

## Removal of Viruses by Soil Passage: Overview of Modeling, Processes, and Parameters

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**ABSTRACT:** In this article, the modeling of subsurface virus transport under saturated conditions and the factors that affect adsorption and inactivation are evaluated. Both equilibrium and kinetic adsorption are considered. Equilibrium adsorption is found to be of little significance. Adsorption appears to be mainly kinetically limited. At pH 7 and higher, conditions are generally unfavorable for attachment, but viruses may preferentially attach to a minor surface fraction of soil grains that is positively charged. The relation of pH with surface charge and their effects on sticking efficiencies are evaluated. Dissolved organic matter decreases virus attachment by competition for the same binding sites and thus reduces attachment. Bonded organic matter may provide hydrophobic binding sites for viruses and thus enhance attachment. Dissolved organic matter may disrupt hydrophobic bonds. The enhancing and attenuating effects of organic matter are very difficult to quantify and may be responsible for considerable uncertainty when predicting virus removal.

Values of inactivation rate coefficients for attached viruses were calculated using data from some batch studies. Enhanced or reduced inactivation is found to be virus-specific and almost independent of adsorption. Temperature is the most important factor that influences virus inactivation. Probably the inactivation rate coefficients of free and attached viruses change similarly with temperature.

Some frequently used bacteriophages are evaluated as model viruses. MS2 and PRD1 meet the requirements for worst-case model viruses, at water temperatures less than about 10°C, at pH 6 to 8, and if the soil does not contain too many hydrophobic sites and not too much multivalent cations. Bacteriophage  $\phi$ X174 may be a relatively conservative model virus, because of its low hydrophobicity and stability. Together in a cocktail, these three viruses span a range of properties, like size, surface charge, and hydrophobicity. F-specific RNA bacteriophages (FRNAPHs) may be very useful naturally occurring worst-case viruses. FRNAPHs that are present in surface water or treated wastewater that is used for recharging groundwater, consist of stable and poorly adsorbing viruses.

An inventory of parameter values from field studies is made. Attachment appears to be the major process that determines virus removal. Still, only very few data are available on attachment and detachment of viruses under field conditions. Removal of viruses by soil passage,  $\log(C/C_0)$ , appears to decline nonlinearly with distance due to heterogeneities within the soil as well as within the population of transported virus particles. Predictions of virus removal at larger distances are severely overestimated if they are based on removal data from column experiments or from short-distance field studies.

**KEY WORDS:** virus adsorption, virus inactivation, virus transport models, virus removal, model viruses, MS2, PRD1,  $\phi$ X174, F-specific RNA bacteriophages.

**ABBREVIATIONS:**  $d_c$  [L], collector size;  $d_p$  [L], virus particle size;  $f_{oc}$ , fraction of bonded organic carbon;  $k_{att}$  [ $T^{-1}$ ], attachment rate coefficient;  $k_{det}$  [ $T^{-1}$ ], detachment rate coefficient;  $k_{eq}$  [ $T^{-1}$ ], distribution coefficient;  $k_F$  [ $L^{-2}T$ ], pseudo-first-order coefficient for the retarding effect of double layer repulsion on the adsorption rate;  $k_{irr}$  [ $T^{-1}$ ], irreversible attachment rate coefficient;  $m$ , constant for layering in Freundlich isotherms;  $n$ , soil porosity;  $v$  [ $LT^{-1}$ ], pore water velocity;  $t$  [T] time;  $x$  [L], distance;  $A_s$ , Happel's porosity dependent parameter;  $C$  [ $L^{-3}$ ] number of free viruses per unit volume in the aqueous phase;  $C_0$  [ $L^{-3}$ ], initial number of free viruses per unit volume in the aqueous phase ( $t = 0$ );  $C_{eq}$  [ $L^{-3}$ ], number of free viruses per unit volume in the aqueous phase when at equilibrium with attached viruses;  $D$  [ $L^2T^{-1}$ ], hydrodynamic dispersion;  $D_{BM}$  [ $L^2T^{-1}$ ], diffusion coefficient;  $K_B$  [J/K], Boltzmann constant;  $K_L$  [ $L^3$ ], langmuir constant related to binding energy;  $N_{pe}$  Péclet number;  $R$ , retardation or storage coefficient;  $RB$ , relative breakthrough;  $S_{eq}$  [ $M^{-1}$ ], number per unit mass of soil for viruses attached to equilibrium sites;  $S_{kin}$  [ $M^{-1}$ ], number per unit mass of soil for viruses attached to kinetic sites;  $S_{max}$  [ $M^{-1}$ ], maximum number per unit mass of soil for viruses when all active surface sites are occupied;  $T$  [ $^{\circ}C$ ], temperature;  $U$  [ $LT^{-1}$ ], superficial water velocity;  $\alpha$ , sticking efficiency;  $\alpha_L$  [L], longitudinal dispersivity;  $\eta$  collision efficiency;  $\mu$  [ $ML^{-1}T^{-1}$ ], dynamic viscosity;  $\mu_{eff}$  [ $T^{-1}$ ], inactivation rate coefficient for free viruses in suspension with soil;  $\mu_i$  [ $T^{-1}$ ], inactivation rate coefficient for free viruses;  $\mu_s$  [ $T^{-1}$ ], inactivation rate coefficient for attached viruses;  $\mu_{s,eq}$  [ $T^{-1}$ ] inactivation rate coefficient for viruses attached to equilibrium sites;  $\mu_{s,kin}$  [ $T^{-1}$ ] inactivation rate coefficient for viruses attached to kinetic sites;  $\rho_B$  [ $ML^{-3}$ ], bulk density of the saturated soil.

## I. INTRODUCTION

### A. Safe Drinking Water Production

Groundwater is the main source for drinking water production. Groundwater may become contaminated with pathogenic microorganisms from artificial recharge with wastewater or surface water, or from septic tanks or leaking sewage pipes. Therefore, to protect groundwater from contamination, adequate setback distances between these sources of contamination and production wells for drinking water are needed. Surface water is also a source for drinking water production and is becoming increasingly important. Surface water may be contaminated with pathogenic microorganisms, mainly due to discharges of wastewater and by manure run-off from agricultural land. To produce safe drinking water from surface water these pathogens need to be removed. One effective way is found to be passage of surface water, through soil, as is the case in bank filtration, dune recharge, and deep well injection. To assure production of safe drinking water from surface water, adequate travel times and travel distances are needed.

Pathogens of major threat to human health are viruses and the pathogenic protozoa *Cryptosporidium* and *Giardia*. Little is known about the fate of these pathogenic protozoa during soil passage (Hancock et al., 1998), although new information is emerging (Brush et al., 1999; Harter et al., 1999). Much more information is available for viruses. It is believed that the processes that determine removal of viruses during soil passage also apply to protozoa, albeit to a different extent. Therefore, this review is confined to the study of virus removal by soil passage. Viruses have been shown to be able to travel considerable distances

through the subsurface depending on their size, their adsorption characteristics, and their degree of inactivation (Keswick and Gerba, 1980). Nevertheless, soil passage is considered an important barrier against viruses (Schijven and Rietveld, 1996).

Currently, the extent of wellhead protection areas and bank filtration sites are based on the travel time of the groundwater or recharge water. For example, in Germany (Dizer et al., 1984) and in The Netherlands a travel time of 50 to 60 days is required. This is based on the assumption that a groundwater travel time of 60 days is adequate to inactivate pathogenic microorganisms, to the degree that no health risk exists (Knorr, 1937). However, due to the high persistence of *Cryptosporidium*, *Giardia*, and viruses this may not be sufficient. Current knowledge of infection risks of, as a consequence of drinking water consumption, has resulted in using maximum allowable concentrations for pathogenic microorganisms in drinking water. These are based on a maximum acceptable infection risk of one per 10,000 persons per year and dose-response relationships for pathogens (Regli et al., 1991). This approach has formed the basis for the Extended Surface Water Treatment Rule and it is under consideration for the Ground Water Disinfection Rule in the U.S. (Macler, 1996). Based on the maximum level of infection risk, a proposal for drinking water protection policy is being prepared in The Netherlands that leads to similar maximum allowable concentrations (Schijven et al., 1996). In the case of viruses, it is based on the dose-response relationship of rotaviruses as a worst case. This maximum allowable concentration is  $1.8 \times 10^{-7}$  viruses per liter. Obviously, such a very low concentration is not directly measurable. Therefore, the only way to evaluate the effectiveness of soil passage is to calculate virus concentrations at the production point from the concentrations in source water by means of a computational model.

## **B. Modeling Transport and Fate of Viruses during Soil Passage**

After a certain travel time and travel distance through soil, viruses are removed. Virus removal is the disappearance of viruses from the system and is defined in this context as the logarithmic reduction of virus concentration,  $\log_{10}(C/C_0)$ . The processes of major importance for removal of viruses during soil passage are adsorption and inactivation (Keswick and Gerba, 1980; Yates et al., 1987). Advection and dispersion affect spreading of viruses and thereby attenuation of virus concentrations. Modeling is a way to quantify these processes. Adsorption of viruses to soil may be modeled as either irreversible or reversible. In the case of irreversible attachment, there is no detachment. In the case of reversible adsorption, one may have equilibrium and/or kinetic adsorption sites. In general, both kinds of adsorption may occur in a given medium. In this article, when talking of adsorption, the effects of both attachment and detachment are meant. Thus, we consider a situation where viruses can adsorb to two different

kinds of sites on solid grains. There are sites where attachment and detachment are fast relative to the flow velocity, allowing equilibrium to occur. For some other sites, adsorption is kinetically limited relative to flow velocity, with constant attachment and detachment rate coefficients. The governing equations of solute transport, including dispersion, advection, and inactivation for three-dimensional saturated flow are as follows:

$$n \frac{\partial C}{\partial t} + \frac{\partial \rho_B S_{eq}}{\partial t} + \frac{\partial \rho_B S_{kin}}{\partial t} = \nabla \cdot (n \mathbf{D} \cdot \nabla C) - \nabla \cdot (n \mathbf{v} C) - Q \quad (1)$$

$$S_{eq} = k_{eq} C \quad (2)$$

$$\frac{\partial \rho_B S_{kin}}{\partial t} = n k_{att} C - k_{det} \rho_B S_{kin} - \mu_{s,kin} \rho_B S_{kin} \quad (3)$$

$$Q = n \mu_l C + \mu_{s,eq} \rho_B S_{eq} + \mu_{s,kin} \rho_B S_{kin} \quad (4)$$

Here,  $C$  is the number of free viruses per unit volume in the aqueous phase, [ $L^{-3}$ ]. In short, we refer to it as the free virus concentration. The adsorbed virus concentration is given in terms of number of viruses per unit mass of soil; we refer to it as the attached virus concentrations [ $M^{-1}$ ]. The symbols  $S_{eq}$  and  $S_{kin}$  are used to denote the concentrations of viruses attached to equilibrium and kinetic sites, respectively. Further,  $\rho_B$  is the bulk density of the saturated soil, [ $M.L^{-3}$ ] and  $n$  is the porosity, [-];  $\mathbf{D}$  is the hydrodynamic dispersion tensor, [ $L^2.T^{-1}$ ];  $\mathbf{v}$  is the pore water velocity vector, [ $L.T^{-1}$ ];  $k_{eq}$  is a distribution coefficient, [ $L^3.M^{-1}$ ];  $k_{att}$  and  $k_{det}$  are the attachment and detachment rate coefficients, respectively [ $T^{-1}$ ];  $\mu_l$  is the inactivation rate coefficients for the free viruses, [ $T^{-1}$ ];  $\mu_{s,eq}$  and  $\mu_{s,kin}$  are the inactivation rate coefficients for attached viruses to equilibrium and kinetic sites, respectively [ $T^{-1}$ ].

### C. Studying Virus Adsorption and Inactivation at Batch, Column, and Field Scales

Adsorption of viruses to soil and concurrent inactivation can be studied at different scales: in batch, column, and field experiments. In batch experiments, almost always only equilibrium adsorption is studied. Advection and dispersion cannot be investigated in batch experiments. In columns, transport of viruses is studied often as a one-dimensional process. Columns can be made of packed soil material or undisturbed soil. In the latter case, as in the field, effects of dispersion should be considered. In uniformly packed columns, dispersion is usually small and may be neglected. In field studies, the actual situation is investigated. Depending on the hydrologic situation, transport may be modeled as 1-, 2-, or 3-dimensional. The effect of dispersion can be very important in the field.

In batch and column studies, any combination of soil and virus may be considered, whereas in field studies many restrictions apply. Removal of pathogenic viruses under field conditions can be studied only if contamination levels are high enough. Usually this is not the case. Only in exceptional situations, permission may be obtained to seed pathogenic viruses in the field. At a site that is in use for drinking water production, this will never be allowed. Therefore, model viruses that are not pathogenic but are still representative for the transport behavior of pathogenic viruses are needed. A model virus is suitable if its inactivation and adsorption are similar to that of pathogenic viruses under given conditions. This implies that it should be possible to predict removal of pathogenic viruses by passage through soil from the removal of the model virus.

Usually bacteriophages are used as model viruses. Bacteriophages offer the following advantages:

- Bacteriophages are not pathogenic to human, but infect a specific host bacterium.
- Bacteriophages can be prepared in large quantities ( $10^{10}$  to  $10^{12}$  phages per  $m_l$ ), allowing seeding of high numbers. This makes it possible to show removal up to  $11 \log_{10}$ .
- The assay of bacteriophages is relatively easy, whereas analysis of pathogenic viruses is much more complex, time consuming, and sometimes not possible at all.

#### **D. Purposes and Outline of the Article**

Several reviews on the transport and fate of viruses through the subsurface have appeared. Keswick and Gerba (1980) reviewed reports on virus isolation from groundwater sources for drinking water production and from recharged groundwater sites. Hydrogeological, biological, and meteorological factors affecting the survival and transport of viruses in groundwater were identified. The following research needs were recommended: (1) investigation of virus-surface interactions and virus inactivation in groundwater; (2) development of experimental methods and predictive models; (3) development of criteria for adequate groundwater protection; and (4) performing field studies at land treatment sites, septic tanks, and on disease outbreaks by groundwater contamination. Gerba (1984) presented a comprehensive review on the factors influencing virus adsorption and inactivation, which were studied mostly in batch experiments. Yates et al. (1987) also reviewed the factors affecting transport and inactivation of viruses through soils, including modeling of batch studies and of transport. They concluded that modeling of virus transport was constrained by a lack of quantitative information on virus behavior during transport. Yates and Yates (1992) presented an overview of the way inactivation, equilibrium adsorption, advection and dispersion determine transport

of viruses. These factors were summarized in a quantitative manner by Gerba et al. (1992). Also of interest are the reviews on the transport of colloids in groundwater by Swanton (1995) and Ryan and Elimelech (1996).

The main purposes of this article are to:

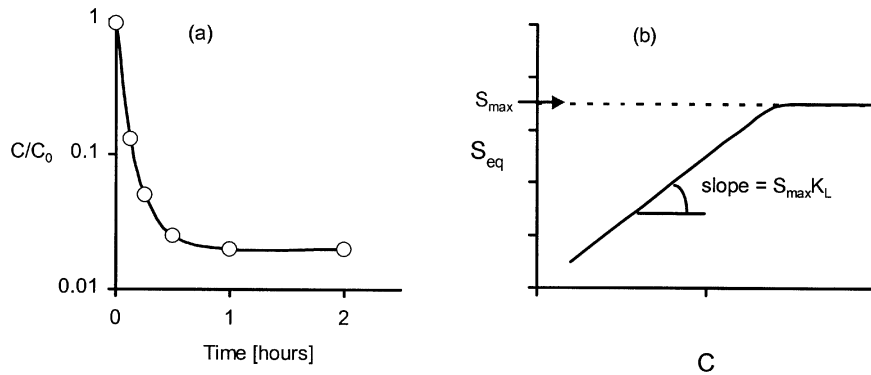
1. Evaluate the modeling that is used to describe and quantify adsorption and inactivation of viruses during subsurface transport;
2. Review the major factors that affect these processes;
3. Evaluate model viruses as representatives of subsurface transport and behavior of pathogenic viruses; and
4. Discuss the relative contributions of adsorption and inactivation to the removal of viruses during subsurface transport.

In Section II, modeling of equilibrium adsorption of viruses to soil in batch experiments and during transport through soil in column and field experiments is discussed. A similar study but for kinetic adsorption will be presented in section III. Also, in Section III, colloid filtration theory, Derjaguin-Landau-Verwey-Overbeek (DLVO) theory, hydrophobic interactions, and blocking are discussed. In Section IV, the factors that affect adsorption of viruses to soil are reviewed. In Section V, modeling of virus inactivation is reviewed, and the major factors that affect inactivation are discussed in Section VI. This article focuses mainly on saturated flow conditions; however, because unsaturated conditions have a significant impact on inactivation, this is discussed also. Section VII discusses the effects of advection and dispersion on virus transport in the field. In Section VIII, some model viruses are evaluated. In Section IX, the relative contribution of adsorption and inactivation to virus removal are evaluated, and removal of viruses with distance is discussed. Finally, a summary and conclusions are presented in Section X.

## **II. EQUILIBRIUM ADSORPTION**

### **A. Equilibrium Adsorption in Batch Experiments**

In batch experiments, a suspension of viruses is agitated with a quantity of the solid material of interest in a container. Concentration of viruses present in the water phase of the container is measured as a function of time. Concentration of viruses in a control container with water but without soil is measured to calculate inactivation, but also to be able to compensate for possible losses due to attachment to the walls of the container. A typical semi-log plot of virus adsorption to soil in a batch suspension is given in Figure 1a. Initially, free virus concentrations decline with time, but after a short time, they remain almost constant. At that point, a distribution of viruses between solid and liquid phase is obtained, because of reversible adsorption. This apparent equilibrium is rapidly reached but is not



**FIGURE 1.** Adsorption of a virus to soil in a batch suspension: (a) Decreasing concentration of free viruses with time; (b) Example of Langmuir isotherm.

instantaneous. It depends on the actual attachment and detachment rates. The time to equilibrium has been reported to vary from 30 min (cf. Gerba and Lance, 1978; Goyal and Gerba, 1979; Taylor et al., 1980; Gerba et al., 1981; Singh et al., 1986; Gantzer et al., 1994) to 60 to 90 min (cf. Moore et al., 1981, 1982; Taylor et al., 1981; Bales et al., 1991; Sakoda et al., 1997). In all these studies, inactivation was either neglected or found to be insignificant within the time scale of the experiment. At larger time scales, the free virus concentration continues to decrease at a steady rate due to inactivation.

Commonly in batch experiments, the kinetic behavior, which is operative before steady state is reached, is not considered, and values of attachment and detachment rate coefficients are not determined. In batch studies, the apparent steady-state concentrations are used to construct Langmuir or Freundlich isotherms (Yates et al., 1987). The Langmuir model assumes that maximum attachment corresponds to a saturated monolayer of solute molecules on the adsorbent surface, that the active sites for attachment are all the same, and that there is no interaction between attached molecules. The Langmuir equation reads:

$$S_{eq} = \frac{S_{max} K_L C_{eq}}{1 + K_L C_{eq}} \quad (5)$$

Where  $S_{eq}$  is the concentration of adsorbed viruses and  $C_{eq}$  is the concentration of free viruses after apparent equilibrium has been reached.  $S_{max}$  is the maximum adsorbed concentration when all active surface sites are occupied;  $K_L$  is a constant related to the bonding energy. A typical example of a Langmuir isotherm is plotted in Figure 1b. It shows that the slope of the increasing part of the curve equals  $S_{max} K_L$  and that the curve finally reaches  $S_{max}$ . When  $K_L C_{eq} \ll 1$ , the Langmuir equation may be linearized to obtain a linear isotherm:

$$S_{eq} = S_{max} K_L C_{eq} \quad (6)$$

Freundlich isotherms have also been applied to describe attachment of viruses to soil (Gerba, 1984). Here, no assumption is made on the homogeneity of active sites for attachment. The Freundlich formula reads:

$$S_{eq} = k_{eq} C_{eq}^m \quad (7)$$

Here,  $m$  is a constant. For many systems with low free virus concentrations,  $m$  is not significantly different from unity, whereby Equation 7 reduces to a linearized form similar to Equation 6 (Vilker and Burge, 1980; Yates et al., 1987).

Moore et al. (1981) showed that adsorption of poliovirus 2 to Ottawa sand could be described by the Langmuir equation:  $S_{max}$  appeared to be  $2.5 \times 10^{12}$  virus particles per kg of sand. At lower surface coverage, adsorption was successfully described by the Freundlich equation. Vilker and Burge (1980) summarized several examples where Freundlich and Langmuir isotherms were applied. In these examples,  $S_{max}K_L$  was shown to vary between 2 and 640,000 liter/kg. In some of these examples,  $S_{max}$  was shown to be very large (i.e., in the order of  $10^{14}$  to  $10^{15}$  sites per kg of soil) but  $K_L$  was shown to be very small in the order of  $10^{-14}$  to  $10^{-11}$  liter per virus. Vilker and Burge (1980) concluded that virus adsorption is saturation limited, that is to say, the number of adsorption sites is finite. They further concluded that the large values of  $S_{max}$  and the small values for  $K_L$  indicate that virus adsorption is characterized by a large number of sites, but equilibrium strongly favors the liquid phase over the adsorbed phase. Other examples of application of Freundlich isotherms can be found in Gerba and Lance (1978), Taylor et al. (1980), Lipson and Stotzky (1983), Bales et al. (1991), and Sakoda et al. (1997).

In batch experiments, time to reach apparent equilibrium is not only dependent on the virus type and virus concentration, but it also depends on the particle size of the adsorbent and the degree of agitation (Vilker and Burge, 1980; Moore et al., 1981). Adsorption of poliovirus 2 was relatively constant from one experiment to another, but adsorption to a few soils varied greatly. To organic muck 16 to 99% of the poliovirus was adsorbed, and to sandy loam 94 to 99.7% (Moore et al., 1981). High variability in adsorption among different batch studies is thought to depend primarily on the heterogeneity of soil preparations, like a wide range of particle sizes (Vilker and Burge, 1980). Jin et al. (1997) argued that results from batch experiments have been neither consistent nor reproducible, largely due to the fact that there is no standard protocol. That is to say, different sizes and types of containers and different methods of agitation are used. Also, an air–water interface may be present in some, but absent in other experiments. All these differences may influence the equilibrium of a batch system. This makes it very difficult to compare values for adsorption between different batch studies. Nevertheless, batch tests



have been used extensively to investigate the effects of various factors (e.g., pH, organic matter, and soil type) and to compare adsorptive behavior of different viruses in combination with different solid materials under a given set of experimental conditions.

## B. Equilibrium Adsorption of Viruses to Soil during Subsurface Transport

Equilibrium adsorption has been assumed in several column and field studies, neglecting kinetic adsorption (e.g., Park et al., 1994; Powelson et al., 1990; Powelson and Gerba, 1994; Tim and Mostaghimi, 1991). Under these assumptions and for a one-dimensional situation, Equations 1 and 4 are simplified to:

$$n \frac{\partial C}{\partial t} + \rho_B \frac{\partial S_{eq}}{\partial t} = nD \frac{\partial^2 C}{\partial x^2} - n \frac{\partial C}{\partial x} - Q \quad (8)$$

$$Q = n\mu_l C + \mu_s \rho_B S_{eq} \quad (9)$$

Equations 2 and 9 can be combined to:

$$R \frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} - v \frac{\partial C}{\partial x} - Q/n \quad (10)$$

Here, the retardation coefficient is  $R = 1 + (\rho_B/n)k_{eq}$ , which is evidently equal to or larger than 1.

Viruses are not removed by equilibrium adsorption. According to the equilibrium model description given by Equations 9 and 10, removal of viruses during subsurface transport is only due to virus inactivation. Sometimes an extra sink term for irreversible attachment is also included to account for virus removal (Jin et al., 1997; Matthes et al., 1988; Yates and Ouyang, 1992):

$$R \frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} - v \frac{\partial C}{\partial x} - k_{irr} C - Q/n \quad (11)$$

Here,  $k_{irr}$  is the irreversible attachment rate coefficient. It is usually denoted as filtration (see Section III.D).

For solute contaminants and proteins, temporal and spatial variability of the distribution coefficient has been observed, due to subsurface heterogeneities, such as grain size, surface area, pH, temperature, and redox potential (Chrysikopoulos and Sim, 1996). Assuming the same may be the case with viruses, Chrysikopoulos and Sim (1996) developed a transport model with a stochastic time-dependent

distribution coefficient. Simulations with a time-dependent coefficient resulted in an enhanced spreading of the free virus concentration compared with the case with a constant  $k_{eq}$ .

In some cases, retardation coefficients of about 2 to 5 have been reported (Bales et al., 1991, 1997; Powelson et al., 1993; Powelson and Gerba, 1994). However, in most experiments, little or no retardation was found (Bales et al., 1991, 1993; Pieper et al., 1997; Jin et al., 1997; Schijven et al., 1999). Apparently, retarded breakthrough by equilibrium adsorption is of little significance.

Values of the “retardation coefficient” of less than one have also been reported. That is to say, faster virus transport relative to that of a conservative salt tracer has been observed most probably due to pore size exclusion of the virus (see Section VII).

### III. KINETIC ADSORPTION

#### A. Kinetic Analysis of Batch Experiments

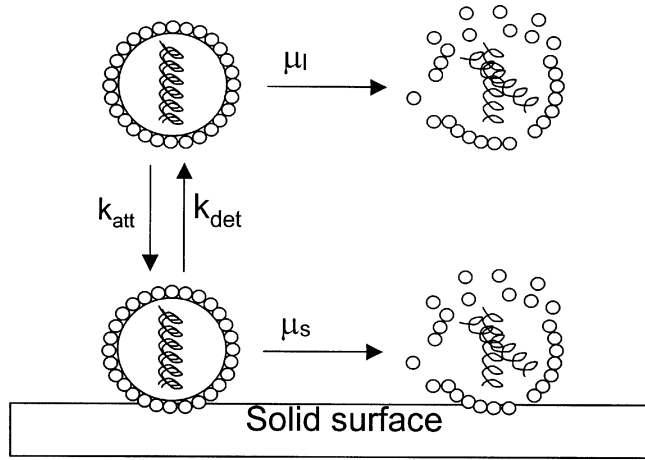
In a batch suspension of viruses and soil, adsorption equilibrium is not reached instantaneously. Instead, virus adsorption at the microscale can be described as the result of two processes, each of which takes a certain time (see Gerba, 1984). In the first process, the viruses are transported close to the solid surface. Here, one speaks of mass transport. In the second process, the viruses are immobilized at the surface by physical and possibly chemical interactions. The overall rate of attachment depends on which of these two processes, mass transport or virus-surface interactions, is the rate-limiting step (Grant et al., 1993). The kinetic behavior that is operative before apparent equilibrium is reached can be described by virus attachment to the soil, virus detachment from the soil, and inactivation of free and attached viruses. Thus, the kinetics of the system can be characterized by four parameters:  $k_{att}$ ,  $k_{det}$ ,  $\mu_f$ , and  $\mu_s$  (Figure 2). The governing equations are

$$n \frac{dC}{dt} = -nk_{att}C + k_{det}\rho_B S - n\mu_f C \quad (12)$$

$$\rho_B \frac{dS}{dt} = nk_{att}C - k_{det}\rho_B S - \mu_s \rho_B S \quad (13)$$

As we have seen in Section II.A, on a time scale of a few hours, inactivation of viruses in a batch system is commonly negligible. In that case, Equations 12 and 13 have the following analytical solution:

$$C / C_0 = \frac{k_{det} + k_{att} \exp[-(k_{att} + k_{det})t]}{k_{att} + k_{det}} \quad (14)$$



**FIGURE 2.** Attachment and inactivation of virus in bulk fluid and at the solid-liquid interface (Grant et al., 1993). Here,  $k_{att}$  is the attachment rate coefficient;  $k_{det}$  is the detachment rate coefficient;  $\mu_i$  is the inactivation rate coefficient of viruses in the aqueous phase and  $\mu_s$  that of viruses attached to the solid surface.

The slope of the  $C-t$  curve at  $t = 0$ , assuming negligible inactivation, is approximately equal to  $-k_{att}$  (Figure 1a). This is evident from Taylor series expansion of Equation 14, which yields:

$$C / C_0 = 1 - k_{att}t + \frac{t^2}{2} k_{att}(k_{att} + k_{det}) - \frac{t^3}{6} (k_{att} + k_{det})^2 + \dots \quad (15)$$

Thus,  $k_{att}$  may be evaluated from early measurements of a batch experiment. Once an apparent steady state is reached (i.e., negligible  $dC/dt$ ) and neglecting inactivation, from Equations 12 and 13 it also follows:

$$\frac{\rho_B}{n} S_{eq} = \frac{k_{att}}{k_{det}} C_{eq} = \frac{\rho_B}{n} k_{eq} C_{eq} = (R-1)C_{eq} \quad (16)$$

Thus, from equilibrium results,  $k_{det}$  can be obtained. Therefore, it is possible to determine both  $k_{att}$  and  $k_{det}$  from batch experiments.

Often, adsorption of viruses to soil in batch experiments is expressed as the fraction of viruses that is adsorbed at equilibrium. The relation between the ratio  $k_{att}/k_{det}$  and the adsorbed fraction  $f$ , at equilibrium, is as follows:

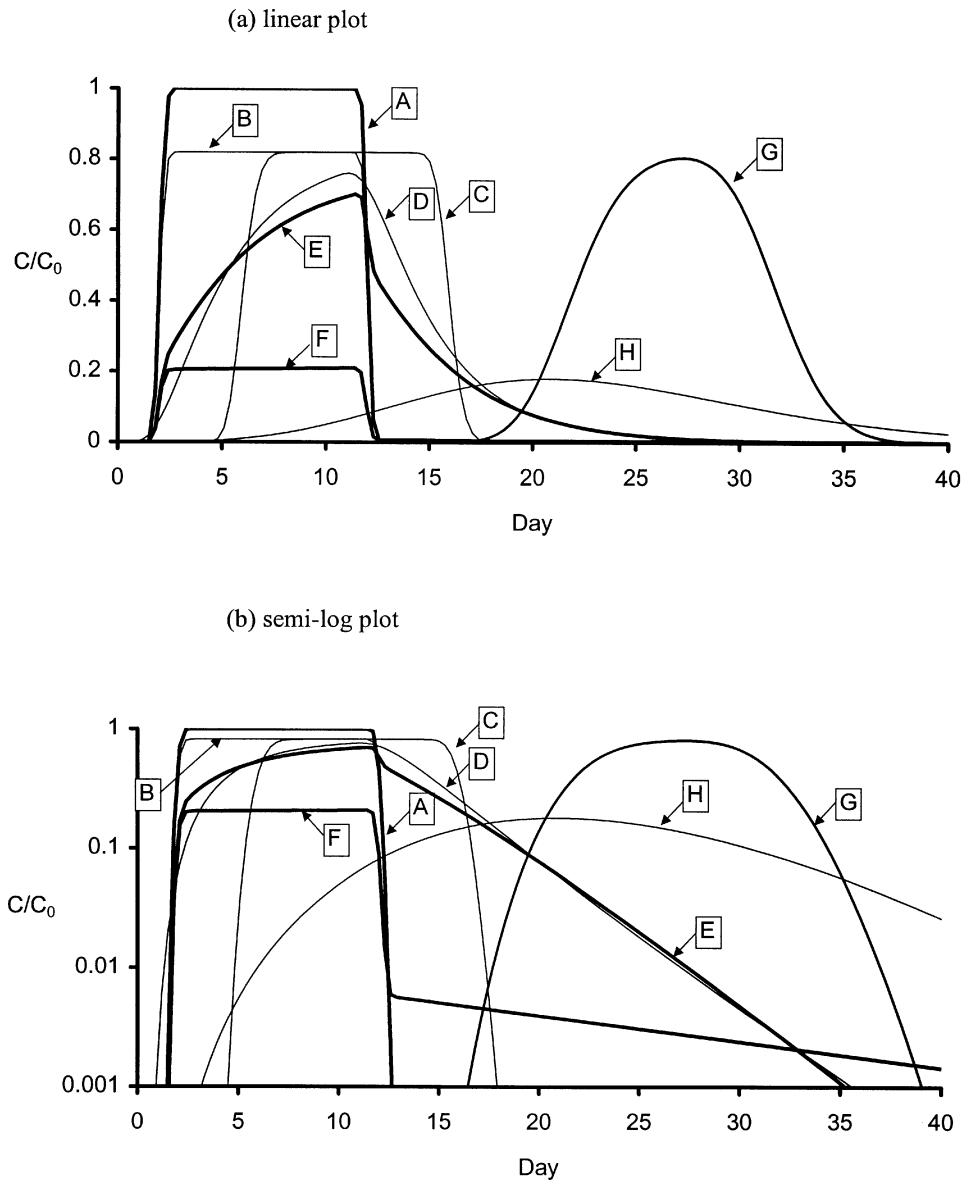
$$\frac{k_{att}}{k_{det}} = \frac{C_0}{C_{eq}} - 1 = \frac{f}{1-f} \quad (17)$$

On longer time scales (e.g., days) virus inactivation will be significant and causes disappearance of viruses from the system. The interplay of kinetic effects and virus inactivation in a batch system is discussed in Section V.C.

## **B. Kinetic Adsorption of Viruses to Soil during Subsurface Transport**

Under transport conditions, adsorption of viruses to soil may be kinetically limited relative to advection. In field studies (e.g., Bales et al., 1997; Schijven et al., 1999), it appeared that attachment rates were relatively fast compared with advection, whereas detachment rates were much slower. Bales et al. (1997) expressed this in terms of time scales for attachment or detachment relative to advection over a characteristic travel distance  $L$  by  $v/(k_{att}L)$  and  $v/(k_{det}L)$ , respectively. It was found that the former term was about two orders of magnitude smaller than one, whereas the latter was about three orders of magnitude greater than one. At very low flow rates (e.g., under some natural gradient conditions) kinetically limited adsorption may successfully be described by equilibrium adsorption. The attachment and detachment rates determine the shape of virus breakthrough curves. To show the differences and similarities between equilibrium and kinetically limited adsorption, various breakthrough curves for a column were simulated using the CXTFIT-code (Toride et al., 1995). This code is based on analytical solutions of equilibrium and kinetic transport models, including governing Equations 1 to 4 for a one-dimensional situation. The breakthrough of virus seeded at a constant concentration on top of a column for a duration of 10 days was calculated and is displayed in Figures 3a and 3b. The parameter values that were used for these simulations are given in Table 1. Dispersion is mainly determined by the characteristics of the porous medium, provided that no size and/or charge effects are present. A virus entering a soil therefore will experience the same dispersion as a conservative salt tracer. This is the case when considering curves A and B. A conservative salt tracer will not be retarded and  $C/C_0$  reaches a plateau of one shortly after breakthrough (curve A). A virus that does not attach to the soil will show the same breakthrough as the salt tracer, but the plateau is lower due to inactivation of the virus (curve B). If a virus is also retarded due to equilibrium adsorption, it will show the same curve as B, but shifted to the right (curve C). If this virus is subject to a higher dispersion, the shape of the curve becomes flatter, as illustrated by curve D. Here, a fraction of the virus breaks through earlier, but the time to peak breakthrough is concurrent with the middle of the plateau of curve C.

Curve E simulates breakthrough of a virus, with the same dispersion as the salt, but now exhibiting kinetically limited adsorption, where the attachment and detachment rate coefficients are of the same order of magnitude. The time to peak breakthrough is retarded. In fact, curve's D and E in Figures 3a and 3b are very similar. Curve F simulates the virus, now exhibiting slow detachment. The shape



**FIGURE 3.** Simulated breakthrough curves of (A) a conservative salt tracer; (B) a virus that does not adsorb, but that is inactivated; (C) a virus that is retarded due to equilibrium adsorption, and that is inactivated; (D) like C with higher dispersion; (E) a virus that adsorbs to kinetically limited sites, and that is inactivated; (F) like (E) but now detachment is much slower; (G) like C with higher  $R$ ; and (H) like E, but high  $k_{att}$  and  $k_{att}/k_{det}$  of 10. See Table 1 for corresponding parameter values.

**TABLE 1**  
**Parameter Values Used to Simulate Breakthrough Curves for a Conservative Salt Tracer and for a Virus Exhibiting Different Types of Adsorptive Behavior**

Parameter	A Salt Tracer	B Virus -Adsorption + Inactivation	C Virus as B +Equilibrium Adsorption	D Virus as C +High Dispersion	E Virus as B +Kinetic Adsorption	F Virus as E Slow Detachment	G Virus as C Higher R	H Virus as E Higher $k_{att}$ $k_{att}/k_{det} = 10$
D	0.02	0.02	0.02	1	0.02	0.02	0.02	0.02
R	1	1	3	3	—	-	11	—
k <sub>att</sub>	—	—	—	—	0.75	0.75	—	10
k <sub>det</sub>	—	—	—	—	0.375	0.00375	—	1
$\mu_l = \mu_s$	0	0.05	0.05	0.05	0.05	0.05	0.05	0.05

Note: Resulting breakthrough curves are shown in Figures 3a and 3b. Pore water velocity = 1.5 m/day and distance = 3 m. Dimension of D is [m<sup>2</sup>.day<sup>-1</sup>], that of  $k_{att}$ ,  $k_{det}$ ,  $\mu_l$  and  $\mu_s$  is [day<sup>-1</sup>].

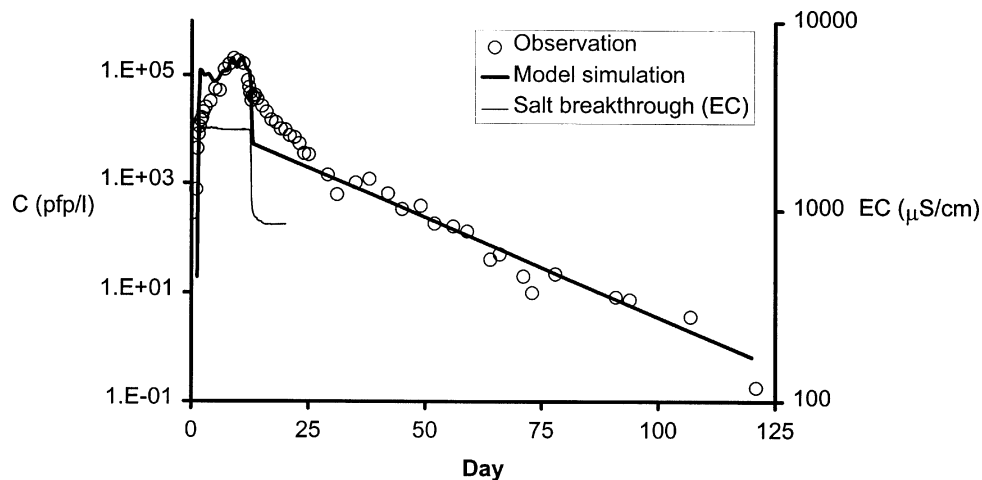
of this curve on linear concentration scale is now the same as A and B; only the maximum concentration is lower due to attachment. However, when plotted on a semi-log scale a tail becomes visible, which makes it distinct from the other curves (Figure 3b). Now, if one stops measurements before the end of the plateau is reached, or if only linear plots are made, one may conclude that there is no reversible adsorption but a very high rate of irreversible adsorption (see Jin et al., 1997). Curve G simulates transport of a virus that is retarded due even more to equilibrium adsorption. Curve H shows that transport of a virus that attaches to kinetic sites may also show significant retardation. In this case the ratio of  $k_{att}/k_{det}$  is equal to  $R - 1$  of curve G. Retardation is less in the case of H compared with G, but the breakthrough curve is much more dispersed.

These simulations show that description of virus transport by an equilibrium model and a kinetic model may lead to similar results, and that investigation of tailing on a semi-log plot is needed to tell the difference. A shortcoming of many virus transport experiments is that the tail of the breakthrough curve is not measured, so that the kinetic behavior cannot be observed (see Powelson et al., 1990; Powelson and Gerba, 1994; Jin et al., 1997).

Modeling of both equilibrium and kinetic adsorption was carried out by Bales et al. (1991, 1997). Adsorption of bacteriophages MS2 and PRD1 was shown to be reversible and kinetically limited (Bales et al., 1991, 1993, 1997; Kinoshita et al., 1993). The kinetic effect was evidenced from the slow rising limbs and the long tails of the breakthrough curves. Bales et al. (1991) showed that in order to fit an equilibrium model to MS2-breakthrough curves, apparent dispersion values of up to 150 times higher than for the salt tracer had to be used to fit the rising limbs of the breakthrough curve. Furthermore, to fit the declining limbs of the breakthrough curve, a small dispersion value and an apparent  $R$  of less than one were needed. This is physically unacceptable and it indicates that the adsorption must be modeled as a kinetic process. Bales et al. (1991, 1997) found that the contribution of equilibrium adsorption to the total adsorption was negligible, and that kinetic adsorption controlled virus attenuation.

In the study of Bales et al. (1993), breakthrough curves of MS2 in silica showed a gradual increase in free virus concentrations toward maximum breakthrough concentration, similar to curve E. This means that the detachment rate coefficient is in the order of the attachment rate coefficient. However, the breakthrough curves of MS2 in columns with hydrophobic bonded silica reached a steady-state value much sooner, similar to curve F. In this case, detachment is much slower than attachment.

Other clear examples of kinetic behavior are shown for bacteriophages MS2, PRD1,  $\phi$ X174, Q $\beta$  and PM2 in a column study by Dowd et al. (1998), for MS2, PRD1,  $\phi$ X174, and poliovirus 1 in field studies by DeBorde et al. (1999), for MS2 and  $\phi$ X174 by DeBorde et al. (1998); and for MS2 and PRD1 by Schijven et al. (1999). In these field studies, phage transport was not retarded by equilibrium adsorption. Figure 4 shows a typical breakthrough curve of MS2 from the field



**FIGURE 4.** Breakthrough curve of MS2 after 2.4 m of passage through dune sand (Schijven et al., 1999). Observed and simulated concentrations are shown. EC is the electrical conductivity that was measured during breakthrough of the sodium chloride tracer.

study by Schijven et al. (1999). It was found that the value of  $k_{att}$  determined mainly the maximum breakthrough concentrations. It was also found that the tail slope was determined mainly by the value of  $\mu_{s}$ , whereas its intercept was affected mainly by the value of  $k_{det}$ . The end of the rising limb and the start of the declining limb of the breakthrough curves could not be simulated completely, due to an as yet unknown process. Possibly, there is more than one type of kinetic sites involved.

There is growing evidence from column and field experiments that removal of viruses during transport is governed mainly by kinetic adsorption. Neglect of kinetic effects and use of equilibrium adsorption models may lead to an estimation of an artificially large dispersion coefficient. If detachment is slow compared with attachment, tailing may be observed only if measurements are carried out for a long enough time and when concentrations are plotted on a semi-log scale.

### C. Batch vs. Column Experiments

Estimates of adsorption parameters from batch experiments appear to be of limited use in the prediction of virus adsorption in column or field experiments. As already pointed out in previous sections, apparent equilibrium distribution is commonly assumed in batch systems, whereas kinetic attachment/detachment is needed to describe adsorption under transport conditions. Also, due to the stirring in a batch experiment, the number of accessible sites for attachment is much higher than in a column. In a column, attachment rates may thus be so low that attachment



rates of various viruses are relatively close to each other. Detachment is limited by diffusion over an energy barrier resulting from virus–soil interactions and by diffusion across a boundary layer near the solid surface (Ryan and Elimelech, 1996). The diffusion coefficient of the virus and the thickness of the boundary layer control the rate of transport across the diffusion boundary layer. The latter is controlled primarily by the velocity of the advecting fluid. If this velocity increases, the thickness of the diffusion boundary layer decreases. Therefore, the detachment rate coefficient in a batch system is presumably smaller than under transport conditions, where there is advective flow. This implies that the observed ratio  $k_{att}/k_{det}$  from a batch experiment is expected to be larger than that obtained from a column or field experiment.

Bales et al. (1991) found that adsorption of MS2 in columns with silica beads was kinetically limited. They found ratio value  $a$  of 0.36 l/kg for  $k_{att}/k_{det}$  in the column experiment, but in the batch experiment  $k_{att}/k_{det}$  was found to be 700 times higher. The 270 times higher specific surface area of the silica sorbent that was used in the batch experiment may only partly explain this difference. Therefore, these findings suggest that  $k_{att}/k_{det}$  from a batch experiment is indeed larger than that from a column or field experiment. Powelson and Gerba (1994) estimated the retardation coefficient  $R$  from column experiments with MS2 ( $R = 1.4$ ), PRD1 ( $R = 2.2$ ), and poliovirus 1 ( $R = 5.2$ ) using the equilibrium model as described by Equation 11. These estimates were 12 to 130 times smaller than those determined from batch studies.

Contrary to expectations, in some batch experiments, values for the ratio  $k_{att}/k_{det}$  are found to be smaller than in corresponding column experiments. Goyal and Gerba (1979) carried out a number of batch experiments where the percentage of adsorption was determined after 30 min. Their results are converted to the ratio  $k_{att}/k_{det}$  using Equation 17, and are reported in Table 2. This ratio is generally found to be less than one at pH 7 to 8. However, as discussed in the previous section,  $k_{att}/k_{det}$  was found to be much larger than one in column studies (Bales et al., 1991, 1993) and field studies (Bales et al., 1997; Pieper et al., 1997; DeBorde et al., 1999; Schijven et al., 1999). This is perhaps an artifact due to the duration of batch experiments being too short. The following comparison between column experiments of Wang et al. (1981) and Lance et al. (1982) and the batch experiments of Goyal and Gerba (1979) also seems to support this. Wang et al. (1981) and Lance et al. (1982) studied the removal of poliovirus 1 and echoviruses 1 and 29 in columns with the same soils. Residence time of the viruses in the columns was about 3 days. It was found that poliovirus 1 and both echoviruses were all removed very well (99 to 99.9%). Lance et al. (1982) suggested that poliovirus 1 does not behave significantly different from other enteroviruses under transport conditions in long soil columns that approximate field conditions. However, in the batch experiments of Goyal and Gerba (1979), poliovirus 1 adsorbed very well to different soils, whereas echovirus 1 and 29 were the least adsorbing viruses (Table 2). Thus, the difference in adsorption that was found in batch experiments

**TABLE 2**  
**Values of the Ratio  $k_{att}/k_{det}$  from Batch Experiments with Viruses from Group I, II, and III and Nine Different Soils**

	pH	4.5	4.9	5.5	7.1	7.1	7.8	8	8	8.2	pI <sup>a</sup>
I	CB4 (V216)	142	2.0	0.79	0.14	0.020	0.43	0.52	0.47	0.52	
	CB4 (V240)	76	12	2.7	1.3	0	0	0.32	0.35	0.54	
	Echo 1 (Farouk)	332	9	3.5	0.39	0.27	1.2	0.14	0.12	0.15	5.1
	Echo 1 (V212)	332	2.3	3.8	0.79	0.30	0.85	0.61	0.23	0.010	6.4
	Echo 1 (V239)	49	1.9	2.1	0.053	0.11	0	0.96	1.1	1.1	5.3
	Echo 1 (V248)	499	999	4.6	0	0.33	0.43	0.33	0.49	0.49	5.0
	φX174	4999	32	4.9	0.22	0.0050	0	0.14	0	0	6.7
	MS2	999	0.89	0.52	2.2	0.20	0.20	1.3	0.64	0.43	3.5 <sup>b</sup>
	<b>Average</b>	929	132	2.9	0.64	0.15	0.39	0.54	0.43	0.40	
	CB3 (Nancy)	9.0	4.9	2.7	1.3	12	24	32	32	0.54	
II	Echo 7 (Wallace)	99	2.1	0.087	0.25	49	999	199	99	0.28	
	Polio 1 (LSc)	999	16	1.3	0.72	19	999	249	99	4.6	6.6
	T2	9999	249	1.8	0.087	9.0	0.56	19	6.1	2.3	4.2
	T4	332	332	6.1	0	49	2.6	124	332	16	4-5
	<b>Average</b>	2288	121	2.4	0.47	28	405	125	114	4.7	
	f2	99	0.064	0	0	0.35	0	0	0.19	0.0030	
III	<b>Soil Characteristics</b>										
	$f_{oc}$ %	0.3	1.4	0.4	3.6	4.2	0.88	0.78	1.7	0.27	
	Clay (%)	39	53	4	3	54	3	36	28	13	
	Silt (%)	13	16	4	8	20	8	24	13	10	
	Sand (%)	48	13	92	89	26	89	40	59	77	

<sup>a</sup> pI-values taken from Gerba (1984).

<sup>b</sup> pI for MS2 from Penrod et al. (1995).

Note: Data derived from Goyal and Gerba (1979).

was not found in column experiments. An explanation for this difference may be that echoviruses attach much slower than poliovirus 1, and thus may not have reached equilibrium after 30 min in the batch tests, whereas in the columns these viruses had sufficient time to attach to a similar extent. The following analysis from a field study by Schijven et al. (1999) also illustrates that a much longer time may be needed to reach equilibrium in a batch experiment. In this study, a value for  $k_{att}/k_{det}$  of about 5000 was found (see also Table 8). Using Equation 14 and the values from this field study, it can be shown that apparent equilibrium would be reached after 40 h in a batch experiment. Now, if one stops this batch experiment after, for instance, only 1 h, a ratio  $k_{att}/k_{det}$  of only 0.18 would be found. Thus, it is believed that in many cases, batch experiments may have been stopped far too soon, thereby causing a strong underestimation of  $k_{att}/k_{det}$ . Also, this would mean that a low value of  $k_{att}/k_{det}$  that is found in a batch experiment after a short period of time is rather a consequence of slow adsorption than of equilibrium.

Nevertheless, it may not always be the case that  $k_{att}$  in batch experiments is larger than in column experiments. An example is reported in Jin et al. (1997). They stopped a batch experiment of  $\phi$ X174 and sand after 3 h and estimated a retardation coefficient  $R$  of 4.5. This means they assumed equilibrium adsorption was reached. Then, based on this value, they predicted a retarded breakthrough curve for their column experiments. However, breakthrough of  $\phi$ X174 was not found to be retarded in any of the column experiments ( $R = 1$ ), implying that the removal process was not due to equilibrium adsorption. As already pointed out in the previous section, measurements in the column experiment were stopped before the end of the plateau of the breakthrough curve was reached. Detachment in the column experiments therefore was not investigated. Thus, the authors interpreted the removal process as a first-order irreversible attachment in both column and batch experiments. This interpretation then indicated that  $k_{att}$  of  $\phi$ X174 obtained from the column experiment was found to be about twice as high as that from the batch experiment. At this point there is no clear explanation for these observations.

## D. Colloid Filtration

Analogous to attachment of viruses to solid surfaces in a batch system, attachment of viruses in flowing water to the surfaces of solid particles in a porous medium involves two processes: mass transport to the surface, and virus-surface interactions (see Section III.A). Therefore, the attachment rate coefficient  $k_{att}$  depends on microscale flow and diffusion characteristics as well as surface properties of viruses and soil grains. These processes are also described by colloid filtration theory, which allows exclusion of the effects of flow and diffusion by expressing the attachment rate of viruses in terms of collision efficiency  $\eta$  and sticking efficiency  $\alpha$ . According to this theory, a suspended particle may come into contact with a particle of the solid medium, the collector, either by interception,

sedimentation, or diffusion (Yao et al., 1971). The attachment rate coefficient is related to the collision efficiency  $\eta$  and the sticking efficiency  $\alpha$  as follows (Yao et al., 1971):

$$k_{att} = \frac{3(1-n)}{2d_c} \alpha \eta v \quad (18)$$

Here,  $d_c$  is the average diameter of collision (grain size), [L]. The fraction of particles that collide with the collector is given by  $\eta$ , the collision efficiency. Viruses can be regarded as colloidal particles because of their size and surface charge. Viruses are small in size and their transport in the immediate vicinity of the collector surface is dominated by Brownian diffusion, whereas effects of interception and gravitation are negligible. In this case the collision efficiency is given by the Smoluchowski-Levich approximation (Penrod et al., 1996):

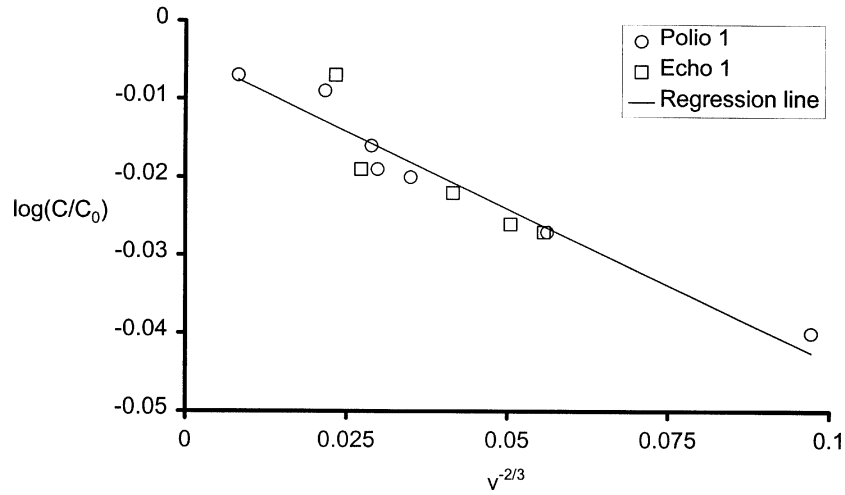
$$\eta = 4A_s^{1/3} N_{pe}^{-2/3} \quad (19)$$

Here,  $N_{pe} = d_c n v / D_{BM}$ , a Péclet number, accounts for diffusion;  $D_{BM} = K_B(T + 273) / (3\pi d_p \mu)$  is the diffusion coefficient, [ $L^2 T^{-1}$ ];  $K_B = 1.38 \times 10^{-23}$  is the Boltzmann constant [J/K]; T is temperature;  $d_p$  is the virus particle size;  $\mu$  is the dynamic viscosity [ $ML^{-1}T^{-1}$ ];  $A_s = 2(1 - \gamma^5) / (2 - 3\gamma + 3\gamma^5 - 2\gamma^6)$  is Happel's porosity dependent parameter, with  $\gamma = (1 - n)^{1/3}$ .

Funderburg et al. (1981), Wang et al. (1981), and Lance et al. (1982) found that virus removal in columns was inversely related to the flow velocity. Data on removal of poliovirus 1 and echovirus 1 in the upper 17 cm of the columns in the study by Wang et al. (1981) offer the possibility to investigate the relation between virus removal and flow rate. In a column with characteristic length L, assuming steady-state conditions, and neglecting dispersion, virus inactivation, and detachment, the solution of Equation 1 is:

$$\log\left(\frac{C}{C_0}\right) = -\frac{k_{att}}{v} \frac{L}{2.3} = -\frac{\Psi}{2.3} v^{-2/3} \quad \text{where} \quad \Psi = \alpha L \frac{3(1-n)}{2d_c} 4A_s^{1/3} \left(\frac{D_{BM}}{d_c n}\right)^{2/3} \quad (20)$$

Thus, colloid filtration theory predicts that virus removal,  $\log(C/C_0)$ , is proportional to  $v^{-2/3}$  (Yao et al., 1971). In the study by Wang et al. (1981), it was found that poliovirus 1 and echovirus 1 were removed to a similar extent at different flow rates. Figure 5 shows removal of both viruses vs.  $v^{-2/3}$ . This relation appears to be linear with a correlation coefficient of 90%. Also, Jin et al. (1997) observed less removal of  $\phi X174$  when flow velocity was increased. The ratio of virus removal



**FIGURE 5.** Removal,  $\log(C/C_0)$  of poliovirus 1 and echovirus 1 vs.  $v^{-2/3}$  (data from Wang et al., 1981).

at the different flow velocities was approximately equal to the ratio of the two flow velocities to the power of  $-2/3$ . This suggests that the relation between virus removal and flow velocity, as described by colloid filtration theory, is valid.

The sticking efficiency,  $\alpha$ , represents the fraction of the particles colliding with the solid grains that remain attached to the collector (Martin et al., 1992). The sticking efficiency reflects the net effect of repulsive and attractive forces between the surfaces of the particles and the collector and depends on the surface characteristics of the virus and soil particles. Therefore, it depends on pH, organic carbon content, and ionic strength. It is believed that  $\alpha$  is independent of hydrodynamic effects (velocity and dispersion). So, if for a given set of conditions such as type of virus, type of soil, pH, organic carbon content, and ionic strength  $\alpha$  is known, it is possible to calculate the value of  $k_{att}$  for a different set of physical conditions using Equation 18. This emphasizes the importance of knowing the value of  $\alpha$  under a given set of conditions.

Commonly,  $\alpha$  is derived from experimental values of  $k_{att}$  and calculated values of the collision efficiency  $\eta$  using Equation 18 (Bales et al., 1991, 1993; Kinoshita et al., 1993; Penrod et al., 1996; Redman et al., 1997; Schijven et al., 1998, 1999). Alternatively,  $\alpha$  has been estimated from relative breakthrough (RB) of the mass of viruses relative to that of a conservative salt tracer (Pieper et al., 1997; DeBorde et al., 1999; Ryan et al., 1999) and assuming irreversible adsorption using the following equations:

$$\alpha = \frac{d_c \left[ \left[ 1 - 2(\alpha_L / x) \ln(RB) \right]^2 - 1 \right]}{6(1-n)\eta\alpha_L} \quad \text{where} \quad RB = \left[ \int_{t_0}^{t_f} \frac{C_{virus}}{C_{0,virus}} dt \bigg/ \int_{t_0}^{t_f} \frac{C_{salt}}{C_{0,salt}} dt \right] \quad (21)$$

The sticking efficiency can also be calculated according to the so-called Interaction Force Boundary Layer (IFBL) approximation (Swanton, 1995; Ryan and Elimelech, 1996):

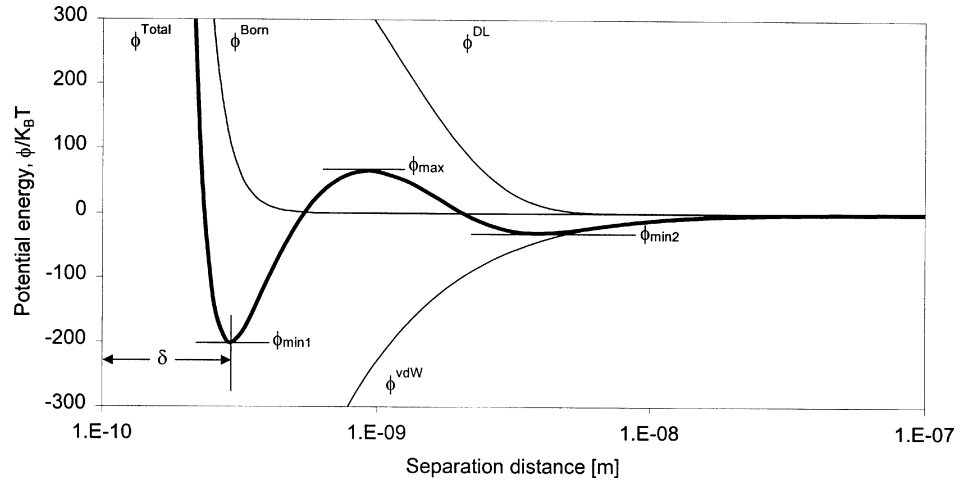
$$\alpha = \left( \frac{\beta}{1+\beta} \right) S(\beta) \quad \text{where} \quad \beta = \frac{1}{3} (2)^{-1/3} \Gamma \left( \frac{1}{3} \right) A_s^{-1/3} \left( \frac{D_{BM}}{U d_c} \right)^{1/3} \left( \frac{k_F d_c}{D_{BM}} \right) \quad (22)$$

Here,  $k_F$  is a pseudo-first-order coefficient that accounts for the retarding effect of double layer repulsion on the attachment rate, [L<sup>-2</sup>T].  $S(\beta)$  is a slowly varying function of  $\beta$  with tabulated numerical values, and  $U$  is the superficial flow velocity, [LT<sup>-1</sup>] (Ryan and Elimelech, 1996). As described in the next section, theoretical values of the sticking efficiency, and thus also of the attachment rate coefficient, considerably underestimate experimental values.

## E. Derjaguin-Landau-Verwey-Overbeek Theory

The attractive and repulsive forces that exist between colloids (e.g., viruses) and grain surfaces determine the interactions between them. These forces can be described in profiles of the intersurface potential energy by DLVO theory. Such profiles are constructed by summing the potential energies of double-layer repulsion or attraction, London–van der Waals attraction, and poorly characterized short-range “non-DLVO” forces such as hydration and steric repulsion (Ryan and Elimelech, 1996). An example of a DLVO-energy profile is shown in Figure 6. The total potential energy curve is characterized by an attractive energy well at a very small separation distance ( $\delta$ ), with the primary minimum ( $\phi_{min1}$ ), a repulsive energy barrier ( $\phi_{max}$ ), and a shallow attractive energy well at a larger separation distance, with the secondary minimum ( $\phi_{min2}$ ).

The van der Waals attraction is an electrical force between instantaneous dipole moments within the different molecules (Gerba, 1984). It is linearly dependent on the value of the Hamaker constant. The Hamaker constant depends on the nature of the interacting materials (Swanton, 1995). The Hamaker constants of most forms of organic matter are similar to those of water, hence the van der Waals interactions between organic colloids are weak. Inorganic matter tends to have large Hamaker constants and thus a stronger inherent attraction for virus (Moore et al., 1981).



**FIGURE 6.** DLVO energy as a function of separation distance between a colloid and a collector. The total potential energy ( $\phi^{\text{Total}}$ ) is the sum of the double layer potential energy ( $\phi^{\text{DL}}$ ), the van der Waals potential energy ( $\phi^{\text{vdW}}$ ), and the Born potential energy ( $\phi^{\text{Born}}$ ). The total potential energy curve is characterized by an attractive well at a very small separation distance ( $\phi$ ), the primary minimum ( $\phi_{\text{min1}}$ ), a repulsive energy barrier ( $\phi_{\text{max}}$ ), and a shallow attractive well at a larger separation distance ( $\phi_{\text{min2}}$ ). The potential energy is normalized by  $k_B T$  (Ryan and Elimelech, 1996).

A double-layer potential energy arises from the overlap of diffuse clouds of ions (double layers) that accumulate near charged surfaces to balance the surface charge. If the interacting surfaces are like-charged, the double-layer potential energy will be repulsive (Ryan and Elimelech, 1996). If the surfaces are oppositely charged, the double-layer potential energy will be attractive. In formulations of the double layer theory, potential energy is considered to be sensitive to variations in the surface potentials of the colloid and the collector, ionic strength of the solution, and colloid size. The dependence on colloid size has not been verified (Ryan and Elimelech, 1996).

It has been shown that the rate coefficients for attachment ( $k_{\text{att}}$ ) and detachment ( $k_{\text{det}}$ ) should increase exponentially as the height of the corresponding energy barriers increases (Ryan and Elimelech, 1996):

$$k_{\text{att}} \propto \exp\left(-\frac{|\phi_{\text{max}}|}{k_B T}\right) \quad (23)$$

$$k_{\text{det}} \propto \exp\left(-\frac{|\phi_{\text{max}} - \phi_{\text{min1}}|}{k_B T}\right) \quad (24)$$

DLVO theory provides a conceptual framework to understand interactions of virus particles and solid surfaces under different conditions, such as pH, ionic strength, and colloid size. The effects of these conditions are discussed in more detail in Section IV.

DLVO theory has, however, several shortcomings in predicting attachment and detachment rates. It has generally been shown that under unfavorable conditions for attachment, DLVO theory underestimates attachment by many orders of magnitude. At pH 7 to 8, as in many aquifers, the net surface charge of most viruses and soils is negative, and thus conditions for attachment are generally unfavorable. Under such conditions, colloid–surface interactions are the rate-limiting process for attachment (Ryan and Elimelech, 1996). Experimentally determined sticking efficiencies are sensitive to the ionic strength of the solution and to the electrokinetic (zeta) potentials of particles and collectors but not to the degree predicted by theory. Experimentally determined sticking efficiencies and attachment are virtually independent of colloid particle size, in contrast with DLVO theory predictions. Also, it has been shown that the sensitivity of attachment rates to ionic strength decreases markedly as the degree of surface charge heterogeneity increases (Ryan and Elimelech, 1996). Furthermore, DLVO theory was formulated for smooth bodies with ideal geometries (e.g., spheres interacting with flat surfaces) and uniform properties. In practice, real particles are irregular, and the surface of the bodies are rough and are likely to be heterogeneous in composition and charge (Swanton, 1995).

Under favorable conditions (i.e., in the absence of repulsive energy barriers or in the presence of attractive double-layer interactions) colloidal transport to the vicinity of the soil surfaces is the rate-limiting step. In this case, particle attachment models within the framework of the DLVO theory are satisfactory in predicting the effect of solution ionic strength, fluid velocity and particle size. Favorable chemical conditions for attachment may develop in groundwaters with high levels of water hardness and ionic strength. Attachment will also be favorable for solid surfaces (or patches on solid surfaces) that are positively charged due to iron, aluminium, or manganese oxide coatings (Ryan and Elimelech, 1996). Because of the electrostatic attractive forces, it is reasonable to assume that attachment to such favorable patches is irreversible (Ryan and Elimelech, 1996).

Bales et al. (1991) suggested that attachment to kinetically limited sites would occur in the primary minimum, and to fast equilibrium sites in the secondary minimum. However, as was shown in their and other studies (Bales et al., 1993, 1997; Kinoshita et al., 1993; Schijven et al., 1999), kinetically limited attachment prevails. Loveland et al. (1996) calculated DLVO profiles for PRD1 and quartz with or without ferric oxyhydroxide coatings and showed that secondary minima were extremely small. So, probably, attachment of a virus in the secondary minimum of a site is not significant. Loveland et al. (1996) suggested that a secondary minimum does need to be deep to affect virus transport. Under strongly repulsive conditions, viruses will not attach in the primary minimum. Instead, viruses may



accumulate in the boundary layer in front of the energy barrier. In time, diffusion and advection will carry them out of the boundary layer and back to the advective flow. Regardless of the depth of the secondary minimum, the transport of these viruses has been retarded by reversible attachment.

## F. Hydrophobic Interactions

As reviewed by Gerba (1984), hydrophobic interactions between viruses and solid surfaces may also contribute significantly to adsorption. Hydrophobic interactions may be seen as a consequence of the thermodynamically unfavorable interaction of hydrophobic substances with water molecules and is not due to interactions among hydrophobic particles themselves (Wait and Sobsey, 1983). Hydrophobic interactions are not described by DLVO theory (Swanton, 1995). Interactions between hydrophobic groups on the surfaces of the virus and the solid may cause an increase in virus attachment. At high pH, when both the surfaces are negatively charged, hydrophobic interactions may be the major factor maintaining virus attachment (Shields and Farrah, 1983; Gerba, 1984; Bales et al., 1991). Bales et al. (1991) showed that MS2 attached much less to silica beads in a batch experiment at pH 7 than at pH 5, but when the silica beads were coated with C<sub>18</sub>-trichlorosilane, 400 times more attachment took place, independent of pH. Bales et al. (1991) concluded that hydrophobic effects are important for adsorption of even relatively hydrophilic viruses. Loveland et al. (1996) argued that the addition of hydrocarbon chains to a silica surface decreases the negative surface charge of the silica surface in addition to providing hydrophobic attachment sites for viruses. According to Loveland et al. (1996), increased virus attachment may, therefore, be more reasonably attributed to a decreased double-layer repulsion rather than to hydrophobic expulsion from solution.

Hydrophobic interactions of viruses with solid surfaces have been suggested to play a role in the interaction between poliovirus 1 and millipore and zeta plus filters (Farrah et al., 1981), and also, among poliovirus 1, echovirus 1, and rotavirus SA 11 and highly organic estuarine sediments (Wait and Sobsey, 1983) and between poliovirus 1 and nitrocellulose membrane filters (Shields and Farrah, 1983). Bales et al. (1991, 1993) suggested hydrophobic interactions of MS2 and PRD1 with octadecyltrichlorosilane coated silica. It was also suggested for MS2,  $\phi$ X174, T7, PRD1, and  $\phi$ 6 with nitrocellulose and cationic polysulfone membranes (Lytle and Routson, 1995).

## G. Blocking

When particles attach to a solid surface, the attachment rate may decrease with time if particle–particle interactions are repulsive; the solid surface becomes

progressively occluded as particles accumulate. This surface exclusion phenomenon is termed blocking. Ryan and Elimelech (1996) have reviewed the blocking process of colloids and how it is modeled. Blocking may prevail in groundwater having low ionic strength and low levels of hardness. Because the majority of the available surface area of solids in groundwater has chemical characteristics unfavorable for particle deposition, colloidal attachment in groundwater is thought to be largely restricted to a minor patch-wise distributed fraction having energetically favorable charge characteristics (Ryan and Elimelech, 1996). The same restrictions apply to attachment of viruses. Jin et al. (1997) observed blocking of  $\phi$ X174 in a sand column. Initially, significant removal of  $\phi$ X174 took place, but with increasing amounts of attached phages, the attachment rate dropped and an increase of  $C/C_0$  up to one was observed. However, under field conditions, virus concentrations in recharging water are so low that blocking of binding sites with viruses is not likely to occur. In case of contamination of an aquifer with wastewater, viruses represent only a very minor fraction of the wastewater organic matter. In that case, organic matter will probably block binding sites and a progressive blocking effect of viruses will not be the case.

#### **IV. FACTORS AFFECTING ADSORPTION OF VIRUSES TO SOIL**

##### **A. Introduction**

The interactions that take place between viruses and soil particles are determined by their surface characteristics. Virus–soil interactions are electrostatic and hydrophobic in nature. Surface characteristics may be altered by changes in pH, ionic strength, multivalent ions, and organic matter (Gerba, 1984). Changes in these interactions are quantified by changes in attachment and detachment rate coefficients. As we have seen in Section III.E, DLVO theory provides a conceptual framework to understand many of these changes. In the following sections, the effect of virus and soil type, pH, ionic strength, multivalent cations, and organic matter on attachment and detachment of viruses to solid surfaces is discussed in more detail.

##### **B. Effect of Virus Type**

The behavior of different viruses in their interactions with solids is believed to be the result of differences in the electrical charge and the hydrophobicity of the virus surface (Shields and Farrah, 1987). Most viruses have a size in the range of 20 to 200 nm and consist of nucleic acid encapsulated in a protein capsid. The amino acids in this protein coat contain weakly acidic and basic groups, like

carboxyl and amino groups, that determine the amphoteric nature of the virus particle. The isoelectric point (pI) of a virus particle is the pH value at which it has a zero net charge. However, localized pockets of positive and negative charges may still exist across the virus surface. The isoelectric point of a virus may vary not only by the type of the virus but also by the strain (Gerba, 1984). At pH values above 5, the electrophoretic mobility of MS2, a measure of its surface charge, remains constant (Penrod et al., 1995). At pH values above 6, electrophoretic mobilities of vaccinia virus, reovirus and  $\lambda$  are also relatively insensitive to changes in pH (Penrod et al., 1995). At higher pHs, PRD1 (Loveland et al., 1996) and recombinant Norwalk-like virus particles (Redman et al., 1997), however, show a further decrease in their negative charge.

The coat proteins of a virus may contain spans of amino acids that are hydrophobic. Dependent on the way these proteins are folded, such hydrophobic parts may either be on the inside or the outside of the virus coat (Gerba, 1984). Viruses differ in hydrophobicity, as was shown by Shields and Farrah (1987), who determined the hydrophobic nature of 15 viruses by means of octyl-sepharose chromatography. Echovirus 5 and MS2 were found to be the most hydrophobic of the viruses tested, whereas echovirus 7 and  $\phi$ X174 were found to exhibit little if any hydrophobic character. According to Lytle and Routson (1995) in a study on the retention of viruses by nitrocellulose and cationic polysulfone membranes,  $\phi$ X174 was the least hydrophobic of the viruses studied, but hydrophobicity of MS2, T7, PRD1, and  $\phi$ 6 was not much different. A detailed description of the coat proteins of MS2, including its hydrophobic outer regions, is presented by Penrod et al. (1996).

Adsorption behavior of viruses is very complex, and even under relatively well-defined conditions of batch experiments a nonunique behavior is observed. Several studies (Burge and Enkiri, 1978; Goyal and Gerba, 1979; Singh et al., 1986) have clearly shown that most viruses, even strains of the same type, may behave differently under similar conditions. Goyal and Gerba (1979) compared attachment of a large number of different types and strains of human enteroviruses, bacteriophages, and a simian rotavirus to nine different soil types. From these data, it was possible to distinguish two groups of viruses according to their mean percentage of adsorption at equilibrium (Gerba et al., 1981). It was remarked that this grouping depended on the choice of viruses and soils that were studied. Viruses of group II adsorb very well to most soils, sludges, and marine sediments, whereas those of group I adsorb to a lesser degree. Mean percentage of adsorption of viruses of group I was 44% and that of group II 78%. Bacteriophage f2 is probably a special case because its mean adsorption of 16% was significantly lower than that of all the other viruses. Table 2 summarizes the batch experiments of Goyal and Gerba (1979). Recall that instead of giving percentages of adsorption at equilibrium, the ratio  $k_{att}/k_{det}$  is calculated using Equation 17. Adsorption of group I viruses is most affected by pH, exchangeable iron content and organic

carbon content of the soil. Adsorption is inversely correlated with pH, which explained 83% of the variation in the mean attachment. Adsorption of group I viruses is also inversely correlated with the organic matter content of the soil, but positively correlated with exchangeable iron. Adsorption of group II viruses was not found to be significantly affected over the range of soil characteristics studied here. Gerba et al. (1981) suggested that the net charge of group I viruses was influenced more over the range of pH values and organic matter concentrations found in nature than that of group II viruses.

Several studies indicate that knowledge of the pI of a virus makes it possible to predict the likelihood of its attachment to a charged surface (Gerba, 1984). Also, Gerba (1984) suggested that group I viruses might have lower isoelectric points (pI) than group II viruses. However, the data in Table 2 are not in agreement with that suggestion. Dowd et al. (1998) observed a linear relationship between the amount of adsorption to soil and the isoelectric point of five different phages, MS2, PRD1, Q $\beta$ ,  $\phi$ X174 and PM2 in circulating flow columns (i.e., in these columns the outflow is connected to the inflow). The amount of adsorption was the least for the phages with the higher pI. This is contradictory to the expectation that a virus with higher pI is less electronegatively charged and thus adsorbs better because of weaker repulsion. Besides, in flow-through columns this relation with pI was not found. Possibly, the presence of multivalent cations was the cause that more negatively charged viruses attach more.

The isoelectric point is only an indication of what the net surface charge of a virus may be at a given pH. Penrod et al. (1996) and Redman et al. (1997) showed that  $\lambda$  and Norwalk virus-like particles have a stronger negative zeta-potential than MS2 at pH 5 to 7, although MS2 has a lower pI than the other two. Penrod et al. (1996) found strong evidence that the surface charge of MS2 originates from the charge of the amino acid residues that are present on the exterior of the virus particle, by calculating pI of MS2 correctly from the ionization of these exteriorly located amino acid residues. They also suggested that the attachment rate of MS2 is not only determined by electrostatic repulsion, but also by steric repulsion of hydrophilic loops that protrude from the surface of MS2. In this study, it was also shown that the attachment rate of phage  $\lambda$  was determined primarily by the surface charge on the capsid of this virus, but the tail of  $\lambda$  contributes relatively little to the overall charge of the virus.

Due to the differences in electrical charge and hydrophobicity that exist between different types of viruses, and even between different strains of the same virus type, virus-soil interactions will differ, and thus virus removal by soil passage will be virus-type dependent. Therefore, to predict removal of viruses by soil passage, a combination of viruses may be considered that represent a range of adsorption characteristics. Alternatively, a virus that adsorbs less than other viruses under certain conditions, may be considered as a worst-case model virus. Viruses with a strong negative surface charge and little hydrophobicity meet these requirements.

### C. Effect of Soil Type

Ryan and Elimelech (1996) described that most of the surface area of soil grains has chemical characteristics unfavorable for particle deposition. Therefore, colloidal attachment in groundwater is thought to be largely restricted to a minor fraction of the grain surface having energetically favorable charge characteristics. These favorable sites, resulting from surface charge heterogeneity, are often manifested as positively charged patches on the surface of negatively charged mineral grains. Such patch-wise charge heterogeneities are general to all aqueous geologic settings, originating from inherent differences in the surface properties of adjacent crystal faces on mineral grains, and from minerals having bulk- or surface-bound chemical impurities. Oxides of iron, aluminium, and manganese are the most common sources of surface charge heterogeneity in the groundwater environment. These oxides carry a positive charge at near-neutral pH and are generally present in minor amounts as surface coatings on mineral grains. It has been shown that minor degrees of charge heterogeneity on collector surfaces result in attachment rates that are orders of magnitude larger than similar surfaces having no charge heterogeneity.

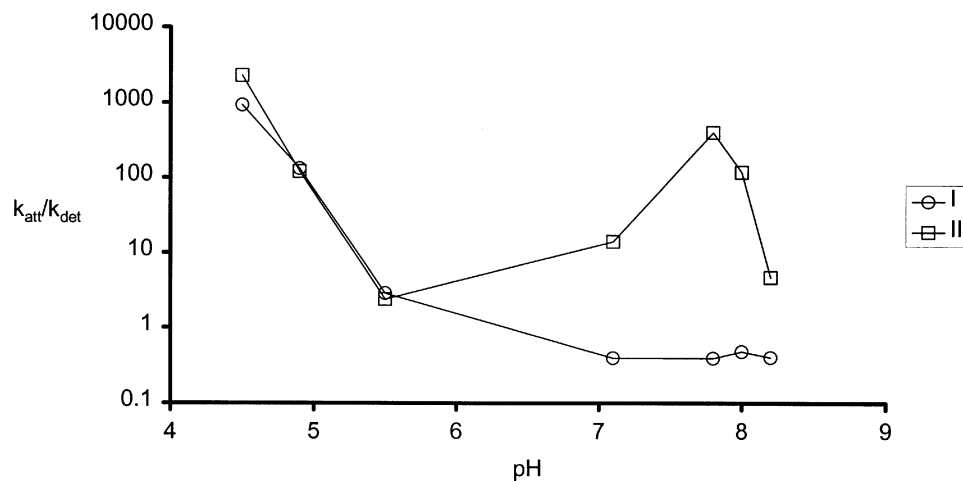
Several studies appear to support this concept that viruses preferably attach to a surface fraction of the soil having favorable charge characteristics. Solids that have high isoelectric points are better virus adsorbents than those with low isoelectric points (Gerba, 1984). Also, favorable for attachment of viruses are a higher cation exchange capacity (Burge and Enkiri, 1978), exchangeable iron (Gerba et al., 1981), and iron oxides (Moore et al., 1981; Lipson and Stotzky, 1983; Grant et al., 1993; Loveland et al., 1996). Attachment of viruses has been shown to be better to soils with higher specific surface areas (Moore et al., 1982). Generally, granular soils are weaker adsorbents than clays and minerals (Sobsey et al., 1980; Moore et al., 1981). Clays may have surfaces that have a very heterogeneous charge distribution. Vilker et al. (1983) suggested that poliovirus 1 adsorbed to the edges of montmorillonite particles, where regions of positive charges are located due to the presence of aluminium ions. Farrah and Preston (1993) modified sand by precipitation of metallic salts. Columns of this modified sand removed significantly more viruses (poliovirus 1, coxsackievirus B5, echovirus 5, and MS2) than columns of unmodified sand. In batch experiments, Moore et al. (1981) showed an apparent surface saturation of poliovirus 2 (about 30 nm in size) to Ottawa sand at pH 7.5 of  $2.5 \times 10^{12}$  virus particles/kg. It was thus concluded that granular materials like Ottawa sand (surface area 0.018 m<sup>2</sup>/g) have an enormous capacity for viruses and are unlikely to become saturated in natural systems. Nevertheless, at this maximum concentration of attached virus particles, only approximately 1% of the total sand surface was covered. Jin et al. (1997) demonstrated blocking of  $\phi$ X174 in columns with Ottawa sand. Ottawa sand contains detectable levels of iron, probably in the form of goethite. By calculation of the surface area that is occupied by a monolayer of  $\phi$ X174 particles, they also showed that the apparent saturation

of the sorption sites was far less than the total surface area of the sand. Therefore, they suggested that the active surface for virus attachment was limited to patches of positive charges on the sand particles formed by goethite.

#### D. Effect of pH

In many batch studies, it has been shown that, generally, viruses attach to a lesser extent at higher pH (Burge and Enkiri, 1978; Goyal and Gerba, 1979; Sobsey et al., 1980; Taylor et al., 1980, 1981; Gerba et al., 1981; Grant et al., 1993). According to DVLO theory, this can be explained by an increased electrostatic repulsion at higher pH. Figure 7 shows a plot of the average values of the ratio  $k_{att}/k_{det}$  vs. pH for viruses of group I and II from the study of Goyal and Gerba (1979). In the range of pH 4.5 to 5.5 there is little difference between viruses of group I and II. Goyal and Gerba remarked that the naturally isolated viruses used in their study were all isolated from field samples by adsorption to membranes at low pH. Thus, viruses that did not have this characteristic would not have been isolated. Within the pH range of 4.5 to 5.5, the ratio  $k_{att}/k_{det}$  declines with increasing pH. The ratio for group I viruses continues to decrease, but at a lower rate, as pH increases. It appears that the ratio  $k_{att}/k_{det}$  of group I viruses becomes less than 1 at a pH equal to or higher than their pI. However, the ratio  $k_{att}/k_{det}$  of a group II virus shows an increase for pH values of up to 8, at which point it also decreases with increasing pH (see also Table 2).

Tables 3 and 4 are an inventory of sticking efficiencies of viruses in column and field studies, at different pH values, respectively. In Figure 8, the sticking



**FIGURE 7.** Average values of ratio  $k_{att}/k_{det}$  vs. pH of viruses of group I and II to soil in a batch suspensions (Goyal and Gerba, 1979). See also values in Table 2.

**TABLE 3**  
**Sticking Efficiency  $\alpha$  for Viruses from Column Studies (6–35 cm)**

Virus	Soil	pH	NaCl [M]	$f_{oc}$	$\alpha$	Ref.
MS2	Glass beads	5.0	0.01	—	0.0015	Bales et al. (1991) <sup>a</sup>
		5.0	0.08	—	0.0015 – 0.0028	
	Silica beads	5.0	0.05	$2.00 \times 10^{-7}$	0.018	Bales et al. (1993)
		7.0	0.05	$2.00 \times 10^{-7}$	0.0022	
	Sand (Cape Cod)	5.7	0.1	$<1.0 \times 10^{-4}$	0.007	Kinoshita et al. (1993)
		7.0	0.1	$<1.0 \times 10^{-4}$	0.01	
8.2	0.1	$<1.0 \times 10^{-4}$	0 <sup>b</sup>			
PRD1	Quartz	3.5	0.01	—	0.12	Penrod et al. (1996)
		3.5	0.3	—	0.16	
	Glass beads	5.0	0.01	—	0.009	Bales et al. (1991) <sup>a</sup>
		5.0	0.1	—	0.09	Kinoshita et al. (1993)
	Sand (Borden)	5.0	0.3	—	0.04	
		5.5	0.01	—	0.0015 – 0.0033	
Polio 1	Sand (Cambridge)	6.5	0.1	$3.0 \times 10^{-4}$	0.17	Bales et al. (1991) <sup>a</sup>
		7.0	0.1	$3.0 \times 10^{-4}$	0.14	Kinoshita et al. (1993)
	Sand (Cape Cod)	7.6	0.1	$3.0 \times 10^{-4}$	0.15	
		7.0	0.1	$5.0 \times 10^{-4}$	1.11	
	Silica beads	5.7	0.1	$<1.0 \times 10^{-4}$	0.62 – $\geq 0.94$	
		7.0	0.1	$<1.0 \times 10^{-4}$	0.63 – 0.82	
8.2	0.1	$<1.0 \times 10^{-4}$	0.58			
$\lambda$	Quartz	5.5	0.05	$2.00 \times 10^{-7}$	0.014	Bales et al. (1993)
		7.0	0.05	$2.00 \times 10^{-7}$	0.0040	
	Glass beads	7.0	0.05	$2.00 \times 10^{-7}$	0.0072	
		3.9	0.01	—	1.25	Penrod et al. (1996)
	Silica beads	5.0	0.01	—	0.045	
		5.0	0.1	—	0.53	
5.0	0.3	—	0.65			

<sup>a</sup> Corrected for the factor  $A_s^{1/3}$ , which was not applied in the original publication.

<sup>b</sup> Set to 0, because no removal was found.

**TABLE 4**  
**Sticking Efficiencies  $\alpha$  for Viruses from Field Studies**

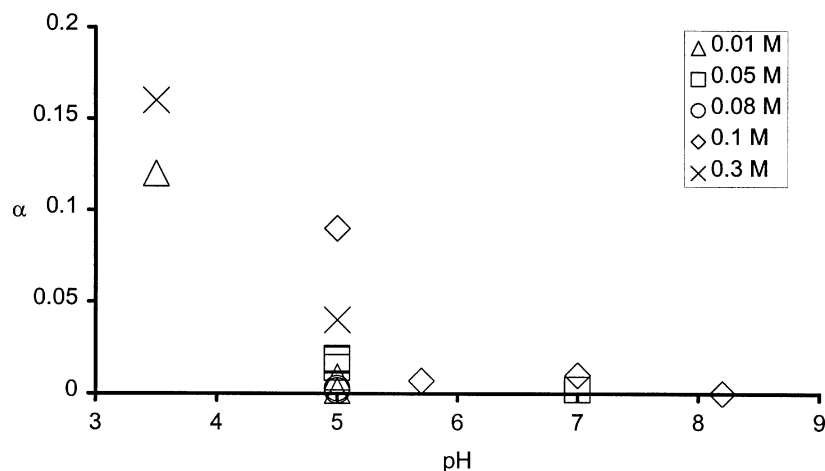
Virus	Soil	Distance [m]	pH	EC [ $\mu\text{S}/\text{cm}$ ] <sup>a</sup>	$f_{oc}$	$\alpha$	Ref.	
MS2	Sand/gravel (Missoula)	7.5	7.2	288	—	0.004 – 0.182 <sup>b</sup>	DeBorde et al. (1999)	
	Dune sand	19.4	7.2	288	—	0.004 – 0.202 <sup>b</sup>	Schijven et al. (1999)	
PRD1	Dune sand	2.4	7.3 – 8.3	900	$1.0 \times 10^{-3}$	0.0014	Bales et al. (1997)	
		30	7.3 – 8.3	900	$1.0 \times 10^{-3}$	0.00027		
	Sand (Borden)	0.94	7.4	311	$3.0 \times 10^{-4}$	0.0028 – 0.0030	Pieper et al. (1997)	
	Sand (Cape Cod)	0.94	8.4	311	$3.0 \times 10^{-4}$	0.00085 – 0.0016		
	Sand (Cape Cod)	1.0	5.0 – 5.7	60 – 100	$< 1.0 \times 10^{-4}$	0.009 – 0.013	Ryan et al. (1999)	
	Sand (Cape Cod)	1.0	6.0 – 6.7	350 – 450	$1.0 \times 10^{-2}$	0.0014 – 0.0026		
	Sand/gravel (Missoula)	0.9 – 1.0	5.4 – 5.6	30 – 40	$< 1.0 \times 10^{-4}$	0.032 $\pm$ 0.016	DeBorde et al. (1999)	
	Dune sand	0.9 – 1.0	5.8 – 6.0	250 – 330	$1.0 \times 10^{-2}$	0.016 $\pm$ 0.005		
	$\phi\text{X174}$	Sand/gravel (Missoula)	7.5	7.2	288	—	0.014 – 0.632 <sup>b</sup>	Schijven et al. (1999)
		Dune sand	19.4	7.2	288	—	0.005 – 0.385 <sup>b</sup>	
Dune sand		2.4	7.3 – 8.3	900	$1.0 \times 10^{-3}$	0.0024		
Polio 1 (CHAT)	Sand/gravel (Missoula)	30	7.3 – 8.3	900	$1.0 \times 10^{-3}$	0.00043	DeBorde et al. (1999)	
	Dune sand	7.5	7.2	288	—	0.006 – 0.311 <sup>b</sup>		
FRNAPH's	Sand/gravel (Missoula)	19.4	7.2	288	—	0.007 – 0.319 <sup>b</sup>	DeBorde et al. (1999)	
	Dune sand	7.5	7.2	288	—	0.047 – 2.108 <sup>b</sup>		
FRNAPH's	Sand/gravel (Missoula)	19.4	7.2	288	—	0.019 – 0.866 <sup>b</sup>	Schijven et al. (1998)	
	Dune sand	2	7.3 – 8.3	900	—	0.0020		
FRNAPH's	Dune sand	4	7.3 – 8.3	900	—	(0.0015 – 0.0029) <sup>c</sup>	Schijven et al. (1998)	
		4	7.3 – 8.3	900	—	0.00078		
FRNAPH's	Dune sand	4	7.3 – 8.3	900	—	(0.000040 – 0.0040) <sup>c</sup>	Schijven et al. (1998)	
		4	7.3 – 8.3	900	—	0.00078		

<sup>a</sup> Corresponding to a range in grain size from 0.00125 – 0.012 m.

<sup>b</sup> Electrical conductivity as a measure of ionic strength.

<sup>c</sup> Ninety-five percent confidence interval.





**FIGURE 8.** Sticking efficiency  $\alpha$  vs. pH of MS2 at different ionic strengths. See also values in Table 3.

efficiency of MS2 is plotted vs. pH within the range of 4 to 9 and at different ionic strengths, using the data from Table 3. The pI of MS2 is 3.5; it is therefore negatively charged within this pH range. The different column studies appear to show a coherent pattern. The value of  $\alpha$  decreases with increasing pH, but reaches a minimum at a pH of about 5.5; then at higher pH,  $\alpha$  remains constant. A similar pattern of electrophoretic mobility of MS2 vs. pH has been shown by Penrod et al. (1995), strongly indicating that surface charge of the virus particles as a function of pH determines attachment. This pattern also strongly resembles that of the ratio  $k_{att}/k_{det}$  of group I viruses with pH (Figure 7). It also resembles that of the zeta potential of quartz as a function of pH (Loveland et al., 1996). The negative charge of quartz decreases from pH 2.3 to 6, and remains minimal at pH values above 6.

MS2 did not adsorb or only poorly adsorbed in columns with sandy soils at pH 5 to 8 (Bales et al., 1991, 1993; Kinoshita et al., 1993). Detachment was slow, but was enhanced when pH increased. This effect was moderate, because few MS2 particles had attached (Kinoshita et al., 1993). Bales et al. (1993) showed that removal of MS2 by attachment to silica beads in columns was much greater at pH 5 than at pH 7. Also, poliovirus 1 attached much more at pH 5.5 than at pH 7. Both viruses detached only slowly. At pH 7, the sticking efficiency of poliovirus 1 was 2 to 3 times higher than that of MS2, but almost the same at pH 5 to 5.5. Penrod et al. (1996) compared the removal of bacteriophages MS2 and  $\lambda$ . Although the net surface charge of  $\lambda$  was stronger negative than that of MS2, attachment of  $\lambda$  was more than that of MS2. It was suggested by Penrod et al. (1996) that a steric repulsive force exists in the case of MS2. This steric repulsive force arises from the hydrophilic polypeptide loops, which extend a maximum of 1 nm off of the MS2

surface. A similar difference in attachment between MS2 and recombinant Norwalk virus particles in quartz bed columns was found by Redman et al. (1997). MS2 has a significantly lower pI but a less negative zeta potential at neutral pH and higher than the Norwalk virus particles. Still, MS2 attached less than the Norwalk virus particles at both pHs 5 and 7.

The sticking efficiency of PRD1 has also been shown to be pH dependent. In columns with silica beads at pH 5.5 (Bales et al., 1991),  $\alpha$  for PRD1 was between 0.006 and 0.013, and at pH 7, PRD1 did not adsorb at all (Table 3). Detachment was very slow compared with attachment, but increased strongly at pH 8. In the same study, MS2 attached even less than PRD1. In a sandy aquifer under natural gradient conditions, attached PRD1-phages released rapidly when pH was increased from 5.7 to pH 6 to 8 (Bales et al., 1995). Also, injection with high-pH-water in a sandy aquifer, which elevated pH from 7.4 to 8.4, resulted in significant remobilization of attached PRD1-phages (Bales et al., 1997). At pH 8.4, attachment rates decreased by a factor of 2 to 3. Concurrently, the detachment rate coefficient increased by a factor  $10^4$  to  $10^5$  to a value higher than the attachment rate coefficient. In a field study by Ryan et al. (1999), it was also shown that an increased pH induced extra detachment of PRD1. This effect was less in a sewage-contaminated zone, where a pH of 8.5 was reached, than in an uncontaminated zone, where a pH of 10 was reached. According to Ryan et al. (1999) an increase to pH 8.5 may not exceed the isoelectric point of some ferric oxyhydroxides. It is likely that in the study by Bales et al. (1997) phosphate adsorbed to the ferric oxyhydroxides thereby augmenting the charge reversal caused by the pH increase.

Also, Loveland et al. (1996) clearly showed that at higher pH, attachment of PRD1 is slower and detachment is faster. In this study, in equilibrium nonflowing columns, PRD1 was attached to quartz and ferric oxyhydroxyde-coated quartz surfaces over a wide range of pH values. At a pH value of about 2.5 to 3.5 pH units above the isoelectric point of the mineral surfaces, a so-called attachment edge existed. Viruses attached at a pH below this edge were irreversibly attached but were reversibly attached at a pH above this edge. DLVO potential energy calculations showed that the attachment edge occurred at the pH at which the potential energy of the primary minimum was near zero, implying that the position of the primary minimum (attractive or repulsive) controlled the equilibrium distribution of the viruses. Virus attachment to a homogeneous mineral surface is either irreversible or reversible, depending on the pH of attachment, but not both (Loveland et al., 1996). However, in a heterogeneous medium, minerals may exist to which attachment is irreversible (e.g., ferric oxyhydroxide, and minerals to which attachment is reversible such as quartz). This is the case when pH lies between that of the isoelectric points of the two types of sites (Loveland et al., 1996).

From batch studies it has become clear that high pH generally favors free virus and low pH favors attached virus (Gerba, 1984). This has been confirmed in column and field studies for viruses like MS2 and PRD1 that have low pIs, but also for poliovirus 1 (pI of 6.6), and phage  $\lambda$  (pI of 5). At higher pH, electrostatic

repulsion increases, reflected in a higher maximum and a higher primary minimum in the DVLO profile. This results in a decreased attachment rate and an increased detachment rate. In most aquifers, surface characteristics of the soil are much more heterogeneous than that of the quartz sand in the columns of Loveland et al. (1996), and also different viruses may be present that have different pIs. Therefore, depending on pH and thus on the charge of the virus and soil particles, adsorption of some of these viruses may be irreversible, whereas that of others may be reversible.

### E. Effect of Ionic Strength and Multivalent Cations

According to DLVO theory, a higher ionic strength compresses double layers, thereby increasing attachment rates. In batch studies, it was shown that viruses tend to adsorb strongly to various materials at high ionic strength (Lipson and Stotzky, 1983; Grant et al., 1993). In a column study by Penrod et al. (1996), it was found that the sticking efficiencies of bacteriophages MS2 and  $\lambda$  increase with ionic strength in the range of 0.01 to 0.1 M NaCl at pH 5 (Table 3). A similar increase of  $\alpha$  for MS2 is the case at pH 3.5 (see also Figure 8). A further increase of salt concentration to 0.3 M NaCl has little impact on the sticking efficiency of phage  $\lambda$ , but causes a decrease in the value of  $\alpha$  for MS2. The findings of Dowd et al. (1998) suggest that more negatively charged viruses attach more than less negatively charged viruses in the case of high concentrations of multivalent cations. Redman et al. (1999) studied adsorption of a filamentous bacteriophage, SJC3, in quartz columns at different ionic strengths and cation valence. SJC3 is a bacteriophage that has been isolated from highly treated wastewater. Experiments were carried out at pH 7, where surface charge of the bacteriophage and the quartz are negative. SJC3 attached more to the quartz when the ionic strength was increased by increasing the concentration of a particular salt from 1 to 100 mM. One exception was observed when using 1 mM NaCl. Contrary to the expectation, removal of the bacteriophage was higher in this case. The authors suggested that at this low concentration of NaCl the filamentous bacteriophage stiffened. Due to this stiffness, SJC3 may be retained in the column by straining.

If DLVO profiles are repulsive at all separation distances, DLVO theory predicts increased detachment rates at lower ionic strength (Ryan and Elimelech, 1996). In agreement with this prediction, Gerba and Lance (1978) found that detachment of poliovirus 1 was enhanced by deionized water that was applied to simulate heavy rainfall. Redman et al. (1999) also showed that attached particles of bacteriophage SJC3 easily detach when the salt concentration was changed from 1 mM  $\text{CaCl}_2$  to 1 mM NaCl. However, if the DVLO profile is attractive near the solid surface, DLVO theory will predict an increasing detachment rate at higher ionic strength (Ryan and Elimelech, 1996). Penrod et al. (1996) found that with increasing ionic strength there was a similar increase of both the attachment and

detachment rate coefficients. This suggests that in the case of the experiments of Penrod et al. (1996), forces between the virus and the solid surface were attractive at a very short separation distance.

Multivalent cations can link virus and adsorbents of like charge by forming salt bridges between them (Sobsey et al., 1980; Moore et al., 1982; Lipson and Stotzky, 1983) or by charge reversal (Grant et al., 1993). Bales et al. (1991) showed in a batch experiment at pH 5 that attachment of MS2 to silica beads was at least 10 times higher in the presence of  $\text{Ca}^{2+}$  than without  $\text{Ca}^{2+}$ . These effects of multivalent ions are of electrostatic nature. Redman et al. (1999) also showed that bacteriophage SJC3 attached more to quartz when the ionic strength was increased by using a multivalent ( $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$ ) instead of a monovalent ( $\text{Na}^+$ ) cation. In a field study by Bales et al. (1997) with the same soil type, pH, and velocity as in the column study of Kinoshita et al. (1993), PRD1 attached much less than in the column study. Pieper et al. (1997) suggested that a possible explanation for the difference might have been the presence of calcium phosphate affecting surface charge in the column study of Kinoshita et al. (1993) by cation bridging or charge reversal. However, as Farrah (1982) showed, calcium phosphate, which is an antichaotropic agent, may also promote hydrophobic interactions.

## F. Effect of Organic Matter

The major occurrence of dissolved and/or suspended organic matter is in the form of humic substances (Shimizu et al., 1998). Commonly, humic substances are, similar to viruses, negatively charged, and hence they compete with viruses for the same binding sites (Gerba, 1984). Among dissolved and/or solid organic matter in the aquifer matrix, humic substances have the highest affinity to nonionic hydrophobic organic compounds (Ouyang et al., 1996; Shimizu et al., 1998). Other forms of dissolved organic matter consist of proteins, polypeptides, and amino acids. From equilibrium batch experiments, it has become clear that dissolved and/or suspended organic matter tends to compete with viruses for attachment sites on the soil surface and thereby reduce virus attachment (Gerba, 1984). Dissolved or suspended organic matter can also be used to elute previously attached viruses (Sobsey et al., 1980). Thus, detachment of viruses may be strongly increased by dissolved or suspended organic matter.

Dizer et al. (1984) showed that in sand columns at pH 7, attachment rates of poliovirus 1, coxsackieviruses A9 and B1, echovirus 7, and rotavirus SA11 were the lowest in secondary effluent when compared with groundwater, tertiary effluent, and distilled water. Dizer et al. (1984) also found that virus attachment to sand in a batch test was reduced by dissolved surfactants. It was suggested that these surfactants disrupted hydrophobic bonds between the sand and the viruses. Such surfactants may also have been present in the secondary effluent, which decreased virus attachment in the columns.

The effect of dissolved organic matter may not always be apparent (e.g., if the nature of the soil is more important for adsorption). Gerba and Lance (1978) found that adsorption of poliovirus 1 and its movement through a loamy sand soil was not affected by the high organic matter content of primary sewage effluent (70 mg/l) compared with that of secondary sewage effluent (10 mg/l). According to the authors, the sewage effluent did not saturate adsorption sites, even after 9 days of flooding. Thus, dissolved organic matter has not influenced adsorption of poliovirus 1 because an excess of adsorption sites was available.

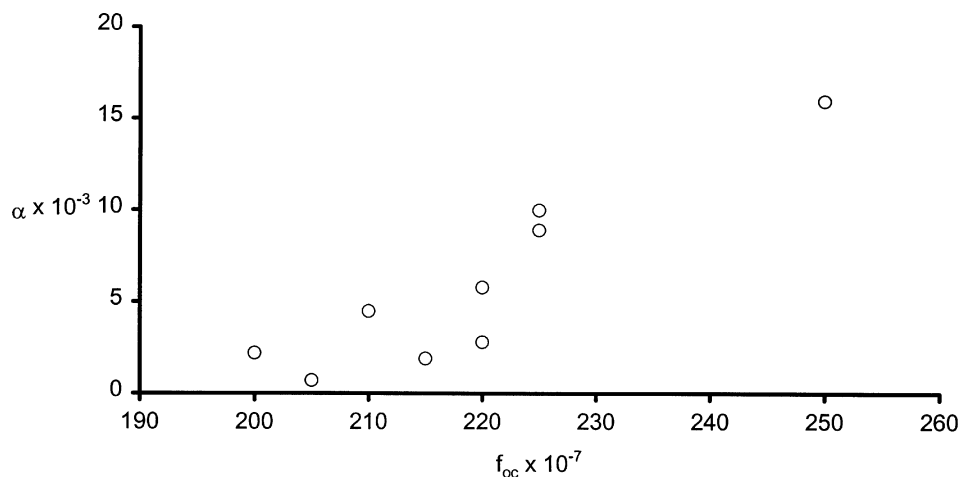
Under unsaturated flow conditions, the effect of dissolved organic matter is even more complex. Powelson et al. (1991) found that natural humic material and sewage sludge organic matter decreased removal of MS2 in unsaturated columns with loamy sand. On the contrary, in a field study, Powelson et al. (1993) found that the effluent type did not affect removal of MS2, whereas PRD1 was removed three times more in secondary treated effluent compared with tertiary treated effluent, which has lower concentrations of dissolved organic matter. Powelson and Gerba (1994) compared behavior of poliovirus 1 and bacteriophages MS2 and PRD1 in columns with a sandy soil under unsaturated conditions with secondary and tertiary effluent, but found no significant influence of the effluent type on removal of any of these viruses.

Similarly, the effect of solid and/or bonded organic carbon content ( $f_{oc}$ ) of the soil is found to be ambiguous. On one hand, a soil with bonded organic carbon has fewer sites for virus adsorption. On the other hand, the bonded and/or solid organic matter may provide hydrophobic adsorption sites in a soil where virus adsorption is otherwise very low. So, the effect depends on the combination of soil type, virus type, and nature of organic matter. Gerba et al. (1981) carried out a statistical analysis on the batch adsorption data from Goyal and Gerba (1979). Adsorption of group-I viruses (Table 2) was inversely related with pH and with  $f_{oc}$ . Variation in adsorption was explained for 83% by pH and for 12% by  $f_{oc}$ . In batch experiments, Moore et al. (1982) observed that adsorption of reovirus 3 to 30 different types of soils and minerals was found to decrease significantly with increasing  $f_{oc}$ . However, when the soil with the highest organic content was omitted from the analysis, there was no significant correlation with  $f_{oc}$ . The authors concluded that  $f_{oc}$  has a significant effect only at high values. The value of  $f_{oc}$  of the organic muck was 3.4%. So, probably, at low  $f_{oc}$ , organic matter does not occupy all adsorption sites for viruses. In another batch study, Moore et al. (1981) found a strong negative correlation between  $f_{oc}$  and adsorption of poliovirus 2 to 34 different types of soils and minerals. The authors described that soil organic matter is a weak adsorbent for poliovirus because of its low pI, and therefore negative charge, and because organic matter exhibits weak van der Waals attraction. They further reasoned that some of the soil organic matter, particularly lower-molecular-weight fractions, is soluble and may pass into solution. The dissolved organic matter may be adsorbed onto other surfaces, and thereby compete for the same binding sites as viruses. On the other hand, in batch studies reported by Burge and Enkiri (1978), adsorption

of  $\phi$ X174 to four different kinds of soil was found to increase with increasing  $f_{oc}$ . Nevertheless, adsorption of  $\phi$ X174 to a fifth soil with a much higher  $f_{oc}$  was found to be much lower. According to the authors, this was due to blocking of adsorption sites by organic matter. They did not explain the positive correlation of adsorption with  $f_{oc}$  when considering only the four soils. However,  $f_{oc}$  of these four soils was also negatively correlated with pH. Because the effect of pH on virus adsorption may be expected to be stronger than that of  $f_{oc}$  (Gerba et al., 1981), pH probably acted as a confounder. That is to say, the observed effect on adsorption was rather an effect of increasing pH than of decreasing  $f_{oc}$ .

An example of cases, where bonded organic material may provide hydrophobic adsorption sites for viruses, is clearly shown by Bales et al. (1993). They used columns with negatively charged silica beads at pH 7, which were artificially coated with very small amounts of hydrophobic C<sub>18</sub>-chlorosilane. Attachment of MS2 could be increased considerably by even very small amounts of this bonded hydrophobic organic carbon material (Table 4 and Figure 9). Bales et al. (1993) also showed that the binding sites in a column of silica beads with only 0.00215% bonded C<sub>18</sub>-chlorosilane could not be saturated with even 70 pore volumes of 10<sup>5</sup> MS2 particles per ml. The concentration of bonded MS2 particles was approximately 10<sup>7</sup> pfu/g.

Yet, an opposite effect was observed in a field study by Pieper et al. (1997) in a sand and gravel aquifer with positively charged iron-oxide coating. They showed that attachment of PRD1 was less in soil with higher  $f_{oc}$ . They injected <sup>32</sup>P-labeled PRD1 into sewage-contaminated ( $f_{oc}$  of 1%) and uncontaminated zones ( $f_{oc} < 0.01\%$ )



**FIGURE 9.** Sticking efficiency  $\alpha$  of MS2 in 15-cm columns of silica beads (pH 7) with an increasing fraction bonded organic matter,  $f_{oc}$  (C<sub>18</sub>-chlorosilane). Data from Bales et al. (1993). The highest value of  $\alpha$  is actually  $> 0.016$ .

of the aquifer (see also data in Table 4). In the contaminated zone, only 42% of PRD1 attached over the first meter, whereas in the uncontaminated zone 83% removal was found. Corresponding estimates of sticking efficiencies were found to be about six times smaller in the contaminated zone than in the uncontaminated zone. Injection of linear alkylbenzene sulfonates (LAS) remobilized 87% of attached PRD1 in the contaminated zone, but only 2.2% in the uncontaminated zone. The authors suggested that LAS caused charge reversal of ferric oxyhydroxide coatings on the soil surface to which PRD1 was attached. They reasoned that the injected amount of LAS was insufficient to cause this charge reversal in the uncontaminated zone. However, removal of LAS itself in both zones was similar (12 to 14%), which implies that this explanation is probably not correct. In another study at the same field site, Ryan et al. (1999) showed that anionic surfactant dodecylbenzenesulfonate (DBS) was also much more effective at mobilizing PRD1 in the contaminated zone than in the uncontaminated zone. They argued that abundant organic matter in the contaminated zone reduced the amount of DBS needed to reverse the charge of ferric oxyhydroxides. Their results suggested that PRD1 attached strongly in the uncontaminated zone.

Another explanation is that PRD1 in the uncontaminated zone primarily attached to sites with ferric oxyhydroxide coatings, whereas these sites were already taken by organic matter in the contaminated zone. Therefore, attachment of PRD1 in the contaminated zone may have been through mainly hydrophobic interactions with bonded organic matter. This kind of attachment is weaker than attachment to sites of positively charged iron oxides (Moore et al., 1981). Thus, it may be reasoned that in the study by Pieper et al. (1997) and Ryan et al. (1999) injected LAS and DBS, respectively, increases detachment of PRD1 in the contaminated zone much more than in the uncontaminated zone by disrupting hydrophobic bonds between the soil and PRD1. This mechanism is consistent with the suggestion of Dizer et al. (1984) that surfactants can disrupt hydrophobic bonds (see also Gerba, 1984). In the vicinity of a hydrophobic site, one may expect less electrostatic repulsion, especially if that site consists of bonded polymeric organic matter. Such polymers may interact in two ways: by steric hindrance or by forming bridges between surfaces. According to Ryan and Elimelech (1996), surfactant attachment may cause colloid release by some “non-DLVO” force like steric repulsion.

To summarize this section on the effect of organic matter, dissolved organic matter may compete for the same binding sites as viruses, and because they are usually present in higher concentrations than viruses, they can decrease virus attachment. Dissolved organic matter, like surfactants, may also disrupt hydrophobic bonds between soil and virus, resulting in an increased detachment rate. At the same time, viruses and many organic materials contain hydrophobic groups on their surfaces. Therefore, once adsorbed, organic matter may provide hydrophobic binding sites for viruses. Also, the presence of solid organic matter may result in an increased attachment rate through hydrophobic binding. The hydrophobic

adsorption effect will be most pronounced in soil with negatively charged grain surfaces. The enhancing and attenuating effects of organic matter on adsorption of viruses and their dependence on soil and virus properties make their quantification very difficult, especially under field conditions. Effects of organic matter are, probably, responsible for considerable uncertainty in predicting virus removal.

## V. VIRUS INACTIVATION

### A. Introduction

In Equations 3 and 4 virus inactivation was described by the inactivation rate coefficients  $\mu_f$  and  $\mu_s$  of free and attached viruses. Modeling of the inactivation of free viruses is described in Section V.B. Section V.C discusses the modeling of  $\mu_s$  in a batch suspension with soil. In Section V.D modeling of virus inactivation during subsurface transport is discussed.

### B. Virus Inactivation in Water

Virus inactivation is usually assumed to follow first-order kinetics, as in Equations 3 and 4. However, nonlinear inactivation curves, resulting from external and internal factors, have also been found. External factors that influence virus inactivation are the environmental factors that will be discussed in Section IV and affect the population of viruses as a whole. Hurst et al. (1992) analyzed the effects of different environmental factors on virus inactivation by means of different regression equation formats and evaluated these formats. Hurst et al. (1992) considered environmental factors such as temperature, conductivity, and turbidity of the water, and ability to support bacterial growth. According to Hurst et al. (1992), virus inactivation is not a first-order but a time-dependent process.

Internal factors that influence virus inactivation may be the presence of virus aggregates or the existence of significant variations in sensitivities among the virus population to the factors that cause inactivation (Yates et al., 1987). Grant (1994) presented a mathematical model for first-order inactivation of total infectious units of viruses, including Brownian coagulation of virions. This model predicted that virion–virion coagulation is negligible in most aquatic environments because virus concentrations are too low or because inactivation occurs too quickly. The predicted coagulation in highly concentrated laboratory suspensions was too high also, because laboratory studies had indicated that at neutral or alkaline pH coagulation of poliovirus was minimal. Grant (1994) reasoned that to the extent that viral aggregates actually exist in aquatic environments, they are probably formed intracellularly and not by coagulation outside the host cell. Furthermore, in aquatic environments, virus aggregates may be dispersed by changes in solution



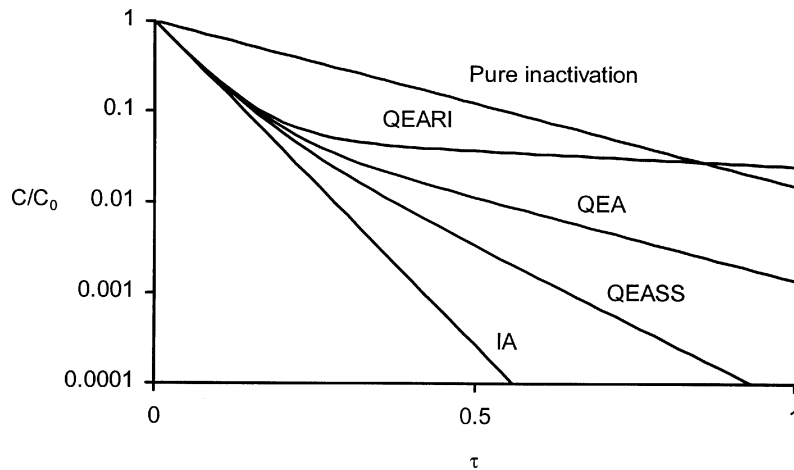
chemistry. Grant (1995) also developed a kinetic model for the inactivation of viruses that exhibit a range of initial aggregate sizes. Aggregates of viruses are assumed to be more resistant to inactivation because all virus particles within an aggregate must be inactivated before the aggregate as a whole is considered inactive. Also, undamaged components of inactive virus particles within an aggregate may recombine by a process called multiplicity reactivation (MR). Grant's kinetic model successfully fitted experimental data from a study on inactivation of vaccinia virus. Usually data on inactivation of individual virus particles, as well as the level of initial aggregation and the number of active components per virus particle needed for MR are not available, but in this study on the inactivation of vaccinia virus they were. The model correctly predicted the relatively slow inactivation that was observed for the aggregated suspension. None of the models of Grant (1994, 1995) address higher-order inactivation, due to variation in individual viruses within a population of viruses, association with other types of viruses, or association with other nonviral organic or inorganic material. These associations, however, are known to be important (see Section VI.C).

Grant et al. (1993) showed a biphasic decline of the logarithmic concentration with time of phage  $\lambda$ , indicating that a population of phage  $\lambda$  consists of two subpopulations with different sensitivity toward inactivation. Rossi (1994) argued that such a biphasic course of inactivation was possibly caused by interaction of virus particles with the walls of the synthetic vials that were used. He showed that this was the case for phage T7, but in a glass vial its inactivation was first order. He also mentioned the existence of very fine colloidal clay particles, to which the viruses were attached, as a possibility that may have caused nonlinear inactivation.

### C. Virus Inactivation in Water with Soil

According to Grant et al. (1993), batch experiments involving viruses and soil are fundamentally nonequilibrium systems, because viruses will also disappear from the liquid phase by inactivation. Inactivation of viruses that are attached to soil may proceed at a different rate than that of free viruses. Grant et al. (1993) developed a numerical model that enables estimation of  $k_{att}$ ,  $k_{det}$ ,  $\mu_t$ , and  $\mu_s$  for viruses in a batch suspension with soil, and demonstrated this for phage  $\lambda$ . No other study has been reported where values of each of  $k_{att}$ ,  $k_{det}$ ,  $\mu_t$ , and  $\mu_s$  were estimated by kinetic analysis of batch experiments. In a batch system, viruses are subject to adsorption and inactivation, simultaneously. Depending on the relative strength of these processes, Grant et al. (1993) distinguished the following four possible behaviors (Figure 10):

1. Quasi-equilibrium adsorption (QEA) is the case when virus inactivation is not influenced by the presence of a solid; thus, the inactivation rate coefficient of detached viruses equals that of attached viruses ( $\mu_t = \mu_s$ ).



**FIGURE 10.** Semi-log plot showing four possible kinetic behaviors in a batch suspension of viruses with soil compared with pure inactivation of viruses in a suspension without soil (Grant et al., 1993).  $C/C_0$  is reduced concentration of viruses in aqueous phase;  $\tau$  is nondimensional time. See Section V.C for an explanation of behaviors. QEA:  $\mu_s = \mu_f$ ; QEARI:  $\mu_s < \mu_f$ ; QEASS:  $\mu_s > \mu_f$ ; IA:  $k_{\text{det}} = 0$ .

2. Quasi-equilibrium adsorption and reduced inactivation (QEARI) is observed when virus particles that are attached to solid materials inactivate slower than those in the aqueous phase ( $\mu_s < \mu_f$ ).
3. Quasi-equilibrium adsorption and surface sink (QEASS) is the case when the solid surface acts like a sink for virus particles if virus inactivation is either accelerated in the presence of the surface ( $\mu_s > \mu_f$ ), or a portion of reversibly attached viruses becomes irreversibly attached with time. These two possibilities are indistinguishable.
4. Irreversible attachment (IA) is observed when viruses are attached irreversibly directly from solution ( $k_{\text{det}} = 0$ ). The curves for IA and pure inactivation have the same intercepts, but the slope of the IA curve is steeper (Figure 10).

There is another way of examining inactivation rate coefficients from batch experiments, as demonstrated by Rossi (1994) and Formentin et al. (1997). Viruses that are attached to clay particles are still capable of infecting their host, and thus can still be enumerated. In such batch experiments with clay particles, it is thus possible to count the total number of viruses (i.e., the number of attached and free viruses together from samples taken at different times). By subtracting the counted number of free virus particles from that of the total number, the number of attached infectious virus particles is obtained. In this way, inactivation of free and attached viruses can be followed and compared directly.

Commonly in batch studies, only the free virus concentration is measured with time. Several of such studies exist that have compared inactivation of viruses in a batch suspension with and without soil added (Sobsey et al., 1980, 1986; Nasser et al., 1993; Gantzer et al., 1994; Blanc and Nasser, 1996; Sakoda et al., 1997). From the data of these studies it is still possible to calculate a value of  $\mu_s$ . In these studies, the percentage of adsorption and the decay rate of the free virus concentration in the suspension with soil,  $\mu_{eff}$ , were measured. The value of this decay rate lies between that of  $\mu_l$  and  $\mu_s$ . In these studies, an apparent equilibrium was reached within a short period of time, whereas inactivation was measured over a much longer period of time. Therefore, it is possible to calculate  $\mu_s$  from the ratio  $k_{att}/k_{det}$ ,  $\mu_l$  and  $\mu_{eff}$ . Applying Equation 10, but eliminating the terms for dispersion and advection, and substituting Equations 9, 2, and 16 gives:

$$R \frac{dC}{dt} = -(\mu_l + (R-1)\mu_s)C \quad (25)$$

This equation has the following solution:

$$C = C_0 \exp\left[-\frac{\mu_l + (R-1)\mu_s}{R} t\right] \quad (26)$$

Now,  $\mu_s$  can be calculated as follows:

$$\mu_s = (R\mu_{eff} - \mu_l)/(R-1) \quad \text{where} \quad \mu_{eff} = -\frac{1}{t} \ln\left(\frac{C}{C_0}\right) \quad (27)$$

Values of  $\mu_s$ , calculated using Equation 27 from the batch studies of Sobsey et al. (1980, 1986) and Blanc and Nasser (1996), are presented and evaluated in Section VI.

#### D. Virus Inactivation under Transport Conditions

Several virus transport models have been developed that incorporate constant first-order inactivation of free ( $\mu_l$ ) and attached ( $\mu_s$ ) viruses (Chrysikopoulos and Sim, 1996; Sim and Chrysikopoulos 1995, 1996, 1998; Toride et al., 1995). Also, models exist that do not distinguish between inactivation of free and attached viruses (Park et al., 1994, 1995; Tim and Mostaghimi, 1991; Yates et al., 1986; Yates and Ouyang, 1992; Yates and Yates, 1987, 1988). Based on the existence of

two or more subpopulations of viruses in a suspension with different inactivation rate coefficients, Sim and Chrysikopoulos (1996) also developed a transport model, incorporating kinetic reversible adsorption and different time-dependent inactivation rate coefficients for suspended and attached viruses. The multiphasic sequential inactivation was approximated by a pseudo-first-order expression with a time-dependent inactivation rate coefficient. This way, inactivation of viruses in some batch studies was simulated better. Model simulations showed that this approximation of virus inactivation implies extended survival of viruses, and consequently more distant migration. This more sophisticated way of modeling virus inactivation during subsurface transport under saturated conditions may also be useful when considering heterogeneous populations of different viruses that exhibit multiphasic inactivation.

The contribution of virus inactivation to the removal of virus under unsaturated conditions is much greater. Furthermore, daily fluctuations in temperature in the upper unsaturated soil layers are considerable. Therefore, Yates and Ouyang (1992) developed a model (VIRTUS) that simultaneously describes the transport of water, heat, and viruses through the unsaturated zone of the soil. VIRTUS was only tested with data from one unsaturated column experiment (Powelson et al., 1990), where model predictions were close to measured breakthrough concentrations. The temperature-dependent inactivation rate capabilities of the model were not tested, simply because experiments were carried out at constant temperature.

In experiments with saturated columns, inactivation of viruses is usually insignificant, and therefore, neglected within the time scale of the experiment (Bales et al., 1989, 1991, 1993; Dowd et al., 1998; Jin et al., 1997; Penrod et al., 1996; Redman et al., 1997). At field scale, virus inactivation may be significant because of much longer timescales. To date, the field study by Schijven et al. (1999) is the only field study where both  $\mu_l$  and  $\mu_s$  were estimated (Table 8). The inactivation rate coefficient  $\mu_l$  for MS2 and PRD1 was estimated from inactivation rates in suspensions with recharge water from the field. The inactivation rate coefficient  $\mu_s$  for each of these phages could be estimated from the slope of the tails of the breakthrough curves (Figure 3).

## **VI. FACTORS AFFECTING VIRUS INACTIVATION IN THE SUBSURFACE**

### **A. Introduction**

Viruses lose their ability to infect host cells with time by inactivation. Viruses are inactivated because of disruption of coat proteins and degradation of nucleic acids (Gerba, 1984). The factors that influence inactivation of viruses have already been reviewed by Yates et al. (1987). They mention three reports on inactivation rates for viruses in groundwater (Keswick et al., 1982; Bitton et al., 1983; Yates

et al., 1985). Since then a number of new studies have been carried out. Table 5 shows an inventory of the values of  $\mu_t$  for viruses in groundwater and sewage water from several studies. Table 6 gives an inventory of  $\mu_s$ -values that were calculated using Equation 27 and data from the studies of Sobsey et al. (1980, 1986) and Blanc and Nasser (1996). In some cases, the value of  $R\mu_{\text{eff}}$  was less than that of  $\mu_t$ . This would result in an estimate for  $s$  of less than zero, which is unrealistic. These estimates were, therefore, omitted. Possibly, the value of the ratio  $k_{\text{att}}/k_{\text{det}}$  was higher than measured. This implies that equilibrium was not reached in these cases, because adsorption was too slow. As already pointed out in Section III.C, this leads to an underestimation of the ratio  $k_{\text{att}}/k_{\text{det}}$  and thus of  $R$ .

Inactivation is usually regarded as a first-order process (see Section V). The most important factors that influence virus inactivation rates are temperature, adsorption to particulate matter and soil, unsaturated conditions, and microbial activity. These factors are discussed in the following sections.

Effects of other factors are found to be of insignificant importance, although some exceptions are reported. Yates et al. (1985) found that pH, ammonia, calcium hardness, magnesium hardness, total hardness, nitrate, total dissolved solids, and turbidity did not significantly affect inactivation of MS2, poliovirus 1, and echovirus 1. Inactivation of MS2, however, increased significantly with calcium hardness. Mathess et al. (1988) reported that the inactivation rates of coxsackievirus A9 and B1, echovirus 7, and poliovirus 1 were larger in deionized than untreated groundwater. The type of water (groundwater, secondary, and primary effluent) was not found to significantly affect inactivation rates of poliovirus 1, echovirus 1, HAV, MS2, and PRD1 (Sobsey et al., 1986; Blanc and Nasser, 1996). However, Nasser et al. (1993) found a greater value of  $\mu_t$  of poliovirus 1, HAV, and F-specific RNA bacteriophages (FRNAPH's) in primary effluent than in groundwater.

## **B. Effect of Particulate Matter and Soil on Virus Inactivation**

Viruses in the environment are often associated with particulate matter or other surfaces and this has a major effect on their inactivation and transport in the environment (Gerba, 1984). Adsorption to organic matter or clays, like kaolinite and montmorillonite, or aggregation in the environment has been demonstrated for bacteriophages T2 (Gerba and Schaiberger, 1975), T7 (Bitton and Mitchell, 1974), and f2 (Armon and Cabelli, 1988) and for poliovirus 1 (Landry et al., 1983; Metcalf et al., 1984) and poliovirus 2 (Moore et al., 1981). Natural colloids, which are usually defined by their size of 1 to 1000 nm, are present in relatively large concentrations (ranging from  $10^8$  to  $10^{17}$  particles per liter) in waters of diverse geological environments (Swanton, 1995). Gerba et al. (1978) found that in treated sewage effluent, the largest quantity of solid-associated coliphages are attached to particles greater than 8.0  $\mu\text{m}$  and less than 0.65  $\mu\text{m}$ . However, Hejkal et al. (1981) indicated that in treated wastewater the majority of enteric viruses are free or

**TABLE 5**  
**Inactivation Rate Coefficient  $\mu_T$  [ $\text{day}^{-1}$ ] of Viruses in Groundwater and Wastewater**

$^{\circ}\text{C}$	Water	MS2	FRNAPHs	PRD1	Polio 1	Echo 1	HAV	Ref.
4	GRW	0.063						Yates et al. (1985) <sup>b</sup>
7	GRW	0.0058		0.01				Yahya et al. (1993)
7	GRW	0.10		0.10				
10	GRW	0.11		0.025			0.19	Blanc and Nasser (1996)
10	GRW <sup>a</sup>				0.11			Matthess et al. (1988)
10	GRW-deionized				0.010			
10	GRW				0.032			
10	GRW				0.013			
10	PE <sup>a</sup>		0		0		0.10	Nasser et al. (1993)
10	PE		0		0.046		0.17	
10	SE		0.031		0.0077		0.12	
10	TE			0.054	0.03			Blanc and Nasser (1996)
10	GRW	0.077		0.051	0.11		0.17	
12	GRW	0.091			0.18	0.24		Yates et al. (1985) <sup>b</sup>
13	GRW	0.16			0.20	0.25		Yates et al. (1985) <sup>b</sup>
16	GRW (5.4 mg/l O <sub>2</sub> )	0.22			0.21			Jansons et al. (1989b)
16	GRW (0.2 mg/l O <sub>2</sub> )				0.069			
17	GRW	0.18			0.31	0.28		Yates et al. (1985) <sup>b</sup>
20	GRW		0.0077		0.038		0	Nasser et al. (1993)
20	PE <sup>a</sup>		0.038		0.077		0.15	
20	PE		0.084		0.14		0.15	
20	SE <sup>a</sup>				0.054			Sobsey et al. (1980)
20	SE				0.14			
22	GRW (0.06 mg/l O <sub>2</sub> )				0.16			Jansons et al. (1989)
22	GRW				0.10			Bitton et al. (1983)
23	GRW	0.36		0.035	0.17		0.18	Blanc and Nasser (1996)
23	GRW	1.3		0.12				Yahya et al. (1993)
23	GRW	0.58		0.30				
23	GRW	0.73						
23	SE	0.38		0.18	1.2	0.92	0.025	Yates et al. (1985) <sup>b</sup>
23	TE	0.28		0.069	0.23			Blanc and Nasser (1996)
25	GRW <sup>a</sup>				0.15		0.082	Sobsey et al. (1986)
25	GRW				0.10	0.10	0.33	
25	PE <sup>a</sup>				0.33	0.33	> 0.055	
25	PE						0.055	

°C	Water	Reo 3	CA9	CB1	CB5	Echo 6	Echo 7	Echo 11	Echo 24	Ref.
25	SE <sup>b</sup>					0.13	0.082	0.055		
25	SE					0.13	0.13	0.073		
30	GRW			0.031		0.12		0.054		Nasser et al. (1993)
30	PE <sup>a</sup>			0.038		0.12		0.20		
30	PE			0.015		0.21		0.18		
	<b>Water</b>	<b>Reo 3</b>	<b>CA9</b>	<b>CB1</b>	<b>CB5</b>	<b>Echo 6</b>	<b>Echo 7</b>	<b>Echo 11</b>	<b>Echo 24</b>	<b>Ref.</b>
10	GRW <sup>a</sup>		0.019	0.012			0.032			
10	GRW		0.027	0.019			0.019			Matthess et al. (1988)
10	GRW-deionized		0.031	0.040			0.038			
16	GRW (2.3 mg/l O <sub>2</sub> )							0.23		Jansons et al. (1989b)
16	GRW (1.6 mg/l O <sub>2</sub> )								0.12	
19	GRW (1.2 mg/l O <sub>2</sub> )				0.12					
20	SE <sup>a</sup>									Sobsey et al. (1980)
20	SE									Jansons et al. (1989b)
22	GRW (0.2 mg/l O <sub>2</sub> )				0.25					

Note: **GRW**, groundwater; **PE**, primary effluent; **SE**, secondary effluent; **TE**, tertiary effluent.

<sup>a</sup> Sterilized

<sup>b</sup> Average values.

**TABLE 6**  
**Inactivation Rate Coefficient  $\mu_s$  of Viruses in Water with Soil**

	Water	°C	$k_{air}/k_{det}$	Polio 1		Reo 3		$k_{air}/k_{det}$	$\mu_s$	$k_{air}/k_{det}$	$\mu_s$	
				nst	st	nst	st					
				Polio 1		Echo 1						HAV
<b>Sobsey et al. (1980)</b>												
Lakeland sand	SE	20	3.2	0.086	0.022	1.2	—	—	0.037			
Ponzer muck	SE	20	0.5	1.2	0.65	12	0.25	0.024				
Bentonite	SE	20	2.3	0.12	0.056							
Kaolinite	SE	20	99	0.093	0.048							
<b>Sobsey et al. (1986)</b>												
FM sand	GRW	25	5.7	0.23	0.063	0.042	0.95	—	0.47	0.38	0.18	
	SE	25	99	0.13	0.16	0.064	—	0.29	0.39			
Corolla sand	GRW	25	0.52	0.71	0.021	0.031	0.13	0.082	3.0	1.0	0.51	
	SE	25	0.72	0.068	0.39	0.042	—	0.39	0.064			
Ponzer muck	GRW	25	0.11	2.2	1.6	0.087	2.1	1.6	0.12	—		
	SE	25	0.075	—	0.96	0.075	—	0.20	0.69	—		
Bentonite	PE	25	0.16	—	—	0.12	—	0.21	0.30	0.22	0.21	
	GRW	25	0.087	—	—	0.15	0.38	—	0.39	0.39		
	SE	25	0.32	—	0.15	1.5	—	0.15	0.56	0.72		
Kaolinite	GRW	25	99	0.33	0.065	99	0.13	0.060	99	99		
	SE	25	99	0.22	0.094	0.087	0.33	—	99	0.066	0.066	
Cecil sandy clay	GRW	25	99	0.074	0.083	99	0.17	0.13	0.56	99		
	SE	25	99	0.092	0.064	99	0.16	0.22	99	99		
	PE	25	99	0.092	0.064	99	0.16	0.22	99	99		



Blanc and Nasser, 1996		Polio 1		MS2		PRD1		
		nst	st	nst	st	nst	st	
Loamy sand	GRW	10	199	0.03	0.18	0.17	4.8	0.07
	SE	23	142	0.16	0.18	0.46	15	0.15
Sand	SE	10	23	0.03	1.34	0.27	37	0.09
	GRW	23	3.5	0.17	11	1.34	199	0.12
	GRW	10	23	0.019	3.3	0.30	199	0.030
	SE	23	0.38	0.022	0.64	0.44	199	0.081
	SE	10	23	0.013	0.64	0.16	199	0.088
	SE	23	0.014	0.64	0.64	0.16	199	0.13

Note: **GRW**, groundwater; **SE**, secondary effluent; **PE**, primary effluent; **nst**, not sterilized; **st**, sterilized.

attached to particles smaller than 0.3  $\mu\text{m}$ . Metcalf et al. (1984) also showed that enteroviruses and rotaviruses in estuarine water adsorb preferentially to particles smaller than 0.3  $\mu\text{m}$  in diameter. Particles less than 0.3  $\mu\text{m}$  include clays, cell fragments, waste products, and other miscellaneous debris (Levine et al., 1985). Furthermore, Payment et al. (1988) showed that in river water 77% of indigenous enteric viruses and 66% of coliphages were probably free or associated with particles with a diameter of less than 0.25  $\mu\text{m}$ . Thus, a substantial fraction of viruses is attached to wastewater effluent solids and other colloidal particles with a size of less than 0.3  $\mu\text{m}$ .

Virus association with solids in natural waters has been observed to generally reduce virus inactivation, but some exceptions have been reported too (Gerba, 1984). Reduced inactivation due to adsorption to suspended matter or marine sediment in seawater or estuarine water has been reported for poliovirus 1, echovirus 1, coxsackieviruses A9 and B3, rotavirus SA11, HAV, FRNAPHs, and phages of *Bacterioides fragilis* (Smith et al., 1978; Liew and Gerba, 1980; Metcalf et al., 1984; Rao et al., 1984; Chung and Sobsey, 1992; Gantzer et al., 1994). Mathess et al. (1988) showed that addition of sand to groundwater at 10°C reduced the inactivation rate of coxsackievirus B1, but the inactivation rates of coxsackievirus A9 and echovirus 7 were not affected by the addition of sand. Grant et al. (1993) showed a reduced inactivation of phage  $\lambda$  in a batch suspension with Ottawa sand at pH 10, but not at pH 5 or 7. Sakoda et al. (1997) also reported significantly reduced inactivation of bacteriophages MS2 and Q $\beta$  in PBS (pH 7.2) by addition of cellulose, DEAE-cellulose, cellulose phosphate, kaolin, carbon, suspended solids, and sediment from a river.

In some studies increased inactivation in the presence of soil was reported. Sobsey et al. (1980) found this for poliovirus 1 in Ponzer muck. Blanc and Nasser (1996) reported this for MS2 and PRD1 in sand and loamy sand. In the study of Formentin et al. (1997), it was observed that bacteriophage f1, a filamentous phage with a length of 100 nm, attached massively to attapulgitic and was subsequently inactivated more rapidly. In this study, it was found that concentrations of free and attached MS2 particles increased after the addition of attapulgitic. Possibly, existing aggregates of MS2 disintegrated when attachment to attapulgitic took place.

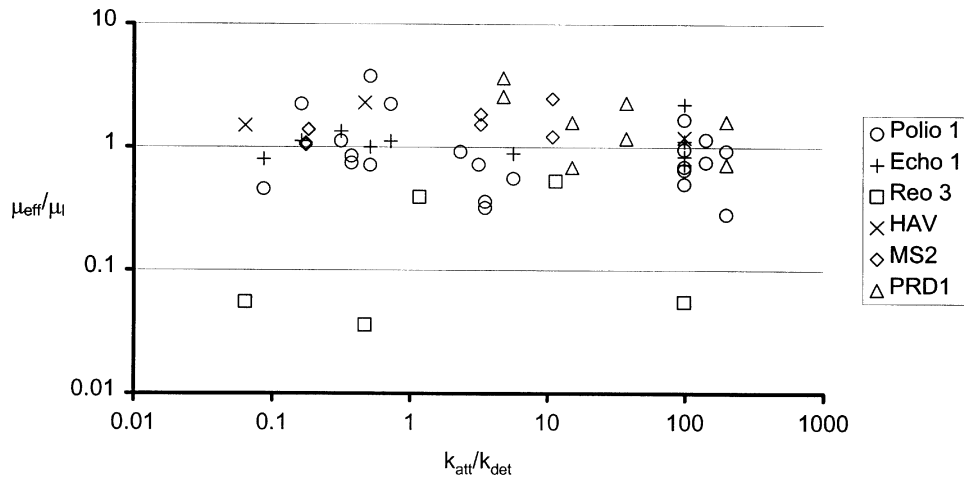
Reduced inactivation was found to be more pronounced in case of stronger attachment, especially to clays (Hurst et al., 1980). Babich and Stotzky (1980) found that in the presence of clay minerals, the inactivation rate of phage  $\phi$ 11M15 in natural lake water was greatly reduced, with the sequence of protection being attapulgitic > vermiculite > montmorillonite > kaolinite. Whether reduced inactivation in the presence of clay will be observed may also depend on its concentration. Gerba and Schaiberger (1975) did not find any increase in the survival duration of phage T2 in the presence of low kaolinite concentrations in natural seawater. Also, Gantzer et al. (1994) showed that the inactivation rate of poliovirus 1 in seawater was not significantly reduced in the presence of low concentrations of montmorillonite (3 and 15 mg/l) compared with that without this clay. These low concentra-

tions of montmorillonite are representative of the density of suspended matter in a natural seawater. However, with 500 mg/l of clay, virus inactivation was significantly less.

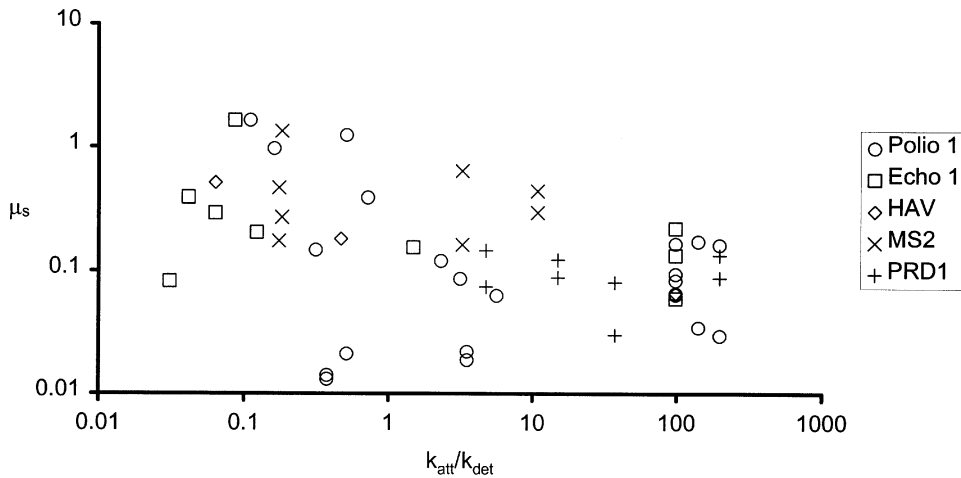
In Figure 11, the ratio  $\mu_{eff}/\mu_s$  is plotted as a function of the ratio  $k_{att}/k_{det}$  using the data from the studies that are summarized in Table 6. Within this context, a value of  $\mu_{eff}/\mu_i$  of less than one defines reduced inactivation (see Equation 27). It appears that inactivation of poliovirus 1, echovirus 1, and HAV is usually reduced but in some cases enhanced, independent of  $k_{att}/k_{det}$ . Inactivation of reovirus 3 is reduced in all cases, but that of MS2 is enhanced in all cases, and that of PRD1 is enhanced in most cases. In Figure 12,  $\mu_s$  is plotted as a function of the ratio  $k_{att}/k_{det}$  using the data from Table 6. It also seems that  $\mu_s$  is independent of  $k_{att}/k_{det}$ . At values of  $k_{att}/k_{det} < 1$ , the values of  $\mu_s$  for poliovirus 1, echovirus 1, and MS2 show more variation and can be relatively high. For values of  $k_{att}/k_{det} > 1$ , the value of  $\mu_s$  is generally less than about 0.25 day<sup>-1</sup> for all viruses that are shown here. The data from Figures 11 and 12 suggest that reduced or enhanced inactivation is very much virus-type specific, and almost independent of  $k_{att}/k_{det}$ .

Gerba (1984) mentioned that reduced inactivation may be the result of protection from proteolytic enzymes or other virus-inactivating substances, increased stability of the virus capsid when attached, prevention from aggregate formation, and blocking from ultraviolet radiation. The latter is of importance in surface waters. Sakoda et al. (1997) suggested that attachment of viruses onto a solid surface prevents them from swelling, and subsequent activity loss.

In the studies analyzed above, the batch suspension was not agitated continuously. Therefore, a protection against forces along the air–water interface probably



**FIGURE 11.** Reduction factor for inactivation rate  $\mu_{eff}/\mu_i$  vs.  $k_{att}/k_{det}$  for viruses in a batch system with soil.



**FIGURE 12.** Inactivation rate coefficient  $\mu_s$  vs.  $k_{att}/k_{det}$  for viruses in a batch system with soil.

does not play a role. This may, however, play a significant role, depending on the degree of agitation (see Section VI.E). In the cases where virus inactivation in the presence of soil was enhanced, presumably this may have been caused by the attachment and detachment processes. Viruses may be degraded because of attachment to sites with metal oxides (Gerba, 1984).

Two field studies exist where virus inactivation was investigated during subsurface transport under saturated conditions. In a field study by Schijven et al. (1999), the value of  $\mu_l$  for free MS2 particles ( $0.03 \text{ day}^{-1}$ ) was found to be three times less than the value of  $\mu_s$  for attached MS2 particles ( $0.09 \text{ day}^{-1}$ ), whereby attachment was found to be very low. This observation is consistent with the analysis from batch experiments with MS2 (Figure 11) that generally showed enhanced inactivation of MS2 in the presence of soil. In the case of PRD1, the estimate of  $\mu_l$  was not clear, but was likely less than that of  $\mu_s$  ( $0.07 \text{ day}^{-1}$ ). On the other hand, DeBorde et al. (1998) found indications for reduced inactivation of MS2 and  $\phi$ X174 during subsurface transport under field conditions. Inactivation was followed for about one half year ( $6$  to  $12^\circ\text{C}$ ). In enclosed tubes,  $\mu_l$  for MS2 and  $\phi$ X174 were about  $0.06$  and  $0.03 \text{ day}^{-1}$  respectively. The tails of the breakthrough curves declined slower in time (i.e.,  $0.016 \text{ day}^{-1}$  for MS2 and negligible for  $\phi$ X174). There is no clear explanation for the difference between the two field studies, where both enhanced and decreased inactivation of attached viruses under transport conditions were observed.

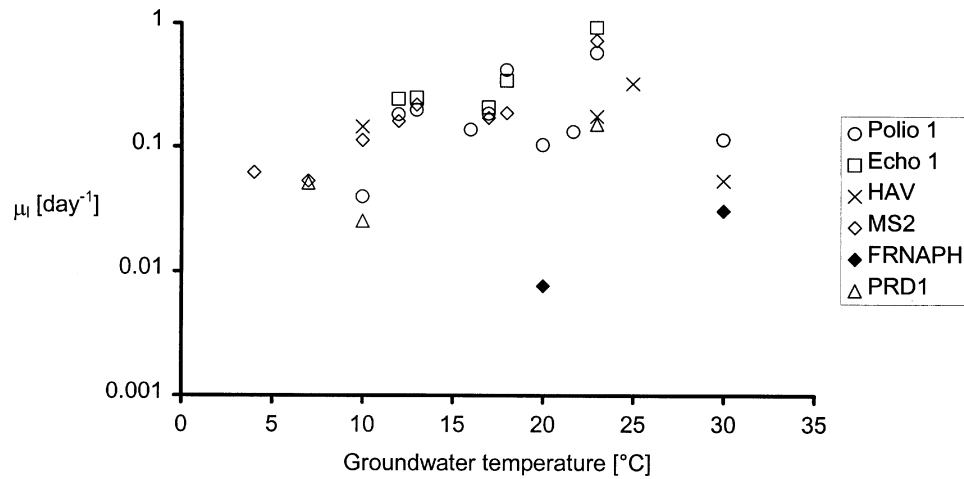
### C. Effect of Temperature

Temperature is the most important factor that influences virus inactivation (Hurst et al., 1980; Yates et al., 1985, 1987). Inactivation rates increase with

temperature (Hurst et al., 1980; Yates et al., 1985; Jansons et al., 1989b; Nasser et al., 1993; Yahya et al., 1993; Blanc and Nasser, 1996). Figure 13 shows how  $\mu_i$  for MS2, FRNAPHs, PRD1, poliovirus 1, echovirus 1, and HAV changes with temperature in nonsterilized groundwater. This figure was constructed using the data from Table 5. Regression analysis suggests an exponential dependence of  $\mu_i$  on temperature in the form:

$$\ln \mu_i = aT + b \quad (28)$$

Values of coefficients  $a$  and  $b$  for six different viruses are given in Table 7. Here, it should be noted that this analysis was based on only a few data for FRNAPHs and PRD1; therefore, the values for these viruses are only indicative. Clearly, the temperature sensitivity of  $\mu_i$  depends on the type of virus. Yates et al. (1985) found



**FIGURE 13.** Inactivation rate coefficient  $\mu_i$  vs. temperature for viruses in nonsterilized groundwater from different studies. See values in Table 5.

**TABLE 7**  
**Regression Analysis of  $\ln(\mu_i)$  for Viruses with Groundwater Temperature ( $T, ^\circ\text{C}$ )**

Equation: $\ln(\mu_i) = aT + b$	Polio 1	Echo 1	HAV	MS2	FRNAPHs	PRD1
Number of observations	10	5	4	8	2	3
Slope $a$	0.033	0.12	-0.024	0.12	0.14	0.09
Intercept $b$	-2.4	-3.0	-1.4	-3.5	-7.6	-4.0
Correlation coefficient	7%	71%	8%	85%	100%	71%

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no significant differences between inactivation rates of poliovirus 1, MS2, and echovirus 1 at different temperatures in groundwater samples from different sites. However, Sobsey et al. (1986) found lower values of  $\mu_l$  for poliovirus 1 and echovirus 1 at 25 and 30°C. Therefore, the data in Table 7 indicate that inactivation of poliovirus 1 is much less sensitive to temperature than MS2 or echovirus 1 (see values of slope  $a$ ). Table 7 further shows that HAV may be regarded as insensitive to changes in temperature. FRNAPHs appear to be very stable as compared with other viruses, but are very sensitive to changes in temperature, as is MS2. In seawater, inactivation rate coefficients for FRNAPHs and HAV were 0.08 and 0.07 day<sup>-1</sup> at 5°C, respectively, and 1.0 and 0.3 day<sup>-1</sup> at 25°C, respectively (Chung and Sobsey, 1992). So, possibly, FRNAPHs are not as stable as the data of Nasser et al. (1993) suggest.

The values of  $\mu_s$  that were derived from Blanc and Nasser (1996) allow evaluation of  $\mu_s$  variation with temperature. It appears that  $\mu_s$  values for MS2, PRD1, and poliovirus 1 that are attached to loamy sand significantly increase with temperature. The same is the case with  $\mu_s$  values for MS2, PRD1 that are attached to sand, but not for poliovirus 1. Probably,  $\mu_s$  values change similarly with temperature as  $\mu_l$  values.

#### D. Effect of Microbial Activity

Inactivation of viruses may be enhanced because of microbial activity (Yates et al., 1987). Hurst et al. (1980) found that the concentration of sewage effluent in distilled water under sterile aerobic and anaerobic, as well as under nonsterile anaerobic incubation conditions did not significantly affect virus inactivation. However, under nonsterile aerobic conditions, inactivation of poliovirus 1 proceeded faster. This indicates a deleterious effect of aerobic microorganisms on the survival of poliovirus 1. Also, inactivation of poliovirus 1 and reovirus 3 in settled sewage (Sobsey et al., 1980) and of poliovirus 1, echovirus 1, and HAV in groundwater, secondary, and primary effluent (Sobsey et al., 1986) was slower under sterile conditions than under nonsterile conditions in most cases (Table 5). The same can be said for inactivation of attached viruses (Table 6). Jansons et al. (1989b) measured inactivation of poliovirus 1 in dialysis bags in different boreholes at a field site. The dissolved oxygen concentration in groundwater was different between boreholes. They found that the inactivation rate of poliovirus 1 was three times higher at a mean dissolved oxygen concentration of 5.4 mg/l compared with 0.2 mg/l. Also, *Pseudomonas maltophilia* was found in large numbers only in the borehole with the higher dissolved oxygen concentration. Inactivation of poliovirus 1 may have been directly affected by a higher dissolved oxygen concentration, or by microbiological activity due to the higher dissolved oxygen concentration.

Seemingly, inactivation rates of viruses are not always affected by the activity of microorganisms. Babich and Stotzky (1980) did not find a significant difference in inactivation rate of bacteriophage  $\phi$ 11M15 between natural, autoclaved, or filtered lake waters. Also, Mathess et al. (1988) found no significant difference between the inactivation rates of coxsackievirus A9 and B1, echovirus 7, and poliovirus 1 in sterile and nonsterile groundwater.

## E. Effect of Unsaturated Conditions

In the calculation of attachment to soil in saturated columns, inactivation of viruses is usually neglected within the time scale of the experiment. However, under unsaturated conditions, the contribution of the effect of inactivation on virus transport is much more important. Powelson et al. (1990) showed that MS2 was neither adsorbed nor inactivated in a saturated 1-m column with a loamy fine sand (pH 8), because after less than two pore volumes the effluent concentration reached that of the influent concentration. However, in a column under unsaturated conditions, the effluent concentration was significantly reduced. By analysis of soil samples from the unsaturated column, it appeared that MS2 adsorbed only poorly, but was removed by enhanced inactivation. Powelson and Gerba (1994) showed that in columns under unsaturated conditions, removal of the MS2, PRD1, and poliovirus 1 was more than three times higher as under saturated conditions. Poletika et al. (1995) showed in a lysimeter with undisturbed unsaturated soil that MS2 was attached stronger and inactivated faster. These results suggested that the air-water interface may be retaining and/or inactivating viruses during transport through unsaturated soil.

Hurst et al. (1980) showed that the inactivation rate of poliovirus 1 increased as the soil moisture content of a sandy soil increased from 5 to 15% and then decreased when the soil moisture content increased from 15 to 25%. Apparently, inactivation was at its maximum near the soil moisture saturation point. Possible explanations for this observation would include soil moisture-level-dependent differences in the extent of virus attachment to the soil and the mechanisms of attachment. Rossi (1994) showed that, in a batch test, inactivation of bacteriophage T7 increased due to the action of strong agitation. During this strong agitation, virus particles have a higher probability to come into contact with the air-water interface. Addition of organic matter (tryptone, humic acid) saturated the air-water interface with organic matter. This lowered the chance of a virus particle entering the air-water interface and resulted in a reduced inactivation rate. Addition of attapulgite clay resulted in very fast attachment of the virus particles to the clay particles, lowering the chance of entering the air-water interface even more and an even stronger reduction of the inactivation rate was observed. Therefore, it was believed that contact with the air-water interface increases inactivation. Also, by addition of attapulgite, H40/1 and H6/1 were very effectively protected against strong agitation (Formentin et al., 1997). Thompson et al. (1998) showed that

bacteriophage MS2 was protected against the air–water–solid contact line when attached to soil particles. In this case, the solid was the hydrophobic polypropylene wall of the tube containing the suspension. At the air–water–polypropylene contact line surface tensions are much higher than at an air–water–glass contact line. A similar effect of a synthetic tube wall on inactivation was also observed by Rossi (1994) for phage T7 (see Section V.A). These findings suggest that physical forces associated with the air–water–solid contact line may be responsible for enhanced inactivation.

## VII. ADVECTION AND DISPERSION OF VIRUSES

It has been shown in both laboratory and field studies that physical heterogeneities of the soil increases the dissimilarities between the transport behavior of microorganisms and conservative solute tracers (Harvey, 1997). Harvey (1997) described movement of microorganisms and solute tracers in relatively uniform granular material, like sand, fractured media, and stratified granular material that consists of adjacent layers of finer and coarser sand. In relatively homogeneous sand, viruses are transported at the same velocity as a conservative salt tracer (Pieper et al., 1997; Schijven et al., 1999). However, in fractured media the kinetically or spatially available flow path can be considerably smaller for microorganisms than for a solute tracer (Harvey, 1997). Many studies of bacterial movement exist that generally show a rapid movement of bacteria due to preferential flow through macropores, cracks, worm holes, and channels formed by plant roots (Abu-Ashour et al., 1994). Even more generally, colloids have been shown to be most mobile in soils with relatively large pores (Ouyang et al., 1996). In fractured or stratified soils, tracers like chloride or bromide are small enough to advect or diffuse into fine fractures that are connected to preferential flow paths that are inaccessible to microorganisms (Harvey, 1997). Breakthrough of a solute in such heterogeneous soils will, therefore, be partially retarded and much more dispersed than a colloidal particle such as a virus. In addition, the solute tracer will fail to reach a steady state value for  $C/C_0$  of 1 as was shown by Bales et al. (1989). Breakthrough curves of solute tracers in heterogeneous soils will typically have the shape of curve E in Figure 3a. Diffusion into a matrix with small pores has the same retarding effect as kinetically limited adsorption. Therefore, time to peak breakthrough of the solute will be later than that of the virus. In these cases, where the average flow velocity of viruses is higher than that of water,  $v$  in Equation 1 should be interpreted as the flow velocity of the viruses. Rehman et al. (1999) developed a stochastic model for virus transport that accounts for effects of spatial variability in aquifer hydraulic conductivity. They could simulate that a high degree of aquifer heterogeneity can lead to virus breakthrough preceding that of a conservative tracer.

Bales et al. (1989) found that 35 to 40% of MS2 was excluded from the pore volume in a column with sandy soil. Bales et al. (1989) also studied transport of



phage f2 and two solute tracers, isothiocyanate and pentafluorobenzoic acid in columns with nonsorbing fractured rock. The breakthrough curve of f2 showed little dispersion, whereas the solute tracers were dispersed, because of diffusion into the porous matrix. The authors concluded that bacteriophages are better tracers for transport of colloidal contaminants than solutes, because they were not subject to matrix diffusion. McKay et al. (1993) showed that MS2 and PRD1 traveled at rates of 2 to more than 5 m/d in a fractured clay-rich till, whereas bromide and  $^{18}\text{O}$  were transported at rates of only 0.01 to 0.07 m/d. Also, in a clay-rich till, Hinsby et al. (1996) showed that MS2 and PRD1 were transported at high rates of 4 to 360 m/day. These high rates were similar to water flow rates in the fractures. The chloride concentration took more than 43 h to approach steady state, because of diffusion into the small pores in the matrix between the fractures. The phages reached steady state in less than 3 h, because of pore size exclusion. Rossi (1994) showed that bacteriophages H40/1 and H6/1 were transported in karst faster than uranine and naphtionate due to preferential flow. Rossi et al. (1994) showed that bacteriophages T7 and f1 migrated about three times faster than naphtionate, based on time to peak breakthrough. Migration rates of the tracers could be correlated with permeability distribution obtained by radio-magneto Tellury measurements. This showed that the tracers followed the more permeable pathways. The same was found with phages H40/1, H6/1, T7, and Psf2 compared with uranine by Rossi (1994) in a karstic aquifer.

Paul et al. (1995) used bacteriophages PRD1 and  $\phi\text{HSIC1}$  as tracers to investigate fate and transport of sewage through a highly porous limestone matrix to marine waters (Key Largo). Bacteriophage  $\phi\text{HSIC}$  was seeded into a septic tank and PRD1 at an injection point for disposal of domestic wastewater. Estimated rates of migration of the two bacteriophages ranged from 14 to 580 m/day, which is over 500-fold greater than the water velocity, which was measured by subsurface flow meters. The phages traveled at least a distance of 800 m. Concentrations of the viruses varied with the falling tides. However, in this study, it was not clear whether the phages really traveled faster than the water because measurement of water flow was not carried out at the same time and place. Heavy rainfall preceded this study and may have caused unusually high flow rates. It was not clear whether PRD1 could reach surface marine waters from the injection point. In a subsequent study, Paul et al. (1997) repeated seeding with bacteriophages MS2, PRD1, and  $\phi\text{HSIC1}$  at Key Largo, but also at another disposal well (Middle Keys). The different phages traveled at similar rates and made their way rapidly to surface marine waters from both injection wells, but transport rates were much greater in Key Largo than in the Middle Keys, 460 m/day and 24 m/day, respectively. This difference in flow rates could be ascribed to differences in tidal pumping, and also to the fact that there exist manmade channels through the limestone at the Key Largo site, but not at the Middle Keys site.

Sinton et al. (1997) studied the movement of somatic coliphages, FRNAPHs and fecal coliforms through an alluvial gravel aquifer where effluent was irrigated.

Breakthrough was followed at 60 m and about 400 m. In a second experiment, MS2, *E. coli* J6-2, and rhodamine WT dye were injected and could also be detected at 400 m. In both experiments, the phages exhibited the shortest times to peak concentration, followed by the bacteria, and then the dye. Sinton et al. (1997) interpreted these data by suggesting that the bacteria were transported faster than the dye because of pore-size exclusion; the phages were transported even faster than the bacteria, because the electrostatic repulsion of the phages is supposedly stronger than that of the bacteria, or that the phages are more often attached to other particles of a size that are transported faster than bacteria. Sinton et al. (1997) based transport velocity of the dye, the phages, and the bacteria on the arrival time of peak concentrations. As was explained in Section III.B, differences in time to peak concentration can also be an effect of different values of kinetic attachment and detachment rate coefficients (curves E and F in Figure 3a). Thus, it may be reasoned that the difference between  $k_{att}$  and  $k_{det}$  was much less for the bacteria than for the phages, which is a more plausible explanation for the observed difference in time to peak concentration between the bacteria and the phages. In addition, kinetic effects may have played some role in the breakthrough of the rhodamine WT dye. A study exists where it was shown that rhodamine WT does not behave conservatively, but exhibited nonequilibrium adsorption (Di Fazio and Vurro, 1994).

To conclude, advection and dispersion are properties of the soil and water, and are commonly determined by means of solute (salt) transport experiments. However, viruses may travel faster than the average water flow and show a smaller dispersion than a solute. Several studies have shown that viruses are transported faster than classic solute tracers, because they can be excluded from small pores and therefore preferentially follow more permeable pathways. This shows that for movement of colloids, bacteriophages are better tracers than solutes.

## VIII. MODEL VIRUSES

### A. Introduction

As pointed out in Section I.D, model viruses are needed to represent the behavior of pathogenic viruses during subsurface transport and to predict their removal. Bacteriophages MS2, PRD1,  $\phi$ X174, and naturally occurring FRNAPHs have been used extensively to study virus transport under various column and field conditions. In this section, the role of these bacteriophages as model viruses is evaluated.

### B. MS2

MS2 is an icosahedral phage with a diameter of 27 nm and a low isoelectric point of 3.5. The three-dimensional structure of its capsid is known at the atomic

level (Penrod et al., 1996). MS2 may be considered as a relatively conservative tracer for virus transport in saturated sandy soils at pH 6 to 8 and with a low organic carbon content, because under those conditions it showed little or no adsorption (Bales et al., 1989; Powelson et al., 1990; Herbold-Paschke et al., 1991; Kinoshita et al., 1993; Jin et al., 1997; Schijven et al., 1999). A low organic content would imply low hydrophobicity of the soil.

In most soils, attachment of MS2 is also relatively low compared with most other viruses (Goyal and Gerba, 1979). Herbold-Paschke et al. (1991) showed that only a few percent of bacteriophages T4, MS2, and  $\phi$ X174 attached in 1-m columns filled with coarse or medium-grade quartz sand. MS2 removal was less than or equal to that of T4 and  $\phi$ X174, and much less than that of simian rotavirus SA11. In columns with a sandy soil (pH 7.5), Bradford et al. (1993) showed that MS2 was removed less (0 to 68%) than poliovirus 1 (99%) and simian rotavirus SA-11 (90%). Farrah and Preston (1993) showed less or equal removal of MS2 compared with poliovirus 1, coxsackievirus B5, and echovirus 5 in column with sand that was or was not modified by precipitation of metallic salts. Bales et al. (1993) showed that the sticking efficiency of MS2 in columns with silica beads was two to three times lower than that of poliovirus 1 at pH 7, but about the same at pH 5 to 5.5 (Table 2). Sobsey et al. (1995) observed that removal of MS2 in 10-cm columns with clay loam (52% clay) at pH 6 to 8 was similar to that of HAV, poliovirus 1, and echovirus 1. Removal of MS2 in columns with organic muck was less than or equal to that of these viruses. Penrod et al. (1996) and Redman et al. (1997) showed that due to steric repulsion, attachment of MS2 to silica was less than that of bacteriophage  $\lambda$  and recombinant Norwalk virus particles to quartz sand at pH 5 to 7, although both had a stronger negative surface charge than MS2. Jin et al. (1997) compared transport of MS2 and  $\phi$ X174 in short sand columns (10 or 20 cm). MS2 did not adsorb but  $\phi$ X174 was retained significantly. In the field studies of DeBorde et al. (1999) and Schijven et al. (1999) removal of MS2 and PRD1 was similar. In the same study of DeBorde et al. (1999) removal of  $\phi$ X174 was slightly more efficient and that of poliovirus 1 (CHAT) even more.

However, possibly due to its hydrophobicity, MS2 may attach more than other viruses to some soils (e.g., to soil K in the batch study of Goyal and Gerba [1979]). Using continuously circulating columns of a sandy soil (95% sand, 7% silt, 2% clay, pH 7.1), Dowd et al. (1998) found that the attached fraction of MS2 was greater than that of four other bacteriophages, PRD1, Q $\beta$ ,  $\phi$ X174, and PM2, possibly due to the presence of multivalent cations. In the same study of Dowd et al. (1998), attachment of MS2 to the sandy soil in a flow-through column was intermediate.

With regard to inactivation, MS2 is less stable than several pathogenic viruses (Table 5). MS2 is inactivated faster at higher temperatures, but at temperatures lower than 7°C, its inactivation rate is very low and similar to that of PRD1 (Yates et al., 1985; Yahya et al., 1993; Blanc and Nasser, 1996; Schijven et al., 1999).

In conclusion, MS2 meets the requirements for a worst-case model virus, provided the water temperature is less than about 10°C, the soil does not contain too many hydrophobic sites, and the concentration of multivalent cations is low.

### C. PRD1

PRD1 is an icosahedral bacteriophage with a diameter of 62 nm with an inner lipid membrane (Bales et al., 1991; Caldentey et al., 1990). Its pI lies between 3 and 4 (Loveland et al., 1997). PRD1 may be considered as a worst-case model virus because of its low inactivation rate between 10 to 23°C (Yahya et al., 1993; Blanc and Nasser, 1996; see also Table 5). Because of its larger size, PRD1 is of interest as a representative of rotaviruses and adenoviruses (Sinton et al., 1997). With regard to attachment characteristics, PRD1 seems to behave less conservatively than MS2 (Bales et al., 1991; Kinoshita et al., 1993; Powelson et al., 1993; Dowd et al., 1998), possibly because it is more hydrophobic than MS2 (Shields and Farrah, 1987; Bales et al., 1991; Kinoshita et al., 1993; Lytle and Routson, 1995). However, in a sandy aquifer (Bales et al., 1997) PRD1 attached at a much lower rate than compared with the column studies (using the same soil) by Kinoshita et al. (1993). Detachment was much slower than attachment, but the detachment rate increased  $10^4$  to  $10^5$  times by a high pH-pulse (Bales et al., 1997). In the field studies by DeBorde et al. (1999) and Schijven et al. (1999) removal of PRD1 was similar to that of MS2. Apparently, PRD1 may also be considered as a relatively conservative model virus, similar to MS2, under field conditions in sandy soils at pH 6 to 8 and with low organic carbon content and low concentration of multivalent cations. In addition, PRD1 is more stable at higher temperatures (12 to 23°C).

### D. $\phi$ X174

$\phi$ X174 is less hydrophobic than MS2 (Shields and Farrah, 1987). In studies on the retention of viruses by barrier materials such as membranes, condoms, and testing gloves,  $\phi$ X174 is regarded as the best model virus, because it exhibits the least electrostatic and hydrophobic interaction (Shields and Farrah, 1987; Lytle and Routson, 1995; Fujito and Lytle, 1996).  $\phi$ X174 essentially has no charge at neutral pH (pI = 6.6 to 6.8) and a size of 27 nm (Fujito and Lytle, 1996; Dowd et al., 1998).

Jin et al. (1997) found that breakthrough of  $\phi$ X174 in columns with Ottawa sand attached significantly, whereas MS2 did not. It was suggested that this difference in adsorption behavior was a reflection of the difference in the isoelectric points of the two viruses. The authors suggested that because the pI of  $\phi$ X174 is the same as that of poliovirus 1, it should have similar attachment behavior. However, Funderberg et al. (1981) already showed that these viruses behave differently. They studied removal of poliovirus 1, reovirus 3, and bacteriophage

φX174 in columns of eight different soils. The strongest positive correlation was found between removal of poliovirus 1 and reovirus 3, and soil cation exchange capacity. Whereas removal of these enteroviruses was correlated more with soil properties, removal of φX174 was affected most by the residence time in the column. Removal of φX174 was usually less than that of poliovirus 1. In columns with quartz sand, removal of φX174 was less than that of bacteriophage T4, and more than or equal to that of MS2. However, removal of all these phages was quite low (Herbold-Paschke et al., 1991). Dowd et al. (1998) showed that in a sandy soil in circulating-flow columns, and in flow-through columns removal of φX174 was less than that of MS2, PRD1, PM2, and Qφ. In field studies of DeBorde et al. (1998, 1999), φX174 appeared to be very stable (i.e., its inactivation was negligible over a period of about one-half year).

φX174 may be a relatively conservative model virus because of its low hydrophobicity (Shields and Farrah, 1987) and stability (DeBorde et al., 1998, 1999). In soils, where hydrophobic interactions would significantly increase virus removal, φX174 would be a better choice as a model virus than for instance, MS2 or PRD1. However, the value of pH will strongly determine whether φX174 will behave conservatively. A pH range of 6 to 8 is very common for most soils, and at pH 6 the net surface charge of φX174 will be positive, but at pH 8 it will be negative.

## E. FRNAPHs

FRNAPHs have similar physical properties as enteroviruses, especially with respect to size (Bitton, 1980; Havelaar, 1993). MS2 belongs to group I of FRNAPHs (Havelaar, 1986). As naturally present model viruses they are of high interest to represent enteroviruses in various treatment processes of surface water, including soil passage. Before entering a treatment like soil passage, enteroviruses and FRNAPH largely have followed the same path (i.e., both have passed the sewerage system, followed by sewage treatment, discharge into surface water, and some kind of pretreatment before recharge into soil). It may be reasoned that along this path from the sewage system to the point of recharge into an aquifer, viruses that are less stable, or that adsorb readily to solid surfaces, have disappeared already. This suggests that a selection has taken place of very stable and poorly adsorbing viruses (i.e., worst-case viruses). This selection has been the same for FRNAPHs and enteroviruses.

In surface water, FRNAPHs occur in numbers of  $10^2$  to  $10^4$  times higher than enteroviruses (Havelaar et al., 1993). Therefore, it has been possible to show 4 to 6 log units removal of FRNAPH's by riverbank filtration (Havelaar et al., 1995).

In an alluvial gravel aquifer where effluent was irrigated, Sinton et al. (1997) studied transport of somatic coliphages, FRNAPH and faecal coliforms in one experiment and MS2 and *E. coli* J6-2 in a second experiment. Concentrations of

all these microorganisms were similarly reduced, about  $9 \log_{10}$  after 400 m of transport. Concentration of the rhodamine WT dye was reduced about  $7 \log_{10}$  over this distance. This shows that removal of FRNAPH and MS2, also an FRNAPH, is similar. Low sticking efficiencies of naturally present FRNAPH have been found by Schijven et al. (1998) under field conditions (Table 3). DeBorde et al. (1998) found a removal rate of naturally present FRNAPHs and somatic coliphages that was half of that of bacteriophages MS2 and  $\phi$ X174. However, the latter two bacteriophages were seeded as slug injections, whereas FRNAPHs were continuously introduced with sewage effluent.

In the study of Nasser et al. (1993), FRNAPH appeared to be very persistent at 10 to 30°C, and not affected by the type of water. They were even more persistent than HAV, which can be regarded as a very persistent virus (Sobsey et al., 1986; Nasser et al., 1993; Blanc and Nasser, 1996). However, Chung and Sobsey (1992) observed that FRNAPHs are less stable than HAV in seawater.

In conclusion, FRNAPHs as a group of naturally occurring viruses are very useful model viruses for the behavior of viruses during subsurface transport. FRNAPHs behave relatively conservatively, like MS2, and they have been shown to be very persistent. Moreover, naturally present FRNAPHs may consist of stable and poorly adsorbing viruses prior to treatment by soil passage.

## IX. VIRUS REMOVAL BY SOIL PASSAGE

### A. Relative Contributions of Adsorption and Inactivation to Virus Removal

Thus far in this article, adsorption and inactivation have been evaluated separately. This section focuses on the relative contributions of these processes to virus removal by soil passage. Virus adsorption to soil is the most important process for attenuation. However, actual removal (i.e., disappearance of viruses), is due to inactivation, and also of irreversible attachment, if it exists. Under steady-state conditions, the relative contributions of inactivation and adsorption to the removal of viruses by soil passage can be compared easily. A steady-state situation occurs when input of virus is continuous. This usually is the case (e.g., during bank filtration, dune recharge, deep well injection, or continuously leaking sewage pipes) and may be seen as a worst-case situation. Considering a one-dimensional steady-state situation, Equation 1 may be simplified to:

$$\alpha_L \frac{\partial^2 C}{\partial x^2} - \frac{\partial C}{\partial x} = \frac{Q}{nv} \quad (29)$$

Here,  $\alpha_L$  is the longitudinal dispersivity, [L]. Under steady-state conditions, it follows from Equation 3 that:

$$\mu_{s,kin} \rho_B S_{kin} = n k_{att} C - k_{det} \rho_B S_{kin} \quad (30)$$

Substitution of Equations 2 and 31 into 4 gives:

$$Q = \lambda n C \quad \text{where} \quad \lambda = \mu_l + \mu_{s,eq} \frac{\rho_B}{n} k_{eq} + \frac{k_{att} \mu_{s,kin}}{k_{det} + \mu_{s,kin}} \quad (31)$$

Now, the solution of Equation 29 can be written as:

$$\log\left(\frac{C}{C_0}\right) = \frac{x}{2.3} \frac{\left(1 - \sqrt{1 + 4\alpha_L \frac{\lambda}{v}}\right)}{2\alpha_L} \quad (32)$$

Where  $C_0$  is the concentration at  $x = 0$ , and  $\log(C/C_0)$  is defined as virus removal. From Equations 31 and 32, the relative contributions of adsorption and inactivation to virus removal can be deduced. Because equilibrium adsorption has been shown to be of little importance in several studies (Bales et al., 1991, 1993, 1997; Pieper et al., 1997; Jin et al., 1997; Schijven et al., 1999), the second term in Equation 31 may be neglected. From Equation 31 it can be seen that if there is no inactivation at all, there will be no removal ( $\lambda = 0$ ) under steady-state conditions. If there is no detachment, removal will be determined by inactivation and irreversible attachment of free viruses ( $\lambda = \mu_l + k_{att}$ ).

In the field study by Schijven et al. (1999), it appeared that  $k_{det} \ll \mu_{s,kin}$  and  $k_{att} > \mu_l$ ; therefore,  $\lambda \approx k_{att}$ . In that case, virus removal is mainly determined by attachment, which appears to work as irreversible attachment. In a field study of Bales et al. (1997), virus inactivation was considered to be insignificant. The authors concluded that virus removal could be described primarily by  $k_{att}$  and  $k_{det}$  and that simply knowing a retardation factor is not sufficient. This is justified only if  $k_{att}$  is much smaller than  $\mu_s$ . Also, Pieper et al. (1997) and DeBorde et al. (1999) regarded virus inactivation as negligible during their field experiments, and considered virus removal to be solely determined by attachment. Although detachment rates were not calculated in these studies, the shapes of their breakthrough curves suggest that detachment rates were much lower than attachment rates.

Table 8 summarizes values of  $k_{att}$ ,  $k_{det}$ ,  $\mu_l$ , and  $\mu_s$  for MS2 and PRD1 calculated from observed virus removal in a few field studies. Values for  $k_{att}$  and  $k_{det}$  have been reported in only two field studies. Bales et al. (1997) reported two values for each (Table 8). During a high pH pulse,  $k_{att}$  was only a factor 2 lower, whereas  $k_{det}$  increased by a factor of  $5 \times 10^4$ . In that case  $k_{det}$  was larger than  $k_{att}$  and probably much larger than  $\mu_s$ , implying that the kinetic term in Equation 31 took the form of the adsorption equilibrium. Schijven et al. (1999) found different  $k_{att}$  and  $k_{det}$  values for different travel distances (see next section).

**TABLE 8**  
**Values of  $\alpha$ ,  $k_{att}$ ,  $k_{det}$ ,  $\mu_l$  and  $\mu_s$ , [day<sup>-1</sup>], for MS2 and PRD1 from Field Studies**

Parameter	MS2	PRD1	Ref.
$k_{att}$	0.8 – 4.1	6.1 – 11	Bales et al. (1997)
$k_{det}$	0.00087 – 0.0030	0.7 – 4.0	Schijven et al. (1999)
$\mu_l$ (5 ± 3 °C)	0.03	0.0003 – 15	Bales et al. (1997)
$\mu_s$ (2 — 5 °C)	0.09	0.00077 – 0.0034	Schijven et al. (1999)
		0.12	Schijven et al. (1999)
		0.07	Schijven et al. (1999)

All in all, it appears that only very few data are available on attachment and detachment of viruses under field conditions. In order to be able to predict virus removal, more values of attachment, detachment, and inactivation rate coefficients for viruses under various conditions are needed. Comparison of the  $\alpha$ -values from Tables 2 and 3 give the impression that, at least for short travel distances, these values are similar for column and field situations. However, this needs verification. Except during perturbations like a high pH, it seems that  $k_{det}$  is generally much smaller than  $k_{att}$ , which is consistent with observations from several column studies (Bales et al., 1991, 1993; Kinoshita et al., 1993; Penrod et al., 1996; Dowd et al., 1998). Possibly, the ratio of  $k_{att}/k_{det}$  from column scale may be extrapolated to field scale under similar conditions. However, this needs to be verified too. The inactivation rate coefficients that were found in a field study by Schijven et al. (1999) are similar in value to those from the batch studies that are summarized in Tables 5 and 6. This suggests that the values for inactivation rate coefficients as found in batch experiments may be used for prediction of virus transport at field scale. However, more information is needed to verify this.

Yates et al. (1986) calculated safe setback distances, solely based on inactivation rates of MS2. To that purpose, they sampled groundwater from 71 sites within a certain area and determined inactivation rates of MS2 in these samples at the corresponding *in situ* temperatures (20 to 30.5°C). Virus inactivation rates of other nearby sites in the area were calculated by means of kriging, a geostatistical technique. A contour map could be made that showed the variation in separation distances assuring 7-log<sub>10</sub> reduction in virus concentrations by inactivation. This approach was extended by combining kriging with a linear relation for  $\mu_l$  of MS2 with temperature. This eliminated the need of having to measure virus inactivation rates in the laboratory (Yates and Yates, 1987). This way, similar maps of setback distances could be constructed. Yates and Yates (1988) extended this model even further to account for alterations in the flow field by pumping. This geostatistical model may, however, underestimate setback distances, because at higher temperatures, the inactivation rate of MS2 is higher than that of other viruses, such as HAV



(Yates et al., 1986; see also Section VI.B). On the other hand, setback distances may be overestimated grossly this way, because the contribution of inactivation of attached viruses was not included (see Equation 31).

## B. Removal of Viruses with Distance

From Equation 32, with constant rate coefficients for attachment, detachment, and inactivation, it can be deduced that in a saturated soil under steady-state conditions, virus removal ( $\log_{10}(C/C_0)$ ) should decline in a linear fashion with travel distance. In several field studies (Bales et al., 1995; Pieper et al., 1997; DeBorde et al., 1998, 1999) viruses were seeded as slug injections, thus no steady state was achieved. In that case, apparent removal rates are expected to increase with distance due to dispersion. However, several column and field studies have shown that the removal rate appears to be initially higher.

Gerba and Lance (1978) have shown that approximately one  $\log_{10}$  of poliovirus 1 was removed ( $C/C_0 = 0.1$ ) from primary sewage during passage through the first 5 cm of a column with loamy sand. An additional 35-cm travel distance was necessary to reduce the virus concentration another  $\log_{10}$ . Similar results were obtained when the column was flooded with secondary sewage. Wang et al. (1981) analyzed removal of poliovirus 1 and echovirus 1 after continuous application to columns with three different sands and a sandy loam for 3 to 4 days at constant flow rates. Removal of both viruses appeared to be very similar. Statistical analysis indicated that the rate of virus removal in the upper 17-cm depth of the soil column was significantly greater than in the lower depths of the soil column. In a field study of Bales et al. (1995), concentration of PRD1 was attenuated about 5  $\log_{10}$  after the first 2 m of passage in a sandy aquifer. Attenuation of PRD1 was estimated relative to that of bromide to account for dilution effects. However, an additional attenuation of only one  $\log_{10}$  during the following 9 m was observed. In another field study of Bales et al. (1997), bacteriophages PRD1 and M1 both were removed about 4  $\log_{10}$  after 0.94 m of passage through a sandy aquifer, but only another one  $\log_{10}$  after the following 1.6 m. A conservative tracer did not decrease similarly and, therefore, dispersion was ruled out as a possible cause. Pieper et al. (1997) presented RB of PRD1 with distance at a field site with a sewage-contaminated and an uncontaminated zone. They found that the strongest attenuation occurred during the first 1.8 m, but concentrations of PRD1 remained nearly constant during the next 1.8 m. Plotting RB of PRD1 on a log scale showed that the removal rate of PRD1 was initially higher. Similar results were obtained by Schijven et al. (1998). They measured efficient reduction of FRNAPHs by 3.8  $\log_{10}$  after 2 m of dune infiltration. However, at 4 m, an additional reduction of only 0.83  $\log_{10}$  was found, albeit with a large uncertainty. Consequently, the corresponding estimated value of the sticking efficiency is much lower at 4 m than 2 m (see Table 3). Schijven et al. (1999) seeded bacteriophages MS2 and PRD1 during 11 days at a field site for

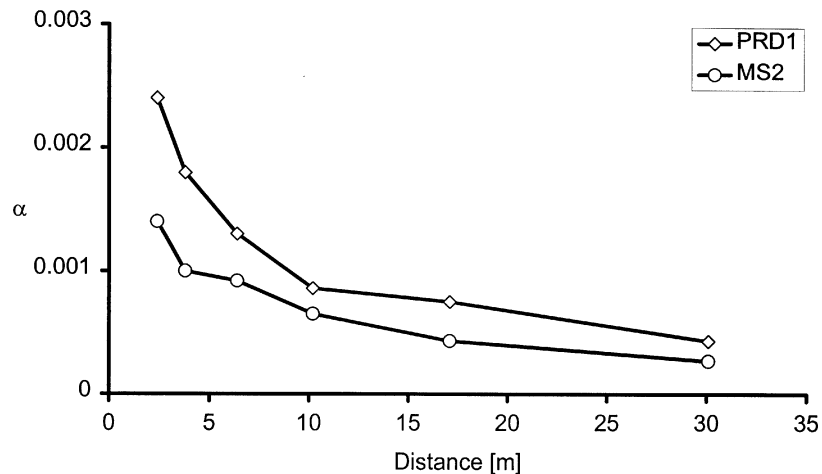
dune recharge. Removal of MS2 and PRD1 was very similar. Bacteriophage concentrations were reduced about 3 log<sub>10</sub> within the first 2.4 m and another 5 log<sub>10</sub> in a linear fashion within the following 27 m. DeBorde et al. (1999) reported removal with distance of MS2, PRD1, φX174, and poliovirus 1 (CHAT) under field conditions. Maximum breakthrough concentrations of MS2, PRD1, and φX174 decreased about 2.5 log<sub>10</sub> with 8 m and an additional 3.5 to 5 log<sub>10</sub> within the next 32 m. Concentrations of poliovirus 1 decreased 4 log<sub>10</sub> within the first 8 m and about 1 log<sub>10</sub> in the following 12 m. In another field study by DeBorde et al. (1998) removal rates of MS2 and φX174 seemed to decrease with distance, but not significantly. MS2 and φX174 were seeded as slug injections. In other words, if the viruses had been seeded for a longer period of time, such that semi-steady-state conditions could have been assumed, the removal rate would likely have declined with distance. However, removal rates of somatic and FRNAPHs were found to be linear over a distance of 18 m. These naturally occurring bacteriophages were present due to continuous infiltration of sewage effluent.

With the exception of FRNAPHs and somatic coliphages in the study by DeBorde et al. (1998), these studies all suggest that initial higher removal of viruses with distance is typical. This phenomenon may be explained by soil and/or virus heterogeneity.

Soil heterogeneity may be a cause, that is if more sites of attachment are available in the first centimeters of passage through a column or in the first meter at a field location. Although this may be the case in field studies, in the column studies that are referred to here, the columns were filled in small increments to obtain uniform packing of the soil. Therefore, it seems unlikely that the first centimeters contained a larger proportion of silt and clays, or smaller grains that could cause more efficient attachment. The field studies of Bales et al. (1995) and Pieper et al. (1997) were performed in the same aquifer that was contaminated by sewage disposal. The soil consisted of stratified, well-sorted, medium to coarse sand with some gravel, and groundwater velocity ranged from 0.2 to 0.7 m/day and porosity from 20 to 40% (Bales et al., 1995). From these studies, it is not clear if injection of the studied bacteriophages took place in finer soil material. Furthermore, nonlinear removal with distance appeared to be independent of the extent of sewage contamination (Pieper et al., 1997). From the field study by Bales et al. (1997), it is not clear whether PRD1 and M1 were injected at a point with finer soil material. Extensive soil analysis in the study by Schijven et al. (1999) showed that the average grain size of the soil was even larger in soil samples from the first 5 and 10 to 15 cm than in soil samples taken at the monitoring wells. Therefore, the possibility that fine-grained sediments at the bottom of the compartment were the cause of high early attachment could be ruled out. On the other hand, higher concentrations of bonded organic matter were found in the soil samples at a shorter distance. This suggests that the bacteriophages may have been removed more efficiently during the first meters of soil passage by hydrophobic interactions.

However, from the above, it appears that soil heterogeneity does not satisfactorily explain initial higher virus removal.

Viral heterogeneity may be a cause for the greater initial removal (i.e., the viruses in the suspensions that were used for seeding a column or a field site may have different affinities for attachment sites), as was suggested by Pieper et al. (1997) and Schijven et al. (1999). Figure 14 shows how sticking efficiency  $\alpha$  for MS2 and PRD1 gradually decrease with distance, as was observed in a field study by Schijven et al. (1999). Albinger et al. (1994) reported a range of sticking efficiencies for bacteria, even in the extreme case of a uniform collector surface and a monoclonal bacterial population. These variations in  $\alpha$  could neither be explained by preferential retention of larger cells nor by intrapopulation genetic variation. Although the outer surface of a virus particle is less complex than that of a bacterial cell, virus particles that are released from their host cell may be still attached to cell debris, which would account for differences in adsorption between virus particles. Furthermore, Goyal and Gerba (1979) have shown that virus adsorption to soil not only depends on the type and strain of virus under consideration, but may also vary between isolates of the same virus type. Viruses may be present as aggregates (see Section V.A), and it has been shown that viruses attach readily to other colloidal particles, like clays that are ubiquitously present in sewage water and surface water (see Section VI.C). It may be reasoned that combinations of viruses and other colloidal particles thus behave as particles with different size and density and therefore different collision efficiencies, but also with different sticking efficiencies. In addition, different types of viruses that are



**FIGURE 14.** Sticking efficiency  $\alpha$  for MS2 and PRD1 with travel distance (Schijven et al., 1999).

attached to the same type of colloidal particles may show the same behavior during subsurface transport. Possibly, transport of a fraction of virus particles may be enhanced, due to their attachment to inorganic colloids such as silicate clays and iron or aluminium oxide clays. Such colloidal particles are released in an anoxic environment and can migrate considerable distances (Ouyang et al., 1996).

Viral heterogeneity is the most likely explanation for the nonlinear removal of FRNAPHs by dune recharge (Schijven et al., 1998). Although FRNAPHs form a rather homogeneous group, they consist of different virus types that differ in size and surface charge. Therefore, they are likely to differ in sticking efficiencies. It can be argued that FRNAPHs with higher  $\alpha$ s attach faster, thereby lowering the average  $\alpha$  of the population of free phages, as they are transported further.

Rehman et al. (1999) developed a large-scale model of virus transport in aquifers using spectral perturbation analysis. A sensitivity analysis showed that aquifer heterogeneity can lead to virus breakthrough actually preceding that of a conservative tracer. Also, use of a heterogeneous colloid filtration term results in higher predicted concentrations than the use of a simple first-order constant attachment rate. It was pointed out that measurements of the spatial variability of the sticking efficiency would be extremely important in predicting virus transport.

In conclusion, removal of viruses by soil passage apparently declines with distance. This has important consequences for prediction of their removal, thus also for the calculation of setback distances to adequately protect groundwater sources and to assure adequate treatment of infiltrated surface water. From the above, it is clear that predictions of virus removal at larger distances are severely overestimated if they are based on removal data from column experiments or from field studies where transport was studied over short distances.

To improve predictions on the removal of viruses by soil passage, knowledge of the soil heterogeneities at the location of interest is clearly needed. Also, heterogeneity of the transported virus particles (i.e., the distribution of sticking efficiencies within the population of virus-particles), including its cause should be investigated further.

## **X. SUMMARY AND CONCLUSIONS**

### **A. Adsorption**

Many batch experiments with suspensions of viruses and soil have been carried out to investigate the effects of various factors on virus-soil interactions. Commonly, on a time scale of only a few hours, virus inactivation is negligible and equilibrium adsorption has been reported to be reached, or has been assumed to be reached. Also, in many column and field studies, adsorption of viruses during transport was assumed to reach equilibrium. This is described by retardation coefficient  $R$ , whereby a value of  $R$  greater than one reflects retarded breakthrough

due to equilibrium adsorption. However, only in some cases, retardation coefficients of about 2 to 5 have been reported. Usually little or no retardation is found. Therefore, retarded breakthrough by equilibrium adsorption is believed to be of little significance. Kinetically limited attachment and detachment mainly govern removal of viruses during transport and determine the shape of breakthrough curves. In field studies, it appeared that attachment rates were relatively fast, whereas detachment rates may be much slower. Dependent on soil heterogeneity, viruses may travel faster than the average water flow and show a smaller dispersion than a solute, because they can be excluded from small pores and, therefore, preferentially follow more permeable pathways.

In a batch suspension of viruses and soil, adsorption equilibrium is not reached instantaneously. Instead, virus attachment at the microscale can be described as the result of mass transport, within a pore, to the solid surface and subsequent immobilization at the surface by physical and possibly chemical interactions. The overall rate of attachment depends on which of these two processes is the rate-limiting step. At pH 7 to 8, as in many aquifers, the net surface charge of most viruses and soils is negative, and thus conditions for attachment are generally unfavorable. Under such conditions, virus–surface interaction is the rate-limiting process for attachment. In the absence of repulsive energy barriers, or in the presence of attractive double-layer interactions, conditions for attachment are favorable. In that case, mass transport to the vicinity of the soil surfaces is the rate-limiting step. Favorable chemical conditions for attachment may develop in groundwaters with high levels of water hardness and ionic strength. Attachment will also be favorable for solid surfaces (or patches on solid surfaces) that are positively charged due to iron, aluminium, or manganese oxide coatings. Attachment to such favorable patches may be irreversible. Several studies support the concept that viruses preferably attach only to a fraction of the soil surface having favorable charge characteristics.

Unfortunately, estimates of adsorption parameters from batch experiments appear to be of little use in predicting adsorption of viruses in column or field experiments. Values for adsorption obtained from different batch studies are even difficult to compare because there is no standard protocol. They are highly variable due to heterogeneity of soil preparations, different sizes and types of containers, different methods of agitation, and differences in the air–water interface. Most batch studies were carried out to measure equilibrium adsorption, whereby attachment and detachment rate coefficients are not determined. Only one batch study exists where the kinetic part (that is operative before equilibrium is reached) was analyzed (Grant et al., 1993). Therefore, values of attachment and detachment rate coefficients from batch studies are not available, but even if they were, they are not applicable for predicting virus removal in transport situations. Due to the stirring in a batch experiment the number of accessible sites for attachment is much higher than in a column. In a column, attachment rates are therefore much lower. The opposite is probably true for the detachment rate. The detachment rate coefficient

for a virus in a batch system is probably smaller than under transport conditions, where there is advective flow. This implies that the observed ratio  $k_{att}/k_{det}$  from a batch experiment is expected to be much larger than that from a column or field experiment. This difference between batch and column experiments has been found in several studies, where equilibrium adsorption was assumed.

A problem with many batch experiments reported in the literature is that the measurements are probably stopped too early. Almost all batch experiments are used to evaluate equilibrium adsorption coefficients. However, in most cases, it is not clear if equilibrium has been reached. The ratio of  $k_{att}/k_{det}$  may therefore have been strongly underestimated. In several column and field studies at pH 7 to 8, where kinetically limited adsorption was considered, the ratio  $k_{att}/k_{det}$  was found to be very large. This is totally opposite to the expectation that  $k_{att}/k_{det}$  from a batch experiment is expected to be larger than that from a column or field experiment. Calculations predicted that equilibrium in a batch system would be reached only after about 40 h. Also, this would mean that a low percentage of adsorption found in a batch experiment after a short period of time is rather a consequence of slow adsorption than of equilibrium.

The major factor that affects adsorption is pH. At higher pH, electrostatic repulsion increases, resulting in a decreased attachment rate and an increased detachment rate. In most aquifers, surface characteristics of the soil are heterogeneous and also different viruses with different pIs may be present. Therefore, dependent on pH and thus on the charge of the virus and soil particles, adsorption of some of these viruses may be irreversible, whereas that of others may be reversible. At pH 7 to 8, adsorption will mainly be reversible.

Dissolved organic matter may decrease virus attachment to soil because of competition for the same binding sites. Dissolved organic matter such as surfactants may disrupt hydrophobic bonds between soil and virus, resulting in an increased detachment rate. At the same time, viruses and many organic materials contain hydrophobic groups on their surfaces. Therefore, once adsorbed, bonded organic matter may provide hydrophobic binding sites for viruses. The presence of solid organic matter also may result in an increased attachment rate through hydrophobic binding. The enhancing and attenuating effects of organic matter on adsorption of viruses and their dependence on soil and virus properties make their quantification very difficult, especially under field conditions. Effects of organic matter will therefore be responsible for considerable uncertainty in predicting virus removal.

## B. Inactivation

Commonly, virus inactivation is considered as a first-order rate process. Inactivation rates of viruses that are attached to solids may be reduced or enhanced. In this article, values of the inactivation rate coefficient  $\mu_s$  for attached viruses were

calculated using data from some batch studies. It appeared that enhanced or reduced inactivation is very much virus-specific and almost independent of the ratio  $k_{att}/k_{det}$ . Reduced inactivation may be the result of protection from proteolytic enzymes or other virus-inactivating substances, increased stability of the virus capsid when attached, prevention from aggregate formation, and blocking from ultraviolet radiation. Attachment of viruses to solids may also significantly reduce inactivation by preventing them from entering into the air–water interface, or the air–water–solid contact line. Enhanced virus inactivation in the presence of soil may also occur in some cases, probably due to repeated attachment and detachment cycles.

Temperature is the most important factor that influences virus inactivation. Inactivation rates increase with temperature. The temperature sensitivity of  $\mu_l$  depends on the type of virus. Probably,  $\mu_s$  values change similarly with temperature as  $\mu_l$  values. Inactivation of some pathogenic viruses, such as HAV, is very insensitive to temperature changes. Microbial activity may increase inactivation rates of viruses under aerobic conditions.

### C. Model Viruses

Adsorption and inactivation are strongly virus dependent. One way to model the removal of pathogenic viruses by soil passage is to use a cocktail of viruses that represent a range of these characteristics. Alternatively, a virus that adsorbs less and is more stable than other viruses under certain conditions may be considered as a worst-case model virus.

MS2 meets the requirements of a worst-case model virus, provided the water temperature is less than about 10°C and the soil does not contain too many hydrophobic sites. PRD1 may also be considered as a relatively conservative model virus under field conditions in sandy soils at pH 6 to 8 and with low organic carbon content. In addition, PRD1 is more stable than MS2 at higher temperatures.

Bacteriophage  $\phi$ X174 may be a relatively conservative model virus because of its low hydrophobicity and stability. Thus, in soils with a high organic carbon content,  $\phi$ X174 would be a better choice as a model virus than for example MS2 or PRD1. However, the pH value will strongly determine whether  $\phi$ X174 will behave conservatively. There is a significant change in its adsorption behavior when pH varies from 6 to 8, a very common pH range for most soils. At pH 6 the net surface charge of  $\phi$ X174 will be positive (high attachment), but at pH 8 it will be negative (low attachment).

FRNAPHs, as a group of naturally occurring viruses, are very useful model viruses. They behave relatively conservatively, like MS2, also an FRNAPH, and they have been shown to be very persistent. Moreover, FRNAPHs that are present in surface water or treated wastewater that is used for recharging groundwater, consist of stable and poorly adsorbing viruses.

In practice, only few viruses have been used as model viruses. Among them, MS2 seems to meet the requirements for a model virus very well. Nevertheless, when carrying out a field experiment, it is advisable to make use of two or three model viruses that span a range of properties, such as size, surface charge, and hydrophobicity. In that regard MS2, PRD1, and  $\phi$ X174 make a promising cocktail.

#### D. Virus Removal

In a recent field study, it was found that virus removal was determined mainly by attachment (Schijven et al., 1999). Similarly, in other field studies, virus inactivation was considered to be insignificant, and therefore virus removal was considered to be solely determined by attachment (Bales et al., 1995, 1997; Pieper et al., 1997; DeBorde et al., 1998, 1999). Although detachment rates were not calculated in these studies, the shapes of their breakthrough curves suggest that detachment rates were much lower than attachment rates. Although large numbers of laboratory studies have been performed to investigate the factors affecting virus transport through the subsurface, only few well-defined field studies have been carried out. Therefore, only very few data are available on attachment and detachment of viruses under field conditions. To be able to quantify virus removal under various field conditions, more values of attachment, detachment, and inactivation rate coefficients are needed. DeBorde et al. (1998) proposed to generate a database of a limited number of field studies that span the range of hydrogeological settings, conditions, and viruses. This article may be considered as a first step toward such a database because it provides an inventory of field studies and parameter values obtained from these studies, even though these data are incomplete. An inventory also has been made of sticking efficiencies, values of attachment, detachment, and inactivation rate coefficients for attached and free viruses both at laboratory and field scale.

Field experiments are costly, complex, and often raise more questions. It may therefore still be useful and desirable to carry out column and batch experiments to obtain parameters that can be extrapolated to the field situation. Possibly, values for sticking efficiencies as found in column experiments are similar to those from field experiments, at least for short travel distances. The ratio of  $k_{att}/k_{det}$  from column-scale possibly may be extrapolated to field-scale under similar conditions. The values for inactivation rate coefficients as found in batch experiments may be used for prediction of virus transport at field scale. The possibility of extrapolating parameter values from laboratory scale to field scale needs to be verified. Furthermore, laboratory scale experiments are needed to compare removal of model viruses with that of pathogenic viruses.

Removal of viruses by soil passage appears to decline with distance. Possible causes for this nonlinear removal may be heterogeneities within the soil as well as within the population of transported virus particles. In the aqueous environment,



viruses appear to be partially attached to other colloidal particles. This may explain heterogeneity of the adsorptive characteristics of transported virus particles. The nonlinearity of removal with distance has important consequences for prediction of virus removal, thus also for the calculation of setback distances that are needed to adequately protect groundwater sources and to ensure adequate treatment of infiltrated surface water. Predictions of virus removal at larger distances are severely overestimated if they are based on removal data from column experiments or from small-scale field studies. To improve predictions on the removal of viruses by soil passage, knowledge of the soil heterogeneities at the location of interest is needed. Also, heterogeneity of the transported virus particles (i.e., the distribution of sticking efficiencies within the population of virus-particles), including its cause, should be investigated further.

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