 1 min at 4°C at $6000 \times g$ and resuspended in that buffer by gentle pipetting. At the beginning of the adsorption experiment, bacteria equilibrated in the relevant medium were mixed with the T2 phages equilibrated with the reference beads to achieve a final concentration of 7×10^3 bacteria/mL.

The bead concentration was used as an internal standard to ensure that flow rates of the device were constant and that the variation in the number of phage (counted in 30 s after flow stabilization) corresponded to the real variation of the concentration of unadsorbed phages. The bacterial concentration was chosen to avoid co-occurrence. The adsorption experiments using this method were performed with 10^5 phages/mL and 7×10^3 bacteria/mL.

The adsorption rate was determined following Delbrück (1940). Briefly, P being the concentration of phages/mL, B the concentration of bacteria/mL and t the time (in min), the adsorption rate k was defined as follows:

$$\frac{dP}{dt} = -kBP$$

$$P = P_0 e^{-kPt}$$

$$\log_{10} \left(\frac{P}{P_0} \right) = -k_B t$$

Hence the coefficient “ $-k_B$ ” is the slope from $\log(P/P_0)$ versus time and the adsorption rate k can be calculated if B is known.

The observed slow coagulation factor was defined for each ionic strength as the ratio of the observed adsorption rate to the maximum observed adsorption rate across all ionic strengths.

2.3. Adsorption experiment based on centrifugation

In order to compare the method based on flow cytometry to the standard method, the adsorption experiment was performed following a slightly modified version of the Delbrück protocol (Delbrück, 1940). All vessels were made of glass to minimize the adsorption of viral particles on wall recipients. Phages and bacteria were incubated together to allow phages to adsorb on bacteria and experiments were conducted in duplicates. The reaction was stopped at various time points by a 50× dilution in the relevant media and the diluted solution was centrifuged to pellet the adsorbed phages (16,000 × g, 1 min, 4 °C).

In parallel, the free phages remaining in the supernatant were counted by the plaque forming unit method. Warm soft agar (0.6%) was mixed overnight with a bacterial culture in LB and serial dilutions of the supernatant were dotted onto the freshly solidified soft agar upper layer (Zemb et al., 2008).

The adsorption experiments using this method were performed with 1.4×10^6 phages/mL and 10^8 bacteria/mL. The adsorption rate was calculated as described above.

2.4. Diffusion coefficient and electrophoretic measurements

The electrophoretic mobility of the bacteria and phages was measured by dynamic light scattering using a zetasizer Z/S (Malvern, UK). The electrostatic potential was calculated for each ionic strength after converting the electrophoretic mobility (U_e) into surface potential (z) using the Henry equation with Smoluchowski's approximation ($U_e = \varepsilon z / \eta$ with ε the dielectric constant and η the viscosity).

Bacteria were washed 3 times in the relevant medium. Phages were concentrated from the supernatant of an infected culture by adding PEG 8000 (1% final concentration, overnight incubation at 4 °C), centrifuged at 16,000 × g for 4 h, re-suspended in water and then mixed with salts to achieve 10^{10} phages/mL in the relevant medium with the correct ionic strength. In order to make sure that PEG was not measured by dynamic light scattering, the same experiment was performed using PEG 20,000 and checking that the results were similar (not shown). Dialysis against water using a 12,000 mol wt membrane gave similar results (not shown). Additional controls were performed using PEG without T2 phages. The diffusion coefficient of the phages was determined by dynamic light scattering with the same device. Experiments were performed at 20 °C and 25 °C. The diffusion coefficient of the bacteria was approximated by assimilating the bacteria to a sphere and using the Einstein–Stokes relation with a viscosity of $1.221 \times 10^{-6} \text{ m}^2 \text{ s}^{-1}$ and a radius of $5 \times 10^{-7} \text{ m}$.

2.5. DLVO interaction potential and theoretical slow coagulation factor

The theoretical encounter rate due to sole diffusion was estimated according to the calculations of Krut (1952), using the following equation:

$$k_{\text{diffusion}} = 4\pi(D_p + D_b)(a_p + a_b)$$

where D_p and D_b are the diffusion coefficients of the phage and the bacteria and a_p and a_b are their respective radii. The hydrodynamic radius of the phage was estimated from its diffusion coefficient by the Einstein–Stokes relation and was found to be 104 nm.

A phage approaching a bacterial cell was assimilated to a spherical particle approaching a flat plane. The Van der Waals and the electrostatic potential of this system were calculated assuming that both surfaces were rigid with a two-dimensionally distributed surface charge. Therefore, the potential due to electrostatic repulsion follows the equation (Elimelech et al., 1995):

$$V_{\text{el}}(h) = \frac{128\pi a_p 1000 N_A c k_B T}{K^2} \gamma_p \gamma_b e^{-Kh}$$

with h as the separation distance between surfaces, a_p as the radius of the phage, N_A as the Avogadro number, c as the concentration of ions (mol/L), k_B as the Boltzmann constant, T as the temperature (Kelvin), K as the inverse Debye length (m^{-1}), and γ_p and γ_b as the reduced surface potential of the phage and the bacteria (which relates to the surface potential ζ by $\gamma = \tanh(\zeta/4)$). The surface potential of phages and bacterial cells were estimated by logarithmic regression between the measured values.

The potential due to the Van der Waals forces was calculated according to the following equation (Elimelech et al., 1995):

$$V_{\text{vdw}}(h) = -\frac{A}{6} \left(\frac{a_p}{h} + \frac{a_p}{2a_p + h} + \ln \left(\frac{h}{2a_p + h} \right) \right)$$

where A is the Hamaker constant taken as the Hamaker constant of proteins interacting across water, i.e. $5.9 \times 10^{-21} \text{ J}$ (Parsegian, 2006).

According to the DLVO theory (Verwey and Overbeek, 1948), the $k_{\text{diffusion}}$ encounter rate should be divided by a so-called slow coagulation factor W to obtain the number of collisions between phages and bacteria by taking into account the Van der Waals and the electrostatic potentials (V_{vdw} and V_{el}) according to the following equation:

$$W = 2 \int_0^\infty e^{\frac{(V_{\text{vdw}}(h)/k_B T) + (V_{\text{el}}(h)/k_B T)}{h^2}} dh$$

3. Results

3.1. Effect of sodium chloride on the adsorption of phage T2 on *E. coli*

Natural freshwater was sampled and used to determine whether the increase of the ionic strength due to sodium chloride is relevant in the brackish environment in situ. The Delbrück method was used to determine the adsorption of the T2 phage on *E. coli* using this freshwater and compared with the adsorption using the same freshwater complemented with 100 mM of sodium chloride.

The number of un-adsorbed phages was significantly lower 20 min after the sodium chloride was added ($p_{\text{test}} = 0.023$) (Fig. 1). The controls without bacteria and the controls without sodium chloride showed that sodium chloride did not affect the survival of the phage and that the phages adsorbed on bacteria rather than on the vessel walls. This shows that sodium chloride and more generally salt (concentration) plays a significant role in the adsorption of T2 phages in natural fresh waters.

3.2. Effect of sodium chloride on T2 adsorption in controlled conditions

When flow cytometry was used instead of the Delbrück method to discriminate the un-adsorbed T2 phages in diluted phosphate buffer complemented with 100 mM (Fig. 2), the adsorption of

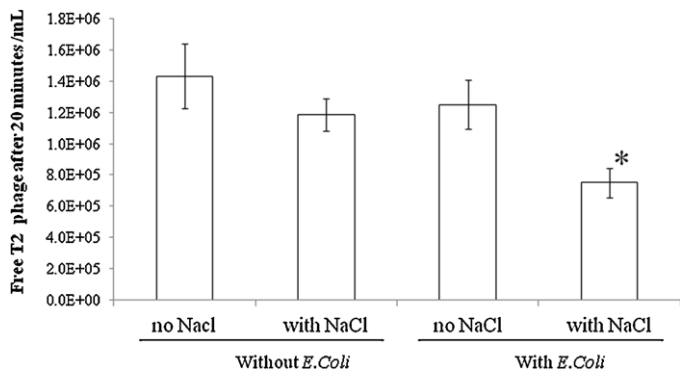


Fig. 1. Effect of the addition of 100 mM of NaCl to natural filtered freshwater on the adsorption of T2 on *E. coli* using the Delbrück method. Error bars represent 6 independent measurements.

the phages on exponentially growing cells followed a first order kinetic during the first 2 min of the experiment (Fig. S1). The initial adsorption rates were negligible in media containing less than 50 mM NaCl but reached 5.12×10^{-6} , 2.28×10^{-5} , 3.48×10^{-5} and $4.51 \pm 0.12 \times 10^{-5}$ mL/min in the dilute phosphate buffers containing 50, 60, 75 and 100 mM NaCl respectively (Fig. 3 and Fig. S2).

Therefore the slow coagulation factor (W) was found to vary between 1 (in diluted PB buffer with 100 mM NaCl) and 8.8 (in diluted PB buffer with 50 mM NaCl). The Delbrück experiment performed on growing cells in diluted phosphate buffer complemented with 10 mM or 100 mM sodium chloride confirmed that the adsorption was close to previously published values for phage T2 and *E. coli* when using the standard protocol: Indeed the adsorption rate of the phages T2 used in the current study was 1.75×10^{-9} mL/min for the 100 mM NaCl buffer while it was not significant for the 10 mM NaCl (Fig. S3), which is consistent with the previously published rate of 2.1×10^{-9} (Puck et al., 1951). Hence, the standard adsorption method based on centrifugation resulted in rates 25,000 fold lower than the flow cytometric method using the same biological material.

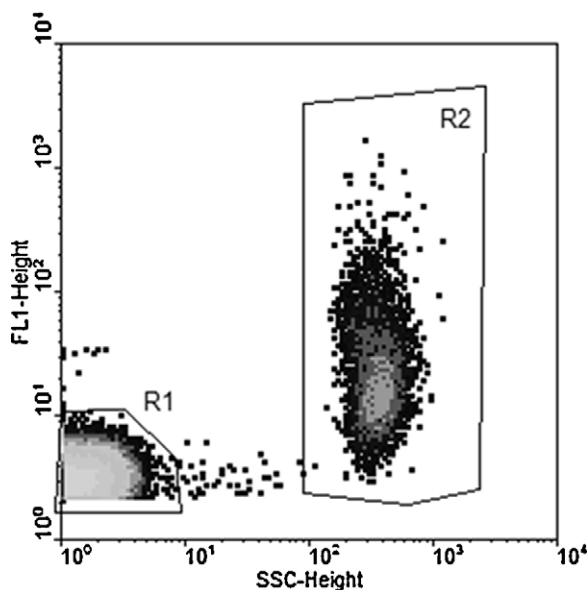


Fig. 2. Typical cytogram of a solution containing phages (R1) and bacterial cells (R2). The X-axis reports the side scatter and the Y-axis reports the green fluorescence related to the content of nucleic acids after staining with the SYBR dye.

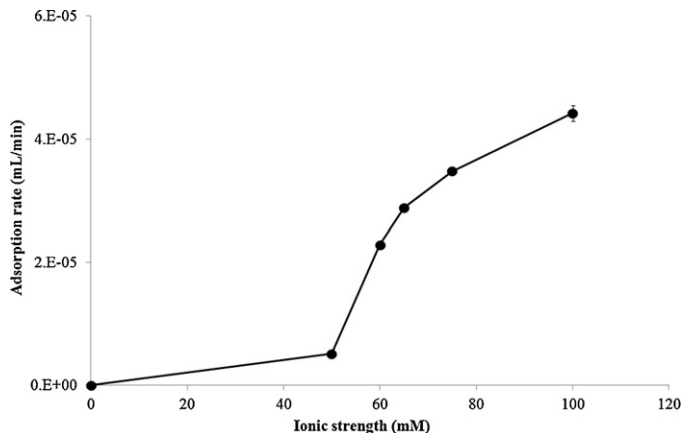


Fig. 3. Adsorption rates in PB buffer 0.1 mM complemented with 1, 50, 60, 65, 75 and 100 mM of NaCl. Error bar might be smaller than the symbol.

3.3. Surface potentials and comparison of the adsorption rates with the DLVO theory

Dynamic light scattering provided accurate measurements of the electrophoretic mobility of the bacteria and the phages. In the dilute phosphate buffer at pH 7 the electrophoretic mobility varied logarithmically with the concentration of sodium chloride (Fig. S4). The zeta potential of the phage T2 calculated from the electrophoretic mobility was -30 mV and -10 mV and depended on the ionic strength (Fig. 4). The zeta potential of *E. coli* bacteria varied between -60 mV and -10 mV. The energetic barrier of the phage–bacteria system increased from $-0.08k_B T$ at 100 mM NaCl to $25k_B T$ at 50 mM NaCl (Fig. 5 and Fig. S4). The DLVO evaluation was examined to relate this increase of the energetic barrier to an increase of the slow coagulation factor (see Section 2). The calculated slow coagulation factor differed from the observed one by several orders of magnitude with a predicted decrease in coagulation which was much more pronounced than the observed one, as shown in Fig. 6. In other words, the DLVO theory predicts almost no adsorption of the phage T2 at 50 mM NaCl, yet adsorption is observed at that ionic strength.

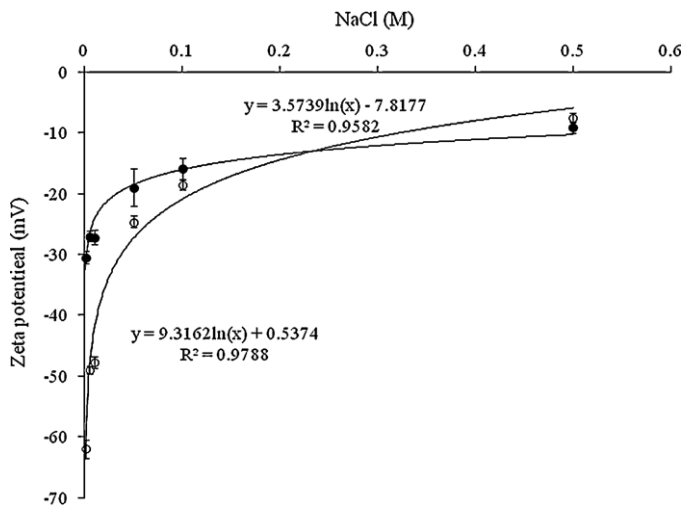


Fig. 4. Zeta potential of *E. coli* and phage T2 in different concentrations of NaCl. The zeta potential (mV) of *E. coli* (open symbols) could be interpolated by the equation $\text{zeta} = 9.31 \ln(c) + 0.53$ and the potential of T2 (closed symbols) could be interpolated by the equation $\text{zeta} = 3.57 \ln(c) - 7.8177$ with R^2 equals to 0.9788 and 0.9582, respectively, c being the concentration of NaCl in mol/L. Error bars represent 6 independent measurements and might be smaller than the symbol.

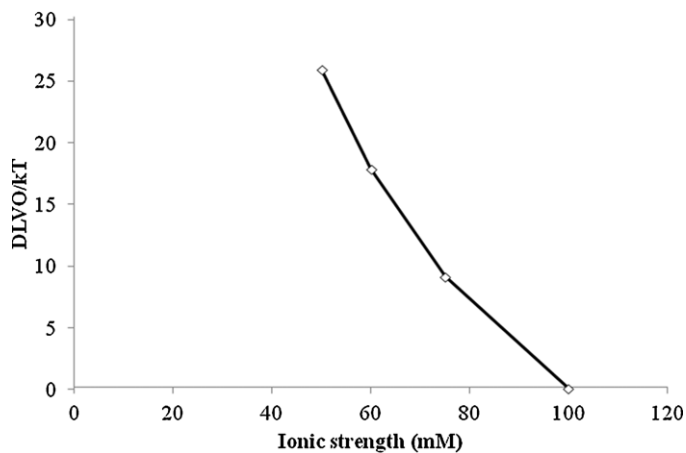


Fig. 5. Energetic barrier evaluated by the DLVO theory restricted to Van der Waals and electrostatics as a function of the ionic strength based on the diffusion rates and the interpolated zeta potentials.

4. Discussion

4.1. High adsorption rates

The current study describes the consequences on phage adsorption of a strong ionic strength increase such as when freshwater mixes with seawater. In order to understand the effects of the sodium chloride on the viral adsorption on living bacterial cells, the adsorption of T2 phages on *E. coli* was measured in controlled conditions using a cytometric method well-suited to weak interactions on biological material which would be destroyed by the method based on equilibrium concentrations used with iron oxides (Murray et al., 1978). The adsorption rates using flow cytometry were around 3×10^{-5} mL/min, which is 1000 fold higher than the highest previously published rate for the T2 phage on *E. coli*, i.e. 3.1×10^{-8} mL/min (Delbrück, 1940). The biological material yielded adsorption rates very close to the rates found previously when the standard method using centrifugation was used (1.75×10^{-9} vs 2.1×10^{-9} mL/min) (Puck et al., 1951). It should be noted that the rates were normalized by the bacterial density, so that this difference is not due to the difference in cell densities, which is over 10^9 cells/mL in the standard method and less than 10^4 in the flow cytometric method.

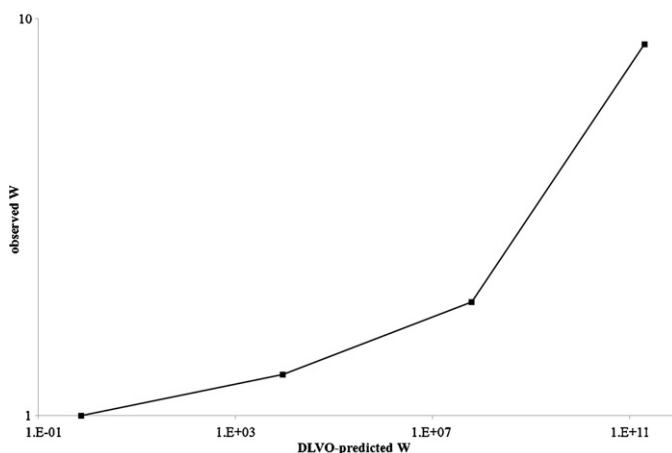


Fig. 6. Comparison of slow coagulation factor of bacteria and phage for salinities between 50 mM and 100 mM NaCl. The axes are in log to encompass the dynamic range involved.

The difference between the flow cytometric and the centrifugation methods might be due to the energies involved in the separation methods between adsorbed and un-adsorbed phages. In that respect, flow cytometry is best suited to the quantification of weak interactions because it represents a gentle mean to separate adsorbed from un-adsorbed phages in liquid suspension. In contrast the Delbrück method may not detect weak adsorptions because of the dilution or the centrifugation steps which can induce desorption of weakly adsorbed phages (Moldovan et al., 2007). Interestingly, phage T2 is inactivated by a mixture of protein Ia-lipopolysaccharide (Hantke, 1978), which suggests that T2 phage adsorbs on a bacterial surface protein and on the bacterial lipopolysaccharide forming the bacterial capsule (Lenski, 1984). Therefore the high adsorption rates observed by flow cytometry could be due to weak adsorption and location of the phage and bacteria in the secondary minimum of DLVO (flocculation) on components of the bacterial capsule, without strong adsorption such as coagulation or any mechanism involving chemical reactions and morphology change of the bacteria-phage interface. Still, the extremely high rates measured by flow cytometry are intriguing because they are 10^4 fold higher than the rate expected from sole diffusion, which is around 2.3×10^{-9} mL/min. Delbrück and coworkers also noted that their measured adsorption rate was about 15 times this theoretical diffusive maximum using previously determined diffusion rates of the phage similar to those found by dynamic light scattering (2.5 vs 2.8×10^{-12} m² s⁻¹) (Delbrück, 1940). This suggests that the diffusion mechanism of the bacteria and phages cannot be easily approximated by extending the Einstein–Stokes relation established for hard spheres to the phage using its measured hydrodynamical radius. If the turning speed is assumed high enough, one could consider that phages actually explore more space with their tail, leading to more frequent encounter without slowing down its diffusion. Assuming a tail of 115 nm and a head of 114 nm (Ackermann and Krisch, 1997), the radius of the phage cannot exceed 230 nm, which corresponds 2.6×10^{-8} mL/min. It should be noted that the flagella can extend the radius of the bacterial cell but that this cannot account for the discrepancy between the observed adsorption and the diffusion maximum since flagella of 10 microns would increase the adsorption rate to 2.1×10^{-8} mL/min, which is still far lower than the measured rate.

4.2. Application of the DLVO theory to the phage–bacteria system

The observed slow coagulation factor can still be compared to the theoretical expectations despite the discrepancy between the observed adsorption rate and theoretical diffusive maximum. From the DLVO theory, it is expected that increasing the ionic strength would enhance the adsorption because screening of the negative surface charge by the cations results in closer approach of the viruses toward the bacterial surface, and increased van der Waals attraction. The distance of approach is further decreased by the contraction of the external macromolecules on the bacterial surfaces, due to charge screening. Without considering these mechanisms, the DLVO-predicted decrease is several orders of magnitude higher than the observed one. This is surprising because the DLVO underestimated the repulsion between phage and ceramic filter, possibly because it does not take into account the hydration forces (Michen et al., 2011).

The first factor that might explain the lack of success of the DLVO theory to predict the adsorption of the phage could root to an error in the estimation of the surface charge by the electrophoretic measurements. Since *E. coli* is not a hard surface, the electrophoretic mobility of microbial particles cannot be rigorously explained by the approaches classically followed for hard and impermeable particles, as demonstrated by Duval and Gaboriaud

(2010). The description of the electrophoretic mobility of bacteria (and to a certain extend of viruses) is described in the diffuse soft particle electrokinetic formalism (DSPE) developed by Duval and Ohshima (2006). In this soft-layer model, electrophoretic mobility is basically described as originating from two terms: the weighted average of potentials over the surface charge layer, and the volume density of membrane-fixed charges (Ohshima and Kondo, 1991). At high ionic strength, where the potentials are low due to the charge screening effect of the electrolytes, the first term decreases so that the mobility is determined mainly by the second term, meaning that a bacterial cell can exhibit a non-zero electrophoretic mobility at high electrolyte concentration. This is observed in the present work, both for viruses and bacteria (Fig. 4). Therefore, the presence of the soft and permeable surface polymeric structures prevents the DLVO theory from predicting better than qualitatively the adsorption of phages on bacteria.

The second factor that might explain the discrepancy between the modeled and the experimental adsorption rates is that *E. coli* is not a smooth surface. Phages might use the external asperities of *E. coli* to adsorb such as pili, fimbriae, fibrils, polymeric fringes, lipopolysaccharides, peptidoglycan, that can extend hundreds of nanometers inside the surrounding solution. These structures probably play a peculiar role in phage adsorption, as shown by cryo-electron microscopy of phages infecting *Caulobacter crescentus*. These phages actively interact with the flagellum before attaching to receptors on the cell pole (Guerrero-Ferreira et al., 2011). The mechanisms causing the preferential attachment to these asperities remain unclear and the interaction potential of the particles toward rough surfaces are difficult to predict despite the extensions of the DLVO theory to encompass rough surfaces. For example, the consideration of the Lewis acid–base interactions using the extended DLVO theory improves the description of the behavior of blood cells in aqueous solution but requires parameters such as the surface tension components (Wu et al., 1999), making it hard to test experimentally.

Finally, the physiological state of the bacterial cells might affect the adsorption rate. For example, Delbrück (1940) found that the adsorption is greater on motile bacteria if their motility is not affected by adverse environmental condition. In this context, the amplified Brownian movement near swimming bacteria (Li et al., 2008) could enhance the adsorption of the phage despite an energetic barrier. This parameter could be of relevance in some experimental situations but is unlikely to cause the large adsorption discrepancies observed because the motility of bacteria probably does not change drastically within minutes at the moderate variation of ionic strength used in the current study. In contrast, the multiplicity of infection used in the current study (i.e. 14) might generate cations fluxes within minutes after exposure to phages (Silver et al., 1968), hence creating “impurities” in the immediate surroundings of the bacterial cells, which might take part to the discrepancies between predicted and observed adsorption rates at low ionic strength.

From a theoretical point of view, the recognition between the phage and the targeted receptor is often described as a key–lock model. Such key–lock recognition is based on short range forces so that the attraction is optimal when the shapes of the key and the lock are matching. For example the binding force between artificial colloids is controlled by how closely the size of the spherical colloidal key particle matches the radius of the spherical cavity of the “lock” site (Sacanna et al., 2010). However, the key–lock model does not take into account the global electrostatic potential as the phage approaches the bacterial host, which might be a limiting factor as they are both negatively charged in biologically relevant solutions. It is unclear how a short range model such as the key–lock model may be relevant despite the electrostatic repulsion that is efficient at long distances in medium with low ionic

strength. The main target of the T2 phage is an outer membrane protein F (OmpF) (Morona and Henning, 1986). Interestingly the Debye length in the medium complemented with 60 mM (where significant adsorption occurs) is 1.23 nm, which close to the size of the protuberance of OmpF measured by atomic force microscopy as 0.6 nm (Philippson et al., 2002). However the protein OmpF shows a high lateral variability of the electrostatic repulsion that depends on the ionic strength (Philippson et al., 2002). This suggests that the approximation of the phage as a sphere equivalent to the hydrodynamical radius and with an average zeta potential is insufficient to predict the adsorption involving interaction between the T2 phage and the outer membrane protein F.

The flow cytometric method has three limitations: firstly, phages with small genomes are challenging to count by flow cytometry due to the limit of detection of the fluorescence (Brussaard et al., 2000). Secondly, the method is restricted to simple media that offer a good signal/noise ratio. Therefore it would be difficult to apply this method to the adsorption in natural media or nutrient-rich media because the detection of the phage separately from other SYBR-labeled material such as other phages could be difficult. Thirdly, the method measures the adsorption of virus-like particles rather than measuring infectious particles as in the classical method based on plaque-forming units. This latter bias is probably minor when high quality viral suspensions are used, in which most of the viral particles are infectious.

5. Conclusion

In conclusion, the method based on flow cytometry to count viral particles described in the current study is well suited to measure weak interactions between phages and bacterial cells in controlled aqueous solutions. The method offers a good signal to noise ratio and is more sensitive than the standard method based on centrifugation.

However, a brutal application of the DLVO theory proposed in 1948 to complement Smoluchowski expressions quantifying flocculation generated by weak interactions between inorganic colloidal particles overestimates the repulsion barrier between biological particles interacting via rough interfaces. A better knowledge of the mechanisms driving the adsorption is needed to understand the consequence of a sudden increase in salinity on the interaction between phage and bacterial cells.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jviromet.2013.02.007>.

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