

MiniReview

Use of PRD1 bacteriophage in groundwater viral transport, inactivation, and attachment studies

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Abstract

PRD1, an icosahedra-shaped, 62 nm (diameter), double-stranded DNA bacteriophage with an internal membrane, has emerged as an important model virus for studying the manner in which microorganisms are transported through a variety of groundwater environments. The popularity of this phage for use in transport studies involving geologic media is due, in part, to its relative stability over a range of temperatures and low degree of attachment in aquifer sediments. Laboratory and field investigations employing PRD1 are leading to a better understanding of viral attachment and transport behaviors in saturated geologic media and to improved methods for describing mathematically subsurface microbial transport at environmentally significant field scales. Radioisotopic labeling of PRD1 is facilitating additional information about the nature of viral interactions with solid surfaces in geologic media, the importance of iron oxide surfaces, and allowing differentiation between inactivation and attachment in field-scale tracer tests.

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1. Introduction

Since its isolation from Kalamazoo, Michigan (USA) sewage [1] and first detailed description by Olsen et al. in 1974 [2], bacteriophage PRD1 (alternative notation PRD-1) has emerged as an important “model virus” to better understand molecular structure [3], phage–host interactions [4], and virus evolutionary history [5] for certain groups of bacteriophages. It also has become an important tracer and viral surrogate for studies involving subsurface viral transport. Some of the same qualities that make PRD1 an important tool in molecular virology contribute to its desirability in environmental field applications. In particular, its high stability in aqueous and geologic media [6,7] and structural and functional similarities to mammalian adenoviruses [8], some of which

are human pathogens, make PRD1 a good surrogate in viral fate and transport studies in groundwater environments. An additional attribute of PRD1 for subsurface tracer applications is its low propensity for attachment to many surfaces relative to other phages [9].

PRD1 is an icosahedra-shaped, 62 nm [10] bacteriophage that has an isoelectric point, pH_{iep} , of 3–4 (<3.2 in Cape Cod groundwater [11] and ~4 in buffer containing 10^{-4} M calcium and phosphate [10]). It shares many similarities with other viruses belonging to the *Tectiviridae* family of icosahedra-shaped, double-stranded DNA-containing bacteriophages. PRD1 contains approximately 25 different proteins and has an internal membrane within its protein capsid outer shell [12]. The presence of an internal membrane makes PRD1 a useful tool for the study of membrane structure and biosynthesis [13] and may contribute to its observed stability in the environment. PRD1 has a number of gram-negative bacterial hosts, which include pseudomonads and strains of *Escherichia coli* and *Salmonella* spp [13]. However,

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Salmonella typhimurium is the host most often employed in the production of PRD1 for the environmental applications described herein. Because two of its hosts are commonly found in sewage, PRD1 may also serve as an indirect indicator of fecal contamination in groundwater environments [14]. However, a number of other phages, particularly some of the coliphages [15] typically are found in higher abundance in sewage and, consequently, are more useful indicators of groundwater quality.

Much has been written about the use of PRD1 in molecular virology. However, this review summarizes the use of this important phage in environmental and groundwater pollution microbiology. We first give a history of the uses of PRD1 as a groundwater tracer and viral surrogate in injection and recovery (seeding) studies in different groundwater environments and its use as a laboratory tool to better understand the effects of physicochemical processes and conditions upon viral transport behavior in geologic and synthetic media. A major impediment in a better understanding and modeling of viral transport in aquifers is a dearth of information about viral inactivation in geologic media. In this review, we examine how PRD1 has been used in field and microcosm studies to assess the roles of solid surfaces and environmental conditions upon viral inactivation that occurs during subsurface transport. Another impediment has been a lack of data needed to better describe viral attachment in physically and geochemically heterogeneous geologic media. This has exacerbated the problem of being able to separate the effects of viral inactivation from that of viral attachment in field studies. We discuss studies in which PRD1 has been used to gather information about the nature of viral interactions with mineral surfaces and, finally, discuss specific needs for future viral transport research and the potential role that PRD1 may play in such studies.

2. History of PRD1 transport studies in geologic media

2.1. Field studies

Both the modern science of virology and the use of microorganisms as groundwater tracers had their origins at the very end of the nineteenth century. In 1897, Loeffler and Paul reported their findings on foot-and-mouth disease [16]. In 1898, Martinus Beijerinck introduced the concept of the virus as a “soluble living germ” [17], based upon research with tobacco mosaic virus. The first papers describing the deliberate injection of microorganisms (pigmented bacteria) into groundwater for the purposes of tracing flow in complex groundwater systems were published during those same years [18,19]. However, it was not until the 1970s that bacteriophages were employed as groundwater tracers (e.g., [20,21]) in subsurface

microbial tracer studies. Bacteriophages often have been the colloidal tracer of choice in field studies designed to assess subsurface transport behavior from a public health perspective [22] because of their small size, non-pathogenic nature, and structural similarities with some of pathogenic viruses that are of groundwater quality concern. Bacteriophages have also been used as groundwater tracers in hydrologic studies. The interested reader is referred to Rossi et al. [23] and Harvey and Harms [22] for more detailed histories involving the use of bacteriophages in groundwater tracer and subsurface microbial transport studies.

Subsurface transport studies involving the deliberate injection and recovery of the PRD1 bacteriophage in the field began in 1990 [24]. This first study employed PRD1 as a surrogate tracer in order to estimate expected virus removal rates occurring during on-land application of sewage during artificial aquifer recharge operations. PRD1 is now commonly used as a viral tracer in transport studies involving a variety of geologic media under both natural- and forced-gradient conditions. Table 1 lists selected studies in which PRD1 was injected into a variety of aquifers and recovered from wells located downgradient from points of injection. In addition to the use of PRD1 as surrogates in subsurface studies designed to delineate virus transport at the field scale, PRD1 also was used in the 1990s as a colloidal groundwater tracer in order to better understand the geohydrology of physically and geochemically complex aquifers characterized by fracture-flow (e.g. [25]). The aquifers chosen for PRD1 tracer studies varied from relatively homogenous dune and well-sorted sand systems to highly heterogeneous systems characterized by preferred flow paths and hydraulic conductivity variations of several factors of ten.

PRD1 also has been employed concomitantly with other phages in a number of field-scale injection and recovery tests. The rationale for using more than one bacteriophage is that there is considerable variation in size, hydrophobicity, and isoelectric point among phages that, collectively, affect their transport through soils [26] and their decay rates [27]. Several injection and recovery field tests performed in the late 1990s involved both PRD1 and the smaller MS2 coliphage [25,28–30]. MS2 is a 20 nm (diameter), icosahedra-shaped, RNA-containing phage lacking a membrane. At ambient groundwater conditions, it has been demonstrated that PRD1 better predicts the persistence of pathogenic viruses than does MS2 [31], particularly at higher temperatures. Nevertheless, the differences in structure, size, and surface characteristics between MS2 and PRD1 allowed additional information to be collected concerning the potential for subsurface viral transport in a variety of hydrologic settings. Although some studies suggest PRD1 sorbs to a much lesser degree than MS2 in aquifer sediments (e.g., [32]), other studies suggest MS2 and

Table 1
Recent field-scale subsurface transport studies involving the bacteriophage PRD1

Media type	Location	Test	Distance (m)	Reference
<i>Fractured</i>				
Shale-saprophyte	Tennessee, USA	NG ^a	35	[25]
Clay-rich till	Sarnia, Ontario, Canada	NG	4	[28,32]
Fractured till	Avedore, Denmark	NG	4 (vertical)	[69]
<i>Granular</i>				
Well-sorted sand & gravel	Falmouth, MA, USA	NG	13	[70]
		NG	1	[53]
		NG	0.9–1.0	[11]
Alluvial sediments	Arizona, USA	FG	50	[24]
Unsaturated fine sand	Tampa, Florida, USA	FG ^b	0.6	[71]
Clast-supported cobbles, gravel, sand (flood plain)	Missoula, Montana, USA	NG	30.5	[49]
Floodplain sediments	Western Montana, USA	FG	21.5	[72]
Dune sand	Castricum, Netherlands	FG	1.4–29	[73]
Layered fluvial sediments	Somerren, Netherlands	FG	8–38	[33]
<i>Karst</i>				
Limestone matrix	Key Largo, Florida, USA	NG	20	[74]
Limestone matrix	Middle Keys, Florida, USA	NG	83	[75]

^a NG indicates natural-gradient conditions.

^b FG indicates forced-gradient conditions.

PRD1 exhibit similar attachment behaviors [33]. The reported differences in relative attachment behavior among study sites may be due, in part, to differences in sediment mineralogy and organic content. However, there is also a possibility that the strains of PRD1 used in the various studies are not structurally identical. Unfortunately, PRD1 is not yet available from a major culture collection (ATTC or NCTCC) that employs rigorous quality assurance protocols and it is uncertain whether the existing PRD1 laboratory stocks are, indeed, genetically identical. At the time of this writing, American Type Culture Collection (ATTC) was in the process of adding this important phage to their collection (strain designation HER-23), but ATCC could not estimate when it would be available.

2.2. Laboratory studies

PRD1 has also been used extensively under more controlled conditions in the laboratory to elucidate the role of selected physicochemical conditions and processes in determining the ability of viruses to be transported through a variety of media. Laboratory microcosms studies that were conducted to examine the attachment, transport, and inactivation of PRD1 in the presence of geologic media or model surfaces are depicted in Table 2. Its well-defined surface and relatively high stability in the presence of saturated geologic media have allowed it to be studied under a variety of chemical conditions. Consequently, the growing environmental data base concerning its attachment, inactivation, and

Table 2
Recent lab-scale studies employing PRD1 to assess the effects of physicochemical conditions and processes upon viral transport in model and real geologic media

Factor studied	Medium	System	Reference
Attachment reversibility	Bonded/unbonded silica	Downflow column	[10]
Detachment, surfactant-induced	Polysulfone and nitrocellulose	Membranes	[76]
Flow-rate	Shale saprophyte	Flow-through column	[77]
Inactivation at surfaces	Aquifer sediments	Static minicolumns	[30]
Isoelectric point	Aquifer sediment	Recirc. Upflow columns	[51]
Non-linear removal	Dune sand	Downflow column	[50]
pH	3 sandy soils	Flow-through column	[48]
pH and attachment reversibility	Quartz/Fe-quartz	Static minicolumns	[52]
Salinity and nonionic surfactant	Membrane filters	Membrane filters	[78]
Salts (mono-,di-,tri-valent)	Variety of synthetic materials	Microporous filters	[79]
Surfactant-induced detachment	Polysulfone and nitrocellulose	Membranes	[76]
Temperature and effluent quality	Soil	Microcosm	[31]
Two-site kinetic attachment	Dune sand	Downflow column	[60]
Water saturation	Coarse soils	Downflow	[80]

transport behavior led to its most recent use as a “model virus” in theoretical viral transport models designed to describe viral transport within physically or geochemically heterogeneous granular media at environmentally-relevant field scales [34,35].

3. Inactivation in solution

Potential mechanisms of inactivation for PRD1 are depicted in Fig. 1. Viruses are composed of labile organic compounds and, consequently, exhibit a natural decay in groundwater. Inactivation in solution is dependent upon both time and physicochemical conditions. Adverse conditions and higher temperatures can

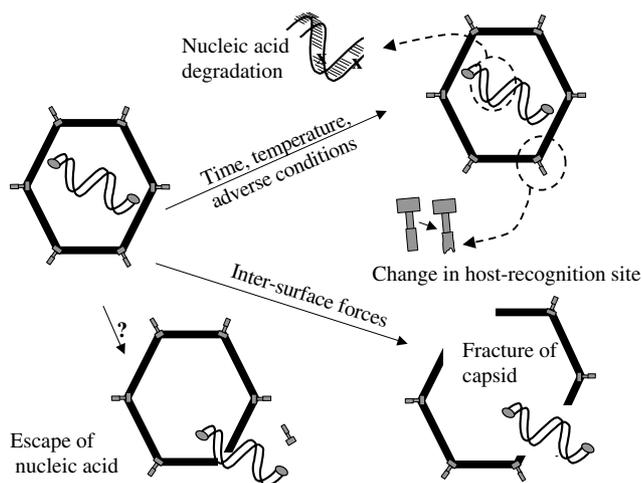


Fig. 1. Schematic depiction of the different potential mechanisms by which PRD1 may lose its ability to infect its bacterial host. The question mark indicates that loss of genetic material resulting from displacement of the vertex structure at a non-host surface is unknown.

accelerate damage to specific viral components that are required for infection, most notably degradation of the viral genome (double stranded DNA in the case of PRD1) and conformational changes in the host recognition site (protein). Because the decline of bacteriophages is exponential with time, the equation relating decay rate (generally expressed in reciprocal days) and virus die-off can be expressed as

$$k = \frac{-2.3 \log_{10} \left(\frac{C}{C_0} \right)}{t},$$

where k is the inactivation rate coefficient, C is the virus concentration at time t , C_0 is the virus concentration at $t = 0$. A negative effect of groundwater temperature upon virus survival was reported for a mixed population of male-specific (F+) bacteriophages concentrated from raw wastewater, poliovirus I, and hepatitis A virus [36].

Published values of inactivation rates for PRD1 in groundwater are given in Table 3. There appears to be a first order increase of decay rate with increasing temperature for incubations of PRD1 in unamended groundwaters collected from different locations. If the inactivation rate measurements reported for the two 7 °C groundwater incubations in the Yahya (1993) study [6] are averaged, PRD1 inactivation rates (expressed as $\log_{10} d^{-1}$) listed for unamended groundwater incubations in Table 3 have the following collective temperature dependence:

$$k = 0.0021T + 0.0029,$$

where T is temperature in °C. The correlation coefficient (r^2) for this relationship is 0.97 ($n = 5$). According to the above relationship, inactivation rates of PRD1 suspended in groundwater at ≤ 10 °C should be quite low; i.e., less than $0.024 \log_{10} d^{-1}$, which facilitated studies involving its use as a groundwater tracer in “cold-wa-

Table 3
Inactivation rates estimated from die-off data for PRD1 suspended in groundwater

Decay rate ($\log_{10} d^{-1}$)	Temp. (°C)	pH	Duration (d)	Groundwater source	Amendments	Reference
<i>Unamended groundwater</i>						
0.052–0.12	23	n/a	36–75	Tucson, Arizona USA		[6]
0	7	n/a	80	Tricel, Canada		[6]
0.038	7	n/a	55	Pinetop, Arizona USA		[6]
0.010 ± 177	5	5–6.5	30	Falmouth, MA, USA		[30]
0.026 ^b	12	7.0	120	Someren, the Netherlands		[33]
0.017–0.021 ^{b,c}	5	7.5–8.0	21	Castricum, the Netherlands ^d		[50]
<i>Amended groundwater</i>						
0.002 ± 0.004^a	5	5–6.5	30	Falmouth, MA, USA	LAS ^e	[30]
0.001 ± 0.005^a	5	5–6.5	30	Falmouth, MA, USA	DBS ^f	[30]

^a Determined for three replicate systems.

^b Inactivation rates converted from ln to \log_{10} values for comparison purposes.

^c 95% confidence interval based upon 30 observations in a soil filled column.

^d Aqueous phase is canal water used to recharge the aquifer.

^e Mixture of linear alkyl benzene sulfonate homologs (anionic surfactants) at 25 mg l⁻¹ final concentration.

^f Dodecylbenzene sulfonate (anionic surfactant) at 25 mg l⁻¹ final concentration.

ter” aquifers (e.g. [28]). However, a great deal of caution must be exercised in using the aforementioned relationship to predict PRD1 inactivation in other groundwater systems. Although a first order dependence of PRD1 upon temperature may be valid for PRD1 for many of the experimental conditions reported, it is not clear that this relationship would hold under more extreme conditions; e.g., pH values outside the range of 4–9, temperatures outside the range of 1–20 °C, or groundwaters with disparate chemistries.

Predicted effects of groundwater chemistry upon inactivation rates for PRD1 are much less clear. In 1985, Yates et al. [27] published results of a detailed study assessing the effects of native groundwater conditions (temperature, pH, ammonia, calcium, magnesium, total hardness, nitrate, total dissolved solids, and turbidity) upon the decay rates of MS-2, poliovirus I, and echovirus I in 11 different groundwaters collected throughout the United States. Aside from the expected dependence upon temperature, none of the aforementioned chemical parameters significantly correlated with decay rate. Nevertheless, it has been shown that PRD1 inactivation rates can be significantly affected by the composition of the liquid medium in which they are suspended. For example, Dowd and Pillai [37] reported a much higher than expected ($0.8 \log_{10} d^{-1}$) inactivation rate for PRD1 when suspended in 21 °C-groundwater containing 750 mg l^{-1} sulfate. However, it should be noted that the incubations in the latter study were continuously shaken for up to 34 days. On the other hand, Schijven et al. [29] reported inactivation to be 34-fold higher when native (Castricum, the Netherlands) groundwater was employed as the suspending medium compared with a peptone/saline solution. Ryan et al. [30] further demonstrated that the decay rates for PRD1 were clearly affected (lessened) by the presence of anionic surfactants (Table 3), a common sewage-derived groundwater contaminant. A protective effect afforded by dissolved organic compounds may also be inferred from results suggesting decay rates for other viruses are less in wastewater than in groundwater incubated at comparable temperature [36]. The latter three studies suggest that inactivation rates of bacteriophages like PRD1 can be substantively diminished by organic contaminants associated with the same point sources that result in their introduction to the subsurface, i.e., septic tanks, leaking sewer pipes, landfills, and on-land sewage disposal facilities.

4. PRD1 attachment and release in geologic media

The attachment and release of viruses to geologic media in groundwater are dominated by electrostatic forces [38]. In addition, other colloidal interactions (steric repulsion, hydrophobic attraction, hydration ef-

fects) potentially play significant roles. The nature of these interactions and the resulting attachment behavior depend on the surface charge of the virus, the surface charge of the geologic media, and the chemistry of the groundwater.

A qualitative context for understanding the electrostatic forces that dominate virus attachment and release has been provided by the Derjaguin–Landau–Verwey–Overbeek (DLVO) theory, which was developed to predict the stability of colloids in suspension [39,40]. The original DLVO theory accounts for London–van der Waals and double layer interactions between interacting surfaces. The London–van der Waals forces, which arise from electrostatic attraction between temporary fluctuating dipoles in the molecules that make up the surfaces of the interacting colloids, are always attractive and fairly weak. For colloids of the same surface charge, the attractive London–van der Waals forces are opposed by repulsive double-layer forces. If the charges of the interacting surfaces are opposite, then the double layer force is attractive. Double layer forces arise from the repulsion (or attraction) between excess ions gathered near the interacting surfaces to balance the excess charge of the surface.

For microorganisms sorbing to a solid surface, a modified or “extended DLVO” theory [41] is needed in order to account for other forces (in addition to the aforementioned London–van der Waals and double-layer electrostatic forces) that come into play as the microbe gets very near to the surface. Steric repulsion, hydrophobic attraction, and hydration forces, which were not included in the original DLVO theory, have been invoked to explain “extra-DLVO” interactions (repulsion or attraction not accounted for by London–van der Waals and double layer forces). Steric repulsion is associated with the interaction of surface polymers with other surfaces [42]. As a polymer-coated particle approaches another surface, repulsion arises from a decrease in the configurational entropy of polymer chains and the exclusion of water molecules around the polymer. Hydrophobic “attraction” refers to the preference of non-ionic surfaces to associate with each other rather than with water. A favorable entropy change resulting from the disorganization of hydrogen-bonding water molecules arranged around hydrophobic surfaces drives this “attraction” rather than any significant attraction between non-ionic surfaces. A thorough treatment of microbial cell surface hydrophobicity may be found in Doyle and Rosenberg [43]. Repulsive hydration forces arise at very small separation distances between interacting colloids [44]. For two hydrated surfaces to approach, the few layers of water molecules hydrating the surfaces must be removed, which results in an interaction sufficiently unfavorable such that actual colloid–colloid contact does not usually occur.

4.1. PRD1 surface charge

The surface charge of viruses is derived from the ionization of carboxyl, amino, and other functional groups on the surface of the protein capsid. Virus surface charge is often characterized by an isoelectric point (pH_{iep}), the pH at which the net surface charge is zero in an aqueous solution of specified ionic strength and composition. At the pH_{iep} , the density of positive and negative surface charge is balanced. Above the pH_{iep} , the number of ionized carboxyl groups ($\text{R}-\text{COO}^-$) exceeds the number of ionized amino groups ($\text{R}-\text{NH}_3^+$); below the pH_{iep} , ionized amino groups are in greater abundance than ionized carboxyl groups. The most detailed analysis of the surface charge of viruses (bacteriophages MS2 and λ) was made by Penrod et al. [45]. For MS2, they catalogued the ionizable amino acids that comprise the capsid polypeptides (glutamic acid, aspartic acid, arginine, and lysine) and examined the three-dimensional structures as determined by X-ray diffraction to locate these amino acids as interior or exterior. Using the exterior amino acids only, they were able to estimate the surface charge of MS2 as a function of pH. The resulting estimate of pH_{iep} for MS2 closely matched the pH_{iep} determined by microelectrophoresis ($\text{pH}_{\text{iep}} = 3.6$ in 0.01 M NaCl). For λ , a bacteriophage with an isometric head of 54 nm diameter and a flexible tail tube of 150 nm length terminated by a 25 nm long tail fiber, a similar analysis was made without the benefit of detailed three-dimensional structural information. The measured pH_{iep} of λ ($\text{pH}_{\text{iep}} = 3.9$ in 0.01 M NaCl) was similar to that of MS2, but the zeta potentials (ζ) were much more negative (~ -50 mV at pH 7 for λ versus -15 mV for MS2). Penrod et al. determined that the isoelectric points of the head and tail of λ must be different, with the head being substantially more acidic than the tail, to match the measured pH_{iep} and produce anionic surface charge greater than that of MS2 at near-neutral pH.

Strictly speaking, the isoelectric point of PRD1 has never been measured. Bales et al. [10] measured negative electrophoretic mobilities for PRD1 over a pH range of about 4.5–7.5 in a calcium phosphate buffer solution containing 10^{-4} M calcium. The electrophoretic mobility range corresponds to a ζ range of about -13 to -30 mV (using the Smoluchowski equation to calculate ζ). In groundwater from the Cape Cod, Massachusetts (USA) site amended with 1.5 mM NaBr (a total ionic strength estimated at 2 mM), Ryan et al. [11] measured ζ of -8 to -25 mV over a pH range of 3.2–6.2. Neither measurement recorded positive electrophoretic mobilities, so the pH_{iep} of PRD1 has not yet been determined precisely. However, extrapolation of the data suggests that it is below pH 3.

4.2. Surface charge of geologic media

PRD1 attachment to geologic media depends strongly on the surface charge density of the geologic media. Geologic media – soils, sediments, and aquifer materials – contain mixtures of oxide, carbonate, and clay minerals and organic matter. The contribution of each of these minerals to the overall surface charge of heterogeneous geologic media must be considered on a fractional surface area basis. For most geologic media, this is a daunting task because of the complex arrangements of the fine-grained minerals that are often present as coatings or pore-filling deposits between grains. The scale of the mineral surface area is also important. At a minimum, the dimension of a patch of a mineral of contrasting surface charge (e.g., a ferric oxyhydroxide coating on a quartz grain) must be at least comparable to the diameter of the attaching virus (62 nm for PRD1). If not, the contrasting electrostatic effect of a heterogeneous patch will merge into the surrounding electric field and will not influence the attachment of a virus approaching the surface.

The surface charge of geologic media is derived from three primary sources: minerals with amphoteric (pH-dependent) functional groups, minerals with permanent charge arising from isomorphous substitution, and ionized functional groups on organic matter. Mineralogical characterization of the geologic media is important for correctly interpreting virus attachment mechanisms and the surface charge of most minerals that make up geologic media depends on the solution pH. At the mineral–water interface of oxide minerals, surface hydroxyls exchange protons depending on the Lewis acid character of the underlying metal. The surface species of minerals other than oxides (carbonates, sulfides, phosphates) also exchange protons. The proton exchange behavior of these surfaces is characterized by a pair of acidity constants and an electrostatic model accounting for the effect of surface potential, ionic strength, and adsorption of potential-determining ions. More simply, the proton exchange can be characterized by a pH_{iep} , which ranges from 2 to 3 for minerals like quartz and manganese (IV) oxides to 8 to 9 for minerals like ferric and Al(III) oxides. The surface charge densities of amphoteric minerals range from about 10^{-8} – 10^{-7} mol m $^{-2}$. Sorbed natural organic matter (NOM) can make up a significant fraction of exposed surfaces in contact with groundwater. This organic matter can be characterized generally as polyelectrolytic macromolecules. Because the major ionizable functional groups are carboxylic and phenolic acids; NOM is negatively charged. The charge density increases with pH to levels of 10^{-3} – 10^{-2} mol m $^{-2}$ as a greater fraction of the ionizable functional groups are deprotonated.

Clay minerals and other phyllosilicates like micas have both edges and faces. The surface charge of the

edges is permanent and amphoteric, having both Al(III) and silicon hydroxyls groups. The overall pH_{iep} values of clay minerals fall in the 3–5 range, but the edge functional groups include Al(III) and silicon hydroxyls with an edge pH_{iep} value in the 7–8 range. The permanent charge is negative owing to the isomorphous substitution, most commonly of Al(III) for Si(IV) in the silica tetrahedral layers; thus, at pH values in the 5–6 range, clay minerals possess both positively and negatively charged surfaces. The positively charged edges are favorable attachment sites for negatively charged viruses [46]. The surface charge densities of clay minerals are dominated by their cation exchange capacities, which range from 10^{-6} to 10^{-5} mol m^{-2} .

4.3. Solution chemistry

PRD1 attachment to geologic media also depends strongly on the pH, ionic strength, ionic composition, and NOM content of the aqueous solution. The key effect of pH on the surface charge of PRD1 and geological media has been discussed in detail in Sections 4.1 and 4.2. At pH values above the pH_{iep} of PRD1, which results in a negative surface charge for PRD1, an increase in pH results in less attachment to a negatively charged geologic medium (e.g., quartz) because double layer repulsion is increased by the increase in surface charge density.

Ionic strength affects the distance at which the electrostatic double layers of the virus and geologic media begin to overlap. At low ionic strength, double layers extend further into the bulk solution because fewer ions are available to balance the charge of the surfaces. At high ionic strengths, double layers are compressed and the electrostatic repulsion between surfaces of the same charge are reduced. At pH values above the pH_{iep} of PRD1, an increase in ionic strength results in more attachment to a negatively charged geologic medium because double layer repulsion is decreased.

A key consideration in the effect of ionic composition on PRD1 attachment is the presence of multivalent cations. The most common multivalent cation of concern is calcium. Calcium is expected to bind to the carboxyl functional groups on the PRD1 protein capsid and organic matter and with surface hydroxyls and cation exchange sites on minerals to reduce negative surface charge density. The monovalent cations typically abundant in groundwater will not bind to these surface functional groups (e.g., sodium, potassium). Instead, they only influence the double layer thickness. At pH values above the pH_{iep} of PRD1, an increase in calcium concentration will result in more attachment to a negatively charged geologic medium because calcium binding will reduce the negative surface charge and, hence, the double layer repulsion. Various researchers have also proposed that calcium increases attachment by

acting as a “cation bridge” that binds to functional groups on the surfaces of both the virus and geologic medium [47] as it has been proposed for the aggregation of organic matter and colloids, but this mechanism has never been confirmed.

Dissolved organic matter is another important solution chemistry factor for virus attachment. Because dissolved organic matter is an anionic macromolecule, its adsorption is favored on positively charged mineral surfaces. Adsorption reduces and, in some cases, reverses the positive surface charge on such minerals. The reduction or reversal of negative surface charge can result in a change from conditions favorable for viral attachment to conditions unfavorable for attachment. At pH values above the pH_{iep} of PRD1, interaction of dissolved organic matter directly with the PRD1 surface is not anticipated owing to double layer repulsion. The lack of interaction between dissolved organic matter and virus surfaces has not been confirmed experimentally.

4.4. Effects of geologic medium and groundwater chemistry on PRD1 attachment

PRD1 attachment to geological media has been examined in in situ field experiments and “static” and flow-through column systems in the laboratory. The examples presented in this section highlight the effects of the virus surface charge, mineral composition of the geologic media, and the presence of organic matter on PRD1 attachment.

Studies comparing the attachment of PRD1 to geologic media with the attachment of other viruses usually show that attachment increases as the virus pH_{iep} increases [29,31,48–50]. These studies involved the bacteriophages MS2, PRD1, and ϕX174 as well as enteroviruses hepatitis A and poliovirus. As the virus pH_{iep} increases, the amount of negative surface charge on the virus decreases, which results in reduced double layer repulsion for geologic media of negative surface charge. The results of one study contradict this trend – Dowd et al. [51] reported viral attachment to an alluvial sediment at pH 7.1 occurred in the following order: $\text{PM2} < \phi\text{X174} < \text{Q}\beta < \text{PRD1} < \text{MS2}$. For this experiment, attachment increased as pH_{iep} decreased. Schijven et al. [47] speculated that this result could be explained by either (1) the presence of ferric oxyhydroxides in the geologic medium (greater attachment of more negatively charged viruses to the positively charged ferric oxyhydroxides) or (2) cation bridging by calcium, which was present at a relatively high concentration in the groundwater used in these laboratory column experiments.

The effect of the surface charge of the geologic media on PRD1 attachment was investigated by Loveland et al. [52] in static columns (columns packed with porous media and filled with one pore volume of solution for a

specified residence time). PRD1 attached to the quartz grains only at pH values of 5 and below. For quartz partially coated by a ferric oxyhydroxide (deposited on the quartz by hydrolysis of ferric iron), PRD1 attached at pH values of 7.5 and below. For both surfaces, the PRD1 “attachment edge” occurred about 2–3 pH units above the pH_{lep} of the grains (quartz, <3; ferric oxyhydroxide-coated quartz, 5.1; in 1 mM sodium nitrate solution). Ferric oxyhydroxides also increased the attachment of PRD1 and other microbes at a deep-well injection site [33]. The introduction of oxygen at the injection well resulted in ferric oxyhydroxide precipitation near the injection well, but not further down-gradient. The extent of PRD1 attachment corresponded to the ferric oxyhydroxide content along the flow path.

The effect of organic matter on PRD1 attachment to ferric oxyhydroxide-coated quartz sand was investigated in two natural-gradient injections of PRD1 into the Cape Cod aquifer [11,53]. The presence of a geochemical gradient in dissolved oxygen, ferrous iron, and organic matter caused by a plume of secondarily-treated sewage below pristine groundwater allowed comparison of PRD1 transport in the presence and absence of organic matter. The presence of the sewage organic matter, both dissolved and adsorbed to the geologic medium, enhanced PRD1 transport (i.e., reduced PRD1 attachment) by a factor of 2–10. The reduction in attachment was attributed to a decrease in the favorable interaction between the negatively charged PRD1 and the positively charged ferric oxyhydroxide patches. The release of PRD1 can be accelerated by changes in solution chemistry that inhibit attachment. An increase in pH to 10 caused a 1000-fold increase in mobile PRD1 in the Borden aquifer [54]. The introduction of a linear alkylbenzene sulfonate mixture at 25 mg l⁻¹ concentration resulted in the recovery of nearly all of the PRD1 attached to the ferric oxyhydroxide-coated Cape Cod aquifer sand [53]. In a similar manner, beef extract is commonly used to elute attached viruses in laboratory experiments [10].

Some aspects of PRD1 attachment have been attributed to hydrophobic interactions owing to the high lipid content of the PRD1 membrane. Kinoshita et al. [48] invoked hydrophobic interactions to explain an increase in PRD1 attachment with organic matter in two different geologic media, aquifer sediment from Cape Cod and Borden, Ontario. Bales et al. [10] reached a similar conclusion by examining MS2 attachment to silica beads and silica beads with 6.5% of their surfaces coated by octadecyltrichlorosilane. The importance of hydrophobicity to PRD1 attachment must be called into question because the iron oxide content of the Borden aquifer sediment is far lower than that of the Cape Cod aquifer sediment. Loveland et al. [52] suggested that the increased attachment attributed to hydrophobic interaction of the PRD1 with the C₁₈ on the silica beads could

be attributed to a decrease in the double layer repulsion at the C₁₈-covered patches. It is now clear from structural data that lipid in the PRD1 membrane could not promote hydrophobic interactions because the membrane is fully contained within the protein capsid. The protein capsid contains hydrophilic and hydrophobic amino acid residues, but measurements of the hydrophobic character of the soluble proteins generally show that hydrophilic amino acids dominate the protein exterior composition [55].

Steric repulsion was determined to play a significant role in inhibiting the attachment of MS2 to quartz sand [56]. An analysis of the DLVO interaction of MS2 with the quartz sand shows that some additional repulsion was required to explain the MS2 attachment behavior. Loops in the structure of the protein capsid fit the accepted model of steric repulsion. Loop compression and the removal of hydration water cause the additional repulsive force. Steric repulsion may be less important for PRD1 than for MS2 because the amino acid loops in the PRD1 protein capsid are short relative to those of MS2.

4.5. Kinetics of PRD1 attachment and release

Over the past two decades, the modeling of virus attachment and release kinetics has evolved from a simple equilibrium distribution approach [57,58], to single-site irreversible and reversible kinetic models [10,29], to a dual-site approach incorporating colloid filtration for a site of irreversible attachment and an equilibrium distribution for a site of reversible attachment [47,59,60] and, finally, to a dual-site approach incorporating a colloid filtration site for irreversible attachment, a kinetic attachment and release site for reversible attachment, and the effect of blocking, a decrease in the attachment efficiency of the geologic medium as its surface is filled with attached viruses [35].

Irreversible attachment is generally considered to be attachment of viruses to sites of opposite surface charge (e.g., PRD1 on ferric oxyhydroxide patches). Loveland et al. [52] demonstrated that the release of PRD1 from ferric oxyhydroxide-coated quartz was negligible. The kinetics of attachment to these sites is modeled as colloid filtration [61]. The first-order rate coefficient for attachment is proportional to two key colloid filtration parameters, the single collector efficiency and the collision efficiency. The single collector efficiency is the rate at which colloids collide with the geologic medium – it accounts for Brownian motion, sedimentation, and interception as mechanisms leading to colloid collisions with grains. The collision efficiency is the probability of a collision resulting in attachment of the colloid to the grain – it depends on surface chemical interactions described by the DLVO theory and extra-DLVO forces.

Reversible attachment has been described by both an equilibrium distribution and a kinetic attachment and

release. In the formulation of some models, the equilibrium distribution has been justified as an adequate representation of virus attachment in the secondary minimum, a feature of the DLVO interaction profile of the potential energy between surfaces and the separation distance [10]. In this way, retardation of virus transport can be attributed to secondary minimum attachment; however, secondary minimum attachment of viruses is regarded as unlikely owing to the shallow depth of the secondary minimum for particles of virus size and surface charge [52]. Portraying reversible attachment as kinetic attachment and release (with a rate coefficient corresponding to both the attachment and release steps) may more accurately portray the interaction of viruses with surfaces of similar charge (e.g., quartz). Viruses may be attached in a shallow primary minimum, which is more likely as ionic strength increases, and their release from this attachment site would be significantly slower than their attachment, a feature that fits most experimental results for PRD1 [10,29]. Bhattacharjee et al. [35] used the two-site (kinetic/irreversible and kinetic/reversible, with blocking) to describe PRD1 attachment to a ferric oxyhydroxide-coated quartz sand. Following the results of Loveland et al. [52], PRD1 attachment to the ferric oxyhydroxide patches was irreversible and PRD1 attachment to the quartz was reversible. Blocking did not occur over long simulation times at virus concentrations considered normal for a septic tank release.

5. PRD1 surface inactivation in geologic media

The possibility of inactivation of viruses attached to geologic media must be considered if some fraction of virus attachment is reversible. Early models of virus transport considered virus inactivation only in solution. Therefore, reversible attachment effectively postponed inactivation. However, viruses may exhibit different inactivation rates in solution and while attached to surfaces. A further complication is that the inactivation rates for viruses coming within close proximity to a surface during attachment or detachment may be different than either the inactivation rate for suspended or attached viruses [30] (see Fig. 2). However, the effect of virus attachment on the rate of inactivation is not definitive – attachment to some geological media accelerates inactivation, while other geological media inhibits inactivation.

5.1. PRD1 inactivation slowed by attachment

Viruses appear to be protected from inactivation by attachment to geological media with high organic matter and clay-sized particle content (e.g., [7,62,63]). In some cases, viruses are protected from disinfectants by attachment to geological media, as Stagg et al. [62] showed for a system containing MS2, bentonite clay, and hy-

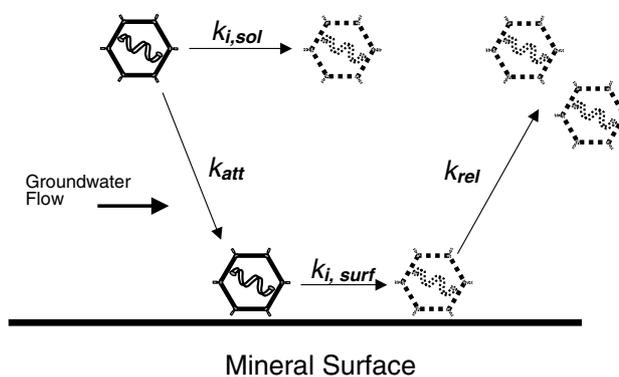


Fig. 2. Schematic depiction of the different conditional rates of inactivation (K) for PRD1 in solution, in close proximity to a mineral surface, on a mineral surface, and detaching from a solid surface in saturated geologic media. The “ k ”s denote kinetics constants. “ k_{att} ” and “ k_{rel} ” are the rate constants that govern PRD1 attachment and release, respectively, “ $k_{i,sol}$ ” and “ $k_{i,surf}$ ” represents inactivation of PRD1 in solution and on a grain surface, respectively. Capsids represented as dashed lines correspond to non-infective (inactivated) PRD1, whereas capsids depicted by solid lines correspond to infective PRD1.

pochlorous acid. Liew and Gerba [63] determined that poliovirus and echovirus survival was prolonged in the presence of clay- and organic matter-rich estuarine sediment in equilibrium with seawater over a temperature range of 24–55 °C, but not at 4 °C. For desert soils amended with sewage sludge, Straub et al. [7] showed that the inactivation of PRD1, MS2, and poliovirus was slower in the presence of a clay loam soil than in the presence of three sandy soils.

For transport of viruses injected into dune sand simulating bank filtration, Schijven et al. [29] reported that PRD1 surface inactivation was slightly less rapid than inactivation in solution. The kinetics of solution inactivation was measured in the dune groundwater and the surface inactivation kinetics were determined by fitting PRD1 breakthrough data with a groundwater transport model. To fit the breakthrough data, surface inactivation was needed as a removal term. The same experiment and model fitting showed that surface inactivation was three times faster than solution inactivation for MS2.

5.2. PRD1 inactivation accelerated by attachment

Inactivation can be accelerated by virus attachment to geological media like iron and aluminum oxides and other materials that bind viruses strongly [29–31,64,65]. Murray and Laband [64] completed the most detailed study of this process. They incorporated radiolabels into the nucleic acid (^3H) and protein capsid (^{14}C) of poliovirus and examined the detachment of the two components from surfaces including quartz, Fe(III) and Al(III) oxides, and aluminum and copper metals. Using divergent detachment behavior of the two virus components

and infective poliovirus as an indicator of surface inactivation, Murray and Laband [64] determined that the surfaces that most strongly bound the viruses caused the most surface inactivation. For example, poliovirus attachment to quartz was relatively weak (the poliovirus and quartz surfaces were both negatively charged) and little inactivation of poliovirus occurred. Poliovirus attachment to aluminum oxide was strong (the surfaces were oppositely charged) and surface inactivation was significant. An analysis of the DLVO interaction between the poliovirus and the mineral surfaces showed that electrostatic forces dominated the attachment mechanism [66].

To further examine the effect of attachment on virus inactivation, Ryan et al. [30] utilized a similar radiolabeling scheme to assess surface inactivation of MS2 and PRD1 in field and laboratory static column experiments. In the field experiment on Cape Cod, Massachusetts, less than 1 percent of PRD1 radiolabeled with only ^{32}P (and measured by infectivity and radioactivity) was trans-

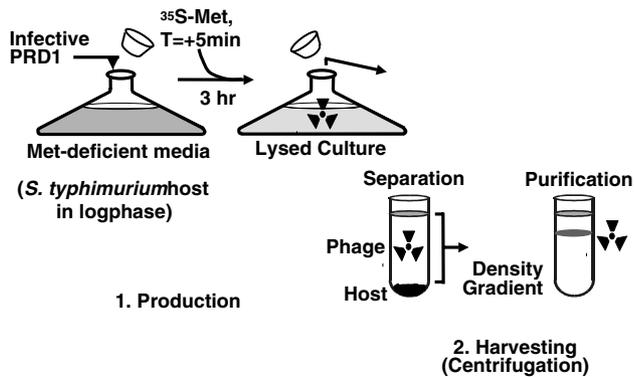


Fig. 3. Procedure for introducing ^{35}S into the protein capsid of PRD1. Met is methionine. *S. typhimurium* is *Salmonella typhimurium*, one of the primary PRD1 bacterial hosts.

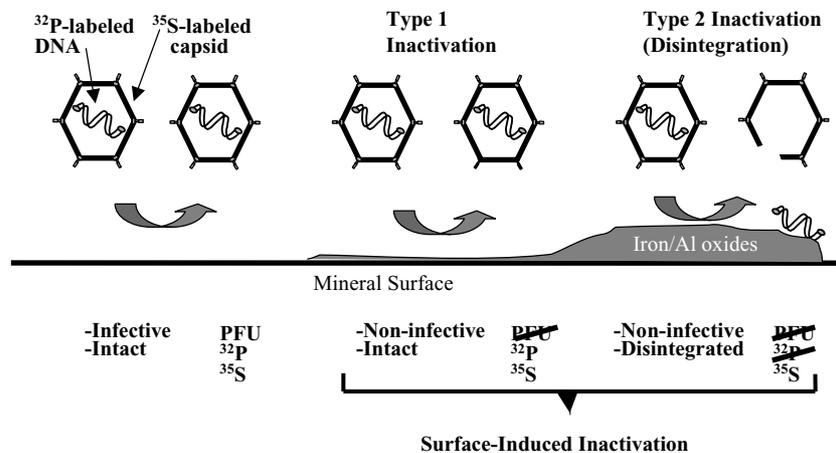


Fig. 4. Schematic depiction of the use of a dual radiolabel (^{35}S -labeled capsid/ ^{32}P -labeled dsDNA) to differentiate between disintegration and intact-type inactivation of PRD1 in the presence of surfaces. Fracture of the ^{35}S -labeled protein capsid and release of the ^{32}P -labeled DNA is shown for interaction with iron-oxide coated surface.

ported over a 1 m distance in the sewage-contaminated zone of the aquifer. After a few days, release of ^{32}P began and persisted for nearly 50 d, with nearly all of the injected ^{32}P ultimately being recovered. No infective PRD1 release coincided with the release of the ^{32}P . This field result strongly suggested surface inactivation occurring at a rate much faster than solution inactivation. In the laboratory static column experiments done to mimic the field results, dual-radiolabeled MS2 (^{35}S , capsid; ^{32}P , nucleic acid) and PRD1 radiolabeled only with ^{35}S in the capsid (^{32}P was not incorporated into the nucleic acid in sufficient quantities) were prepared (Fig. 3). These viruses were attached to Cape Cod sediment in contaminated Cape Cod groundwater and released by successive additions of pore volumes of virus-free solutions. Different behaviors were observed for the release of the radiolabels and infective MS2 and PRD1. Divergent release of the two radiolabels suggested that surface inactivation disintegrated MS2. Surface inactivation rate coefficients were estimated to be at least three times greater than solution inactivation rate coefficients. Strong attachment to the ferric oxyhydroxide patches in the Cape Cod sediment was suggested as the mechanism of surface inactivation. The rate of surface inactivation may have been underestimated by the slow release of inactivated viruses. Separating the kinetics of surface inactivation from the kinetics of the release of viruses back into solution is difficult.

Surface inactivation appears to require strong attractive forces between viruses and surfaces. In the work of Murray and Laband [64], strong attachment was generally the result of the electrostatic attraction between viruses and surfaces of opposite charge. This strong electrostatic attraction appears to be sufficient to disrupt virus structure and cause inactivation. For PRD1, strong electrostatic attraction might disrupt or trigger the response of the receptor proteins at the vertices (Figs. 1 and

4). The interaction of viruses with organic matter, clay minerals, and negatively charged oxide surfaces (e.g., quartz) is generally much weaker and surface inactivation is either not observed or viruses are protected from solution inactivation. The quandary in the application of this surface inactivation model is that the attachment of viruses to surfaces that bind strongly is expected to be irreversible [52]. In spite of this, both Murray and Laband [64] and Ryan et al. [30] measured release of intact, infective viruses as well as the disintegrated components of inactivated viruses from such surfaces.

5.3. Kinetics of virus surface inactivation in geologic media

The kinetics of virus surface inactivation are intertwined with the kinetics of attachment and release in geologic media (Fig. 2). This model reflects contributions of Grant et al. [67] and Ryan et al. [30]. The first step in the surface inactivation process is attachment, which is governed by the first-order rate coefficient k_{att} . We would typically consider attachment to be fast relative to the ensuing steps, especially if the attraction between the virus and surface is strong.

The next step is the inactivation of the attached virus, which can be described by a first-order rate coefficient $k_{i,\text{surf}}$. As stated above, little is known about the mechanism of this step other than the need for strong binding of the virus to the surface. Kinetic data for this step was not collected by Murray and Laband [64] or Ryan et al. [30] for two reasons: (1) inactivation cannot be observed until the inactivated virus components are released and (2) the experiments measured release as a function of number of “flushes,” or pore volumes of solution in contact with the geologic media. Hypothetically, $k_{i,\text{surf}}$ could be related to the strength of virus–surface interactions in the context of DLVO interactions if an experimental means of determining $k_{i,\text{surf}}$ can be implemented.

Following the surface inactivation step may be the release of the inactivated virus, preliminarily described by a first-order rate coefficient, k_{rel} . Potentially, the release step is quite complicated. The inactivated virus may not be released at all because both proteins and nucleic acids can be bound strongly by surfaces that bind viruses strongly. The inactivated virus may be released as an intact virion, in which case the release rate coefficients for components like the capsid and nucleic acid (which can be separately radiolabeled see Fig. 4) would be the same. The inactivated virus may be released as disintegrated debris, which would be detectable by radiolabeling only if the capsid and nucleic acids were released at different rates owing to different binding interactions with the surface. Density-gradient centrifugation of the released virus components could be useful in revealing the details of post-inactivation release.

6. Future perspectives

It is likely that the use of PRD1 as a model and tracer virus in subsurface microbial tracer studies will continue to grow. This is because it is non-pathogenic, relatively stable in a variety of groundwater environments, easy to grow and handle in the laboratory, and structurally similar to adenoviruses of public health concern. Because PRD1 has also become an important model virus in molecular virology, a more detailed understanding of the molecular structure and the manner in which it infects its host are now available. The detailed information from the molecular virology studies, in turn, should facilitate more detailed and mechanistic studies of the nature of viral interactions with defined surfaces in geologic media. Such studies will be able to account more accurately than has been possible in the past for inter-surface forces that come into play as viruses approach, reside on, and detach from surfaces. Also, advances in the understanding of the molecular structure of PRD1 should facilitate more definitive component-labeling, which, in turn, also should lead to a better understanding of PRD1 inactivation studies involving saturated geologic media. A better understanding of surface-induced inactivation is particularly important in view of the fact that temperature-induced inactivation is very slow at groundwater temperatures ≤ 10 °C, typical of many high-latitude aquifers. In such groundwater environments, surface-induced inactivation can be a critical component of natural disinfection. The nature of this latter type of inactivation and, more specifically, its dependence on temperature, mineralogy, and groundwater chemistry needs to be better understood in order to improve predictive capabilities for viral transport on an environmentally relevant field scale.

It is also likely that PRD1 will be employed as a viral tracer in future injection and recovery tests for the purpose of assessing the vulnerability of drinking-water aquifers to microbial contamination, particularly from point sources. The experimental design of vulnerability assessment studies involving PRD1 will be facilitated by the information being gathered concerning its transport, attachment, and inactivation behavior. However, the value of the aquifer vulnerability assessments that employ PRD1 as a tracer will depend, in part, upon how closely the transport and survival characteristics of PRD1 match those of pathogenic viruses that are the common causes of waterborne diseases. It is noteworthy that PRD1 is larger than many enteroviruses and more research is clearly needed that compares the transport and survival characteristics of PRD1 to the viruses that are of primary public health concern in groundwater; i.e., coxsackie, echo, Norwalk, hepatitis A, hepatitis E, rota, enteric adeno, cali, and astro viruses [68]. Although there are striking similarities between the structure of PRD1 and adenoviruses [8], the aforementioned groups

of viral pathogens collectively represent considerable diversity in structure that would be poorly represented by any one virus. Therefore, comparative information collected from studies involving a variety of geologic media concerning the differences in transport and survival behaviors between PRD1 and the aforementioned group of viral pathogens may enhance the value of future injection and recovery tests that employ PRD1 as a surrogate tracer.

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