# Interparticle effects in the cotransport of viruses and engineered nanoparticles in saturated porous media

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### ABSTRACT

The effects of P25 TiO<sub>2</sub> nanoparticles on the transport in saturated porous media of the bacteriophage PP7 was investigated. Two levels of ionic strength were considered, given by monovalent and divalent cations, Na<sup>+</sup> and Ca<sup>2+</sup>. The viruses and the P25 particles were characterized with respect to size and surface charge, by dynamic light scattering and electron microscopy. The breakthrough curves were obtained for suspensions of each particle and both combined, using UV absorbance to quantify P25 and RT-qPCR for PP7. In the single transport experiments, P25 was retained, while the viruses mostly eluted the sand bed. However, in the cotransport experiments, retention increased for the virus and decreased for the TiO<sub>2</sub> nanoparticles under high viral content, due to heteroaggregation among the colloids in the incoming suspension, electrostatic interactions, and ripening. The interparticle interactions were modeled by DLVO theory, resulting in agreement with the experimental observations, which highlights the electrostatic nature.

### 1. Introduction

Water may be a source of infections for humans and animals. Traditionally, the Pan American Health Organization (PAHO) and the European Community (EC) have used bacterial indicators to ensure appropriate surface water quality [1]. However, since viruses are smaller, more difficult to remove by filtration, and more resistant to disinfection [2], their persistence in water should be controlled and superior management strategies developed. A 2019 report by the World Health Organization showed that gastrointestinal viral infections take the lives of around 2.2 million people per year, mostly children in developing countries [3]. Among these infections, those caused by waterborne pathogenic viruses, i.e., adenovirus, enterovirus, hepatitis A and E virus, norovirus, sapovirus, and rotavirus, require specific prevention by the development of affordable potabilization techniques, especially addressed to low-income rural areas.

When suspended in surface water, viruses can travel to depths of up to 64 m and 1600 m in horizontal distance [4] and the removal by soil passage is only partial [5]. Chrysikopoulos and collaborators obtained, by mathematical modelling, that a 40-log removal of MS2 and PRD1 would occur in 153 m of passage through a sandy aquifer [6], but for a fractured aquifer this distance could be as high as  $8000 \pm 4800$  m [7]. To

analyze the fate and transport of viruses in the subsurface, it is necessary to take into account their shape, size, density, net surface charge, electrophoretic mobility, and hydrophobicity [8]. Laboratory experiments showed that transport and retention of viruses in quartz sand depends on the concentration and valence of the present cations [9]. Moreover, the complex environment of natural waters includes dissolved organic and inorganic compounds as well as particles such as clays and other microorganisms that may interact with viral particles and alter the properties that determine their fate and transport. Therefore, comprehensive knowledge about the mechanisms of virus fate and transport in porous media, as well as the influence of the aqueous matrix is of utter importance to be able to estimate exposure scenarios or predict the effectivity of sand filtration in drinking water treatment plants.

A virus resembles an amphoteric colloidal particle [10], with carboxyl and amino groups on its outer protein surface. These chemical groups can gain or lose  $H^+$  depending on the medium pH and ionic strength. Thus, the resulting charged capsid will develop electrostatic interactions with the collector surfaces and other substances, e.g., colloids and ions, present in the medium.

Bacteriophages from *Fiersviridae* family, such as MS2 and PP7 ( $\sim$ 30 nm, RNA genome), were not always suitable surrogates for adenoviruses (90–100 nm, DNA genome) [11,12], which are used as viral fecal

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indicators due to its persistence in the environment [13]. However, they have been used in several studies as surrogates for other enteric viruses, such as enterovirus and norovirus, due to their similar morphological characteristics [14]. Besides, PP7 was used as a standard to test membranes for water filtration, due to its very small size [15]; but was not previously employed to study the transport of viruses through porous media. PP7 belongs to the Fiersviridae family (previously known as the Leviviridae family), Pepesvirus genus, and infects Pseudomonas aeruginosa. Its natural habitat is the same as its host, which includes soil, water, sewage, the intestinal canal, and the human skin [16]. The icosahedral virion of the RNA bacteriophage PP7 (T = 3 shell) is composed of 180 molecules of the coat protein (127 amino acids) and a molecule of the maturation protein bound to the ssRNA genome (3588 bp). Disulfide bonds are formed in the capsid of PP7, facilitating the capsid assembly and stability [17]. The structure of the virion was described [18] and used to model and quantify the effects of pH and salt concentration on the charge regulation of the bacteriophage PP7 capsid [19]. These authors observed that at low pH, the virus capsid charge is positive while at high pH is negative; and also, that the surface charge density is almost constant in between pH range, resulting in a constant electrostatic surface potential as a function of pH that depends on the salt concentration (pI = 3.85 in NaCl 100 mM).

Natural waters are characterized by a pH usually between 4 and 8 [20]. Ionic strength generally varies in an order of magnitude from 1 to 10 mM in soils and from 1  $\mu$ M to 1 mM in surface waters; particularly, Na<sup>+</sup> and Ca<sup>2+</sup> are cations usually present in soils, with average concentrations of 0.45 and 0.8–19.4 mM, respectively, in temperate regions [20]. Furthermore, the amount of divalent cations increases when water percolates through soils, due to chemical weathering of minerals [21].

Engineered nanomaterials are currently manufactured at large scale for diverse applications, such as textiles [22], paints [23], sunscreens [24], catalysts [25], pharmaceuticals [26], water purification [27], solar cells [28], composite materials [29], agents for enhanced oil recovery [30], and energy storage and conversion [31]. TiO<sub>2</sub> nanoparticles are one of the most common materials for industrial applications; in the US alone, their production is expected to grow up to  $2.5 \times 10^6$  metric ton/year by 2025 [32], resulting in soil and natural waters exposure. Due to their widespread use and potential toxicity, TiO<sub>2</sub> nanoparticles arise environmental and public health concern [33,34]. They were reported to produce oxidative stress, histopathological alterations, carcinogenesis, damages in the genetic information within a cell causing mutations, and disruptions in the immune system [34].

The small size, high surface area to volume ratio, and surface chemistry of  $TiO_2$  nanoparticles give them their unique characteristics, for which they are valued. Modifications in the environment may substantially affect their intrinsic behavior as well as their fate and transport in soils [35]. Wang et al. observed that the transport of  $TiO_2$  nanoparticles was increased at higher pH, lower ionic strength and in the presence of natural organic matter [36]. The mobility of  $TiO_2$  nanoparticles in the aquatic environment is related to their particle size and tendency to aggregate and agglomerate; smaller particles can reach groundwater depth, while aggregates are usually retained in the upper soil, clogging the pores [37]. Moreover, we can expect that nanomaterials that are prone to adsorb onto biological colloids [38] will, in turn, alter their transport.

Insight into the mutual influence of viruses and engineering nanoparticles on their transport in porous media is scarce [39–41] and suggests inactivation and increased retention of viruses in porous media, in the presence of  $TiO_2$  nanoparticles [41]. Besides, heteroaggregation between MS2 and  $TiO_2$  was reported [42], which may indicate clogging as a mechanism of virus removal by transport in porous media.

In the present work, we investigated the mutual effects of the presence of nanoparticles in saturated porous media on the transport of viruses, and vice versa. The breakthrough curves for PP7 bacteriophages,  $TiO_2$  nanoparticles, and both together were obtained at different conditions of ionic strength in saturated porous bed. The virus and the nanoparticles were characterized with respect to size and surface charge. Derjaguin, Landau, Verwey and Overbeek (DLVO) theory of colloidal stability was applied for data analysis. Two levels of bacteriophage concentration were considered, representing high concentrations usually found in sewage waters (which is measured by pfu/mL of coliphages) [43]. The selected collector was quartz sand, hydrophilic and negatively charged, to model the typical soil of natural environments. Thereby, we studied the attractive and repulsive electrostatic forces that enhance or inhibit adsorption and, consequently, hinder or facilitate transport.

### 2. Materials and methods

The selected virus and nanomaterial were bacteriophage PP7 (ATCC 15692-B2) and titanium dioxide (Aeroxide TiO<sub>2</sub> P25, Evonik Degussa Corporation, NJ, USA), respectively. PP7 was chosen as surrogate for poliovirus in water [44], since both are naked and single-stranded RNA viruses, with similar diameter (25–30 nm) and icosahedral shape. P25 is fumed hydrophilic TiO<sub>2</sub>, constituted by a mixture of rutile and anatase with an average primary particle size of 21 nm. Milli-Q water, reactive grade NaCl (Anedra, Argentina), and anhydrous CaCl<sub>2</sub> (Cicarelli, Argentina) were employed in all the experiments. The conditions assessed were NaCl and CaCl<sub>2</sub> 0.5 mM and 10 mM ionic strength at pH 5. All materials and reagents were autoclaved at 121  $^{\circ}$ C for 20 min.

The size and zeta potential of the  $TiO_2$  nanoparticles and viruses were measured at the different ionic strengths considered, at pH 5, using a Zetasizer Nano ZS (Malvern, UK) at 25 °C. Particles were also imaged by scanning electron microscopy (SEM) with a Zeiss SUPRA 40 (Zeiss, Oberkochen, Germany).

Bacteriophage PP7 was cultured using the host *Pseudomonnas aeruginosa* (ATCC 15692), as described elsewhere [15]. Briefly, *P. aeruginosa* was first grown overnight in nutritive broth (Britannia, Argentina) at 36 °C until DO<sub>600</sub> of 0.1 was reached ( $\sim 10^8$  cfu/mL), and then, the host culture was infected with a high titer suspension of PP7, in order to achieve a multiplicity of infections (MOI) of 0.05. After 16 h of incubation at 36 °C, PP7 was harvested by centrifugation of cell debris at 8000g for 30 min at 4 °C, followed by filtration through 0.45 and 0.22 µm membranes. The bacteriophage suspension was quantified as genomic copies (gc) or plaque forming units (pfu) using previously described real-time TaqMan PCR [15] and double-layer plaque assay [45], respectively.

The transport experiments were performed using a custom-made acrylic column with 25 mm internal diameter and adjustable end pieces (Omnifit, Cambridge, UK). Two meshes were placed at the upper and lower ends of the bed to ensure an even distribution of the water over the cross-sectional area. The column and tubing were washed with bleach (10% V/V) and thoroughly rinsed with Milli-Q water before each experimental run. The column was filled with Ottawa No. 12 Flint silica sand (U.S. Silica Company, Berkeley Springs, WV). X-ray diffraction confirmed the sand was 100% quartz. An average particle size (d50) of 529 µm was determined by sieve analysis [46]. The sand was conditioned by successive washing under agitation in: ultrapure water, HCl solution (pH 3), ultrapure water, NaOH solution (pH 10), NaHCO3 solution, and ultrapure water. Finally, the sand was dried at 105 °C and autoclaved before use. The sand was gently added to the column previously filled with Milli-Q water and stirred to ensure the saturation of the bed, avoiding air entrapment and layering [47].

Fig. 1 shows the experimental set-up used in the transport experiments. The length of the porous bed was  $5.2 \text{ cm} \pm 0.2 \text{ cm}$ . Milli-Q water was pumped through the column until the electrical conductivity was below  $0.2 \ \mu$ S. The flow rate was set at 2.8 mL/min, giving a mean pore velocity of 1.38 cm/min, characteristic of in-situ remediation processes subjected to forced flow [41]. Experimental data from a tracer break-through curve (BTC) was modeled using CXTFIX21 (U.S. Salinity Laboratory Agricultural Research Service, U.S. Department of Agriculture, Riverside, California) [48], to determine an effective bed porosity of



Fig. 1. Column studies experimental set-up. The inlet was virus,  $\rm TiO_2$  nanoparticles, or both of them together, depending on the experiment that was performed.

 $0.45\pm0.04.$  Afterwards, background solution of the desired ionic strength (NaCl 0.5 mM, NaCl 10 mM, CaCl\_2 0.5 mM, or CaCl\_2 10 mM), was circulated through the sand column for 20 min in order to stabilize the sand bed. A peristaltic pump (Watson Marlow 323 S/U) and an online conductivity meter (VWR International) were used.

For the experiments with TiO<sub>2</sub> suspensions, 30 mL of a 25 ppm suspension at the desired ionic strength was pumped, followed by 30 mL of TiO<sub>2</sub>-free solution of the same ionic strength. The TiO<sub>2</sub>-virus cotransport experiments consisted in three stages in which different suspensions were pumped through the column: first, 50 mL of virus suspension ( $1 \times 10^5$  or  $2 \times 10^6$  gc/mL) at the desired ionic strength; second, 30 mL of a suspension containing both, the virus at the same concentration and 25 ppm TiO<sub>2</sub>; and finally, 30 mL of the virus suspension. This design models the sudden irruption of a nanomaterial into an aquifer, where microorganisms are naturally present.

Effluent TiO<sub>2</sub> concentration was determined by UV absorbance using an UV-Vis spectrophotometer (Shimadzu UV 1650 PC) at a wavelength of 325 nm, chosen from the peak of the absorption spectrum. Virus concentration was determined in an Applied Biosystems 7500 Real-Time PCR system.

Virus genomic RNA was extracted from 200  $\mu$ l of each sample using the High Pure Viral Nucleic Acid Kit (ROCHE Life Science), according to the manufacturer's instructions. The cDNA was obtained from 10  $\mu$ l of RNA using random primers and M-MLV Reverse Transcriptase (Promega). The real-time TaqMan PCR assays, described in [15], were performed using the FastStart Universal Probe Master (ROX) mix (ROCHE Life Science) in an Applied Biosystems® Step-One Plus Real-Time PCR system. All amplification reactions, samples and standards, were carried out in triplicate.

DLVO theory [49,50] was employed to model and predict the attachment of the virus and the nanoparticle to the sand grains, as well as between them. Due to its icosahedral shape, PP7 was considered a sphere [51], as well as TiO<sub>2</sub>. For both, the size was taken equal to their respective hydrodynamic diameters, obtained by Dynamic Light Scattering (DLS) using number-weighted distribution [52]. The average diameter of the sand grains was several orders of magnitude larger than the nanoparticles and the viruses, thus, the collector was regarded as an

infinite plate. The electrical surface potential cannot be determined and was then replaced by the zeta potential, determined by laser Doppler micro electrophoresis. The Hamaker constants employed were: A<sub>vir-us-water-virus</sub> =  $1 \times 10^{-20}$  J [53], A<sub>P25-water-P25</sub> =  $6 \times 10^{-20}$  J [54], A<sub>vir-us-water-quartz</sub> =  $4 \times 10^{-21}$  J [10,55,56], A<sub>P25-water-quartz</sub> =  $1 \times 10^{-20}$  J [57], and A<sub>virus-water-P25</sub> =  $2.45 \times 10^{-20}$  J that was calculated using the expressions in [58] with the aforementioned values and A<sub>water</sub> =  $3.7 \times 10^{-20}$  J [59]. Zeta potential of the quartz was considered: – 40 mV and – 22 mV in the presence of a monovalent cation of concentration 0.5 and 10 mM, respectively [60], and – 34.5 mV and – 18.4 in CaCl<sub>2</sub> solution of 0.5 and 10 mM of ionic strength, respectively [61].

The interactions between two spheres ( $TiO_2$ , virus) were calculated using the following expressions for the van der Waals attractive forces (Eq. 1) and for the electrostatic double layer repulsive forces (Eq. 2) [58]:

$$V_{A} = -\frac{A}{6} \left( \frac{2a_{1}a_{2}}{h^{2} + 2a_{1}h + 2a_{2}h} + \frac{2a_{1}a_{2}}{h^{2} + 2a_{1}h + 2a_{2}h + 4a_{1}a_{2}} + ln \frac{h^{2} + 2a_{1}h + 2a_{2}h}{h^{2} + 2a_{1}h + 2a_{2}h + 4a_{1}a_{2}} \right)$$
(1)

$$V_R = \frac{128\pi a_1 a_2 n_\infty}{(a_1 + a_2)\kappa^2} kT \gamma_1 \gamma_2 e^{-\kappa h}$$
<sup>(2)</sup>

where  $V_A$ : van der Waals interaction potential energy (J),  $V_R$ : electrical double layer interaction potential energy (J), A: Hamaker constant in water (J), a: radius of the sphere (m), h: separation (m),  $n_\infty$ : bulk number of ions (ions m<sup>-3</sup>),  $\kappa$ : Debye-Hückel reciprocal length (m<sup>-1</sup>), k: Boltzmann constant (J K<sup>-1</sup>), T: temperature (K),  $\gamma_i$ : reduced surface potential.

The attractive interaction between sphere and plate ( $TiO_2$  or virus and quartz) was calculated using Eq. 3 [58] and the repulsion interaction with Eq. 4. The latter was derived from Eq. 2, since a large difference in particle sizes causes the largest one to be seen as an infinite plate:

$$V_A = -\frac{A}{6} \left( \frac{a}{h} + \frac{a}{h+2a} + \ln \frac{h}{h+2a} \right)$$
(3)

$$V_R = \frac{128\pi a n_\infty}{\kappa^2} k T \gamma_1 \gamma_2 e^{-\kappa h}$$
<sup>(4)</sup>

DLVO theory considers the total interaction potential energy as the sum of both attractive van der Waals and repulsive electrical double layer potential energies:

$$V_T = V_A + V_R \tag{5}$$

where V<sub>T</sub>: total interaction potential energy (J).

The Debye-Hückel reciprocal length in aqueous solution at 25 °C and the reduced surface potential were calculated as follows [58]:

$$\kappa = 2.32 \times 10^9 \sqrt{\sum C_j z_j^2} \tag{6}$$

$$\gamma = T_g h \frac{z e \xi}{4T} \tag{7}$$

where  $C_j$ : ion concentration (mol dm<sup>-3</sup>),  $z_j$ : valence of ion j including sign of charge, z: valence of symmetrical (z-z) electrolyte, e: electron charge (C),  $\zeta$ : zeta potential (V).

### 3. Results

The measured hydrodynamic diameters and the zeta potentials of  $TiO_2$  and PP7 are shown in Table 1.

The hydrodynamic diameters of  $TiO_2$  showed that the dependence on the salt concentration in the medium is more relevant than the cation charge, at the studied pH.  $TiO_2$  aggregation was very fast in aqueous solutions, due to its reactivity and high surface area [62]. Increased ionic strength enhanced attachment and aggregation, especially for solutions

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$f$ (ydrodynamic diameters (average $\pm$ standard deviation of at least three measurements) and the zeta potentials of TiO <sub>2</sub> and PP7.	

Ionic strength	TiO <sub>2</sub>		PP7		
	Hydrodynamic diameter (nm)	Zeta potential (mV)	Hydrodynamic diameter (nm)	Zeta potential (mV)	
NaCl 0.5 mM	$232.4 \pm 18.8$	$20.9\pm0.2$	$58.0\pm34.4$	$\textbf{-34.8} \pm \textbf{3.3}$	
NaCl 10 mM	$264.8\pm0.4$	$15.7\pm0.3$	$107.1\pm23.9$	$\textbf{-19.4} \pm \textbf{6.2}$	
CaCl <sub>2</sub> 0.5 mM	$234.6\pm26.8$	$16.8\pm0.1$	$160.9\pm55.6$	$\textbf{-26.5} \pm \textbf{1.1}$	
CaCl <sub>2</sub> 10 mM	$361.7 \pm 119.4$	$3.9\pm0.1$	$204.7 \pm 14.6$	$\textbf{-16.5} \pm \textbf{0.5}$	

containing  $Ca^{2+}$ . During preparation, slow mixing was unavoidable, causing collisions among the nanoparticles, which, in turn, resulted in high aggregation [63] and corresponded to the physical transport mechanisms of the classical aggregation theory [64].

In the case of the bacteriophage, aggregation was evidenced, since the primary size was reported to be 27 nm [65]. We can highlight that the obtained hydrodynamic diameters take into account attraction and association with water molecules and electrolytes. Although this amount of aggregation is considerable, there exist previous works reporting relevant viral aggregation, such as in the case of GA phage [66].

BTC for  $TiO_2$ , virus, and their combined presence were obtained at pH 5; the pH was monitored, but changes were always below 0.3 pH units, with no effect on surface properties of the analyzed colloids.

Fig. 2 shows the BTC for TiO<sub>2</sub> in the four different ionic strength suspensions, in the presence and absence of the bacteriophage. When the ionic strength was low (0.5 mM), provided by either NaCl or CaCl<sub>2</sub>, a plateau was reached, at 1.5 injected pore volumes (PV) when the TiO<sub>2</sub> was the only particle in suspension and in the presence of the virus (Fig. 2.a and c). For a higher ionic strength (10 mM), two different situations arose. When the nanoparticles were alone or with the bacteriophage at a concentration of  $1 \times 10^5$  gc/mL, the outlet concentration reached a peak at 1.5 PV, and then decreased (Fig. 2.b and d), which hints to an increasing removal efficiency. However, when the bacteriophage concentration was  $2 \times 10^6$  gc/mL, a plateau was reached again but at a lower value than for the case of low ionic strength, suggesting enhanced retention in the porous media.

BTC for the virus, both with and without the addition of  $TiO_2$ , at the four conditions of ionic strength are shown in Fig. 3. The limits of detection (LOD) and quantification (LOQ) of the TaqMan PCR detection of bacteriophage PP7 were 5 and 6 gene copies per reaction volume

(20–25 µl, equivalent to 200–300 cg/mL) respectively, evaluated by the developing authors of this technique [15]. In this work, those values were  $3.125 \times 10^3$  gc/mL (LOD) and  $3.750 \times 10^3$  gc/mL (LOQ). The four transport experiments showed that the virus retention was enhanced by the presence of P25, which was more notorious for the lowest virus concentration (1 × 10<sup>5</sup> gc/mL). Effluent virus concentration suffered a decrease coincident with the injection of TiO<sub>2</sub>. After this injection stopped, virus concentration at outlet showed an increasing trend indicating that they were transported through the sand bed again.

As it can be clearly seen in Fig. 3, there are unexpected and overestimated values that were obtained from the qPCR method. Virus recovery higher than 100% when using PP7 and other icosahedral viruses as surrogates in different matrices was previously reported [12,67,68]. These empirical observations could be due to the formation of viral aggregates in the feeding solution that tend to disintegrate during the procedure. In fact, some authors argue that this effect biases the results toward higher recovery values. Despite this drawback, the technique proved to be effective in showing the BTC pattern and allowed to establish different trends in the transport of the bacteriophages alone or in presence of the nanoparticles. Overall, qPCR provided a more accurate method for the quantification of the bacteriophage than the traditional double-layer overlay which has been reported to yield irregular values [69–71] and underestimations due to virus inactivation or aggregation.

According to DLVO calculations, the interaction potential energies relative to the thermal energy, given by k T, are shown in Fig. 4 for the four conditions of ionic strength.

 $TiO_2$  was expected to aggregate at the highest ionic strength, where the van der Waals forces outweighed the repulsive interactions, while at 0.5 mM ionic strength repulsion was still expected (Fig. 4.a), though in



Fig. 2. Breakthrough curves for TiO<sub>2</sub> suspended in different media: (a) NaCl 0.5 mM, (b) NaCl 10 mM, (c) CaCl<sub>2</sub> 0.5 mM ionic strength, (d) CaCl<sub>2</sub> 10 mM ionic strength. Blue diamonds indicate the TiO<sub>2</sub> only transport, red circles indicate the transport of TiO<sub>2</sub> in the presence of virus ( $1 \times 10^5$  gc/mL), green triangles indicate the transport of TiO<sub>2</sub> in the presence of virus ( $2 \times 10^6$  gc/mL).



Fig. 3. Breakthrough curves for virus suspended in different media: (a) NaCl 0.5 mM, (b) NaCl 10 mM, (c) CaCl<sub>2</sub> 0.5 mM ionic strength, (d) CaCl<sub>2</sub> 10 mM ionic strength. Red circles indicate the transport of virus  $1 \times 10^5$  gc/mL, green triangles indicate the transport of virus  $2 \times 10^6$  gc/mL.

practice the energy barriers were easily overcome resulting in considerable aggregation. Conversely, in the case of the virus (Fig. 4.b), an important repulsion was predicted for 0.5 mM ionic strength, with energy barriers above 50 k T, and lower repulsion when the ionic strength was augmented, due to compression of the electrical double layer, with energy barriers over 10 k T. A strong electrostatic attraction was predicted when the surface charges were opposite, such is the case of TiO<sub>2</sub> and quartz (Fig. 4.c) and TiO<sub>2</sub> and virus (Fig. 4.e). Accordingly, repulsion was predicted between virus and quartz, which were both negative (Fig. 4.d), with energy barriers over 100 k T for 0.5 mM ionic strength and over 30 k T for 10 mM ionic strength. When repulsion was dominant (Fig. 4.a for 0.5 mM ionic strength, Fig. 4.b and Fig. 4.d), secondary minimums were found; nonetheless, their magnitude was very small and the separation between surfaces considerable. The values of the each energy barrier and secondary minimum are shown in Table 2.

Images of the TiO<sub>2</sub> aggregates obtained by SEM under different conditions of ionic strength (Fig. 5) corroborated the primary particle size of 21 nm. Aggregates displayed irregular shapes with threedimensional, porous structures. An increased NaCl content influenced the conformation of the aggregates (Fig. 5.a and b). The high quantity of NaCl in the original suspension hinders the clear observation of each nanoparticle; however, it was possible to appreciate that the aggregates were irregular, less porous and more compact, that could suggest the presence of denser structures. On the contrary, the effect of  $CaCl_2$  was not so notorious (Fig. 5.c and d).

SEM images of bacteriophage PP7 are shown in Fig. 6; the observed particles confirmed the DLS results that indicated aggregation. As expected, the higher content of salt induced aggregates of a higher number of particles. The aggregates showed compact structures, although the morphology may have been altered during sample preparation.

SEM images of the  $TiO_2$  and virus heteroaggregates are shown in Fig. 7. It was not possible to discriminate the viruses from the nanoparticles. A comparison of Figs. 5 and 7 suggests that the presence of viruses produced aggregates with higher porosity and more open structure, making particle contours more defined to the observer.

### 4. Discussion

The retention of TiO2 by the quartz sand may be explained as a

combination of factors, such as attachment to the clean sand, followed by straining and attachment to the ripe bed. It was previously reported [52] that the size of each TiO<sub>2</sub> aggregate and the amount of aggregates diminished from the top layers of sand to the deeper grains. Therefore, the furthest removal took place in the first centimeters of the porous bed, clogging the pores and hindering the bigger aggregates to exit the column. Under the experimental conditions of pH in this study, the retention was dominated by electrostatic attraction, since the sand was negatively charged and the nanoparticle surface was positive. When the electrolyte concentration was elevated, aggregation was favored. Large aggregates clogged the pores in the sand bed, with the consequent difficulty for new particles to pass through the bed. At last, since TiO<sub>2</sub> deposited onto the sand, the collector surface became gradually heterogeneous and partially covered with same charge sites, so the new incoming particles would be subjected to interactions not only with the sand, but also with previously deposited nanoparticles. When ionic strength increased from 0.5 mM to 10 mM in NaCl and in CaCl<sub>2</sub>, the effluent concentration dropped to some extent (Fig. 2). Based on DLVO calculations, a strong attraction and no repulsion between TiO2 and quartz was expected (Fig. 4.c), due to the opposite sign of the surface charges, allowing deposition and attachment to the available free surface. As the collectors were progressively covered with nanoparticles, the interactions between these TiO2 particles became more relevant. Greater attraction between two nanoparticles was expected for the higher values of ionic strength (Fig. 4.a), thus promoting straining as well as attachment over time [72,73], giving an explanation to the experimental results.

When analyzing the  $TiO_2$  cotransport experiments, it can be seen (Fig. 2) that no relevant difference was found when the nanoparticles were alone or with the virus at the lowest concentration. However, for the highest viral content, the quantity of  $TiO_2$  retained in the porous media was much lower. During the experiments, first, the bacteriophage was passed through the porous bed, followed by the suspension containing both nanoparticles and viruses. Since the bacteriophage and the sand had negative surface charge, the expected retention was minimal. The energy barrier calculated by DLVO modeling confirmed this experimental observation (Fig. 4.d). Besides, a strong electrostatic attraction was predicted between  $TiO_2$  and virus (Fig. 4.e) due to the opposite sign in their surface charges. Hence, when the virus



**Fig. 4.** DLVO predicted interaction potential energy, at the four different ionic strengths, for: (a) two particles of TiO<sub>2</sub>, (b) two particles of bacteriophage, (c) a particle of TiO<sub>2</sub> and quartz sand, (d) a particle of bacteriophage and quartz sand, (e) a particle of TiO<sub>2</sub> and a particle of bacteriophage.

# Table 2 Energy barrier and secondary minimum obtained by DLVO analysis and their locations.

		Virus-Virus		Virus-Quartz		TiO <sub>2</sub> - TiO <sub>2</sub>	
		h (nm)	Energy (kT)	h (nm)	Energy (kT)	h (nm)	Energy (k T)
NaCl 0.5 mM	Barrier	1.1	59.9	0.6	143.9	4.9	51.6
	Sec. min.	247.4	$\textbf{-2.42}\times10^{\textbf{-6}}$	196.6	$\textbf{-3.81}\times10^{\textbf{-4}}$	110.3	$\textbf{-6.43}\times10^{-2}$
NaCl 10 mM	Barrier	1.0	11.7	1.0	35.0	-	-
	Sec. min.	24.7	$-1.45 imes10^{-2}$	26.0	$-3.65 imes10^{-2}$	-	-
CaCl <sub>2</sub> 0.5 mM	Barrier	1.2	50.5	0.7	132.4	5.7	53.3
	Sec. min.	277.0	$-1.38 imes10^{-6}$	218.3	$-2.89 imes10^{-4}$	126.2	$-4.42 imes10^{-2}$
CaCl <sub>2</sub> 10 mM	Barrier	1.0	14.0	1.0	39.4	-	-
	Sec. min.	28.8	$-9.68 imes10^{-3}$	29.6	$\textbf{-2.87}\times10^{\textbf{-2}}$	-	-

concentration was  $1 \times 10^5$  gc/mL, the TiO<sub>2</sub> nanoparticles exited the column in the form of nanoparticle-virus heteroaggregates, but a great amount was still retained on the free collector surface. When the virus concentration increased to  $2 \times 10^6$  gc/mL, a larger proportion of nanoparticles would be first attracted and attached to the bacteriophage. The increased availability of viral particles effectively shielded TiO<sub>2</sub> particles from attractive interactions with sand particles, causing the increased elution of TiO<sub>2</sub>, up to 90–100% of the feed concentration in the presence of NaCl and up to 70–85% in CaCl<sub>2</sub>.

Both surface charge of the virus and the quartz were negative at all ionic strengths, hence retention was not expected. Besides, the DLVO calculations (Fig. 4.d) showed an energy barrier dependent on the salt content: as the level of electrolyte increased, the surface charges were increasingly screened by Na<sup>+</sup> and Ca<sup>2+</sup>, diminishing the energy barrier, due to compression of the electrical double layer and reduction of the zeta potentials in absolute value. Accordingly, the viruses exited the column after 1 PV and reached a plateau when suspended in a NaCl 0.5 mM and 10 mM solution (Fig. 3.a and b). A notorious difference was



Fig. 5. SEM images of TiO<sub>2</sub> with a magnification of 200.00 K X in: (a) NaCl 0.5 mM, (b) NaCl 10 mM, (c) CaCl<sub>2</sub> 0.5 mM ionic strength, (d) CaCl<sub>2</sub> 10 mM ionic strength.



Fig. 6. SEM images of bacteriophage PP7 with a magnification of 400.00 K X in: (a) NaCl 0.5 mM, (b) NaCl 10 mM, (c) CaCl<sub>2</sub> 0.5 mM ionic strength, (d) CaCl<sub>2</sub> 10 mM ionic strength.

found when the suspension was in  $CaCl_2$  solutions; after 1 PV, the bacteriophages eluted from the column, but no plateau was reached; instead, a monotonic increase of effluent concentration was observed (Fig. 3.c and d). When the viruses passed through the column, the dominant repulsion forces among themselves (Fig. 4.b) would lead to

increasing effluent concentration with time, probably due to virus desorption or blocking effects [72,73]. Besides, the divalent cations proved to shield the surface charge more effectively than the mono-valent [56] since the former have higher charge density. When the  $Ca^{2+}$  concentration was increased, a slightly increased adsorption was



Fig. 7. SEM images with a magnification of 100.00 K X of TiO<sub>2</sub> and virus heteroaggregates in: (a) NaCl 0.5 mM, (b) NaCl 10 mM, (c) CaCl<sub>2</sub> 0.5 mM ionic strength, (d) CaCl<sub>2</sub> 10 mM ionic strength.

expected [69], due to compression of the electric double layer [74]. In other words, when the ionic strength was increased, the thickness of the electrical double layer that surrounds both the virus and the quartz diminished, offering the particles to be at a shorter distance, which in turn favors weak attractive forces to develop; consequently retarding the elution. A different perspective may consider a virus as a particle consisting of some ionizable subunits (amino acids) and nonpolar groups, hence, a variation in ionic strength not only modifies the interactions between two different viruses, but also within the virus itself, leading to modifications in the transport and retention patterns, due to outer-sphere complexation [71]. In all cases, when the concentration was increased from  $1 \times 10^5$  gc/mL to  $2 \times 10^6$  gc/mL, a higher amount of viruses eluted from the column. The presence of a small amount of bacteriophages that did deposit onto the sand may have contributed to the already existing electrostatic repulsion between the sand and the viruses; therefore increasing repulsion and enhancing the transport through the porous medium.

The cotransport experiments evidenced that the virus passage was highly modified by the presence of TiO<sub>2</sub>. It can be seen in Fig. 3 that a sharp decay in the effluent concentration was present for all ionic strengths and viral concentrations, coincident with the injection of TiO<sub>2</sub> that started after 5 PV. The retention of the bacteriophages with incorporation of TiO<sub>2</sub> was very high, for as long as the nanoparticles were present in the system. When TiO<sub>2</sub> injection stopped, after 3 injected PV, virus concentration in the effluent showed an increasing trend indicating that the phages were no longer retained in the sand. DLVO theory predicted the attachment between the nanoparticles and the viruses (Fig. 4.e) due to opposite surface charges.

SEM images showed the morphology of the aggregates between nanoparticles (Fig. 5) and viruses (Fig. 6) and helped to corroborate the results obtained by DLS that provided the hydrodynamic diameters. As the particles were aggregated in the medium, their behavior was expected to be different from that in their nanometric size. For example, in the DLVO calculations, the size plays a role in the equations and we used the radius of the stable primary aggregate, measured by DLS, instead of the individual particle size, since the aggregates in the suspension are the ones that interact among them and with the collector walls. Moreover, particle transport to the vicinity of the collector is determined, in part, by particle size. Therefore, diffusion would be less relevant, as ionic strength increases, since it leads to bigger aggregates, as it was in the present work, where the nanometric state of the particles was not prevalent during the experiments.

## 5. Conclusions

The combined presence of TiO<sub>2</sub> nanoparticles and PP7 bacteriophage effectively modified the transport of both particles in the saturated porous media. Single transport experiments showed TiO<sub>2</sub> particles were mostly retained by the sand collectors due to electrostatic attraction and some degree of pore clogging caused by aggregation; while PP7 eluted the sand bed, due to the electrostatic repulsion forces between the virus and the collector. Cotransport experiments displayed a reduced retention of TiO<sub>2</sub> but a sharp increase in that of the virus for all ionic strengths tested.

The inhibition of the transport of viruses in saturated porous media in the presence of  $TiO_2$  suggests their potential use as additives for filtration media or in the design of effective barriers to viruses, which are often difficult to remove due to their small size and resistance to conventional cleaning methods. On the other hand, an accidental or intentional discharge of nanoparticles into aquifers is a concern, due to their ability to affect the natural transport and fate of biological particles, such as microorganisms, with the consequent damage to the ecosystem.

### CRediT authorship contribution statement

Guillermina J. Gentile: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Validation, Writing - original draft. María D. Blanco Fernández: Data curation, Investigation, Methodology, Resources, Validation. María M. Fidalgo de Cortalezzi: Conceptualization, Formal analysis, Methodology, Writing - review & editing.

### **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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