Adsorption of Viruses on Activated Carbon

Equilibria and Kinetics of the Attachment of *Escherichia coli* Bacteriophage T_4 on Activated Carbon

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■ Information is needed about the removal of viruses in waste treatment and water purification. Virus removal has been described as adsorption in the activated sludge, flocculation, and filtration processes. Adsorption of *Escherichia coli* bacteriophage T_4 on activated carbon was studied in an agitated solution containing virus and carbon under controlled conditions of temperature, pH, ionic composition, and reactant concentrations. Surface area and particle size distribution were established for the activated carbon. The kinetics of bacteriophage T_4 adsorption on activated carbon can be described by a reversible second-order equation, first-order with respect to both virus and carbon concentration. Adsorption obeys the Langmuir isotherm and is reversible. Virus is not inactivated by adsorption. Adsorption is unimolecular, with very inefficient use of the available carbon surface area, and is of a physical nature and independent of temperature. The virus is probably completely excluded from pores because of its size.

Viruses occurring in water can be introduced into surface waters and municipal sewer systems by human and animal feces (Kelly, 1957). Considerable numbers of enteric viruses are excreted in feces—for example, 10^5 to 10^6 viruses per gram of feces can be recovered from immune humans 6 weeks after administration of Sabin polio vaccine (Sabin, 1955). Typical densities of enteric viruses in sewage average about 7000 viruses per liter of raw, untreated sewage (Clarke and Kabler, 1964).

Contaminated water was implicated in an infectious hepatitis epidemic in New Delhi, India (Dennis, 1959) and in the United States, and polymyelitis virus has been isolated from well and river water (McLean, 1964; Clarke and Chang, 1959). Enteroviruses have caused major problems in swimming pools and along beaches in the United States (Kelly and Sanderson, 1961). Improved methods of handling water for domestic use are obviously needed.

Poppe and Busch (1930) and Pyl (1931) used carbon to adsorb the virus of foot and mouth disease, rendering dilutions of virus noninfectious. Later, after inoculation, carbon released the virus causing infection in guinea pigs. Activated carbon was recently used to adsorb polio virus and infectious hepatitis virus (Clarke and Chang, 1959).

Materials

West Virginia Pulp and Paper Co.'s Nuchar C-190 activated carbon, derived from a black ash by-product of a chemical pulping process, was used in granular (30-mesh) and powdered form. Coconut charcoal (4- to 16-mesh) was also evaluated; it was not activated and consequently was not as porous as the activated carbons.

Sieving yielded more uniform particles, deionized water rinses removed dust, and the adsorbents were then dried in thin layers at 105°C. for 24 hours. Stock solutions of known carbon concentration were prepared and sterilized. Adsorbent evaluation was based on the capacity for adsorbing virus, ease of handling, and resistance to further fractionation.

Size 26/35 was obtained from Nuchar C-190 granular carbon (particles passed a U.S. standard sieve No. 26 but were retained on No. 35) and size 120/140 from Nuchar C-190 powdered carbon. Coconut charcoal was rejected because of low capacity. Fracturing was visually noticeable in the granular carbon after 10 hours' agitation, exposing new surface areas and complicating interpretation of the adsorption process. The powdered carbon (Nuchar C-190, size 120/140) was selected for the extensive adsorption studies because it had the highest adsorption capacity for bacteriophage T_4.

More complete particle-size distributions were determined on the size 120/140 activated carbon, suspended in water to give uniform dispersions for counting, by microscopic observation, with a Porton's Graticule. The 923 carbon particles measured yielded a geometric mean diameter of 2.5 microns and a standard deviation of 2.00. Average surface area was computed by assuming spherical particles, considered justifiable for this carbon: 98.5×10^7 sq. microns per mg. of carbon, or approximately 1 sq. meter per gram. Particle concentration in the stock solution was determined microscopically with a counting cell.

The specific surface area of Nuchar C-190 was 700 to 900 sq. meters per gram, based on nitrogen adsorption and application of the Brunauer-Emmett-Teller isotherm (West Virginia Pulp and Paper Co., 1964). Total pore volume was 0.9 cc per gram. The pore-size distribution for Nuchar C-190 was not shown but was assumed to resemble like carbons with average pore diameters of 20 A. and pore volumes of 0.6 cc. per gram for pore diameters less than 300 A. Pores with diameters greater than 1200 A. contribute very little to total pore volume.

The main criteria in selecting a virus were feasibility of assay and stability under adverse conditions. Assay techniques were more advanced for bacteriophages than for animal viruses. Properties of *Escherichia coli* bacteriophages are well established and the host is easily maintained. *E. coli* bacteriophage T_4 (Figure 1) was selected for the present study because of its stability to agitation and temperature changes (Table I).

Methods

The assay procedure for T_4 bacteriophage was obtained from Robert S. Edgar. Before assaying, the virus solution was diluted in tryptone broth to yield about 300 plaques per plate (Table II). A liquid tryptone top agar mixture was pre-

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Figure 1. Electron micrograph of *Escherichia coli* bacteriophage T_4

Approximate magnification 3.4×10^5. Negative stain. Provided by Robert S. Edgar, California Institute of Technology

Table I. Properties of *Escherichia coli* Bacteriophage T_4

Size, mμ	
Head	65×80
Tail	100×20
Diffusion constant at 20° C., sq. cm./sec.	0.8×10^{-7}
Specific weight, gram/particle	3.3×10^{-16}

pared from 2 ml. of tryptone top agar, 0.15 ml. of a concentrated solution of *E. coli* B cells in the log growth phase, and 0.05 ml. of the virus solution to be assayed. Solidified agar plates were made using a tryptone bottom agar. Tryptone top agar at 45°C. containing the virus and host cells was poured over the bottom agar. After solidification of the top agar, the plates were incubated at 37°C. for 10 hours. Infection of bacterial cells by the progeny of a single virus caused readily distinguishable clear spots, or plaques, which were counted to determine the initial number of viruses.

Duplicate plates from each sample increased the accuracy. Accuracy and reproducibility varied significantly with the growth phase of the host cells. Host cells in the log growth phase yielded three times as many plaques as cells in the endogenous growth phase.

Reproducibility of the assay also varied with incubation time of the plates. The maximum number of plaques was found between 10 and 14 hours of incubation. For shorter periods plaque formation was not complete and for longer periods some plaques were destroyed by antagonistic properties of the host cell.

Table II. Composition of Media for *Escherichia coli* Bacteriophage T_4

(Constituents per liter of water)

Dehydrated Tryptone	Agar	NaCl
	Tryptone Bottom Agar	
10.0	10.0	5.0
	Tryptone Top Agar	
10.0	6.5	5.0
	Tryptone Broth	
10.0	...	5.0

Stock solutions of T_4 bacteriophage were prepared by mixing virus with *E. coli* cells in a tryptone agar solution, pouring the mixture on a previously prepared agar plate, and allowing it to solidify. After incubation for 24 hours at 37°C., the bacteriophage was harvested by pouring 5 ml. of tryptone broth on the plate. After about 2 hours, all available virus diffused into the broth, and the liquid was poured off and centrifuged to remove bacterial cells. Virus stock prepared in this manner usually titered 10^8 to 10^9 virus particles per ml.

Escherichia coli, strain B, served as host for T_4 bacteriophage. Log growth phase cells were prepared by inoculating tryptone broth with *E. coli* from the stock culture, followed by incubation for 2.5 hours at 37°C. in a Gyrotory shaker. *E. coli* cells were then collected by centrifuging at 3000 r.p.m. for 20 minutes, and resuspended in fresh tryptone broth.

Glassware was washed in a detergent-alkali mixture to remove positive charges, followed by successive rinses in tap water, distilled water, and deionized water. Sterilization was accomplished in a hot air oven at 200°C. for 1 hour or longer.

During experiments adsorbent and virus were continuously mixed by a Gyrotory incubator shaker at constant temperature. Reaction solutions of known composition were made by adding stock solutions to deionized water in 125-ml. flasks. All stock chemical solutions and deionized water were autoclaved and stored at 4°C. The stock carbon suspension was shaken before use. Solutions were buffered with potassium phosphate to give a pH of 6.9 and ionic strengths of 0.08 and 0.10.

The adsorbent was either allowed to settle or centrifuged at 3000 r.p.m. for 3 minutes before sampling. Centrifugation period caused the virus to descend 0.03 cm. (Cookson, 1966). Samples of 0.1 ml. were withdrawn below 0.5 cm. of the surface and immediately diluted in 9.9 ml. of tryptone broth. Control reaction solutions without adsorbent were run in duplicate at identical temperatures, virus concentrations, and chemical compositions.

Preliminary Experimentation

Controls at pH 6.9, ionic strength of 0.10, shaken at 23°C. indicated little loss of virus from natural inactivation (Figure 2). The rate of inactivation in the control was first-order and amounted to 0.004 per hour.

Natural inactivation of virus was not significant in test solutions of pH 7.0 and ionic strengths of 0.02 to 0.20, but was significant at ionic strengths of 0.004. Influence of ionic strength on virus stability has been reported (Sproul, 1957). This phenomenon results from a physical change in the virus

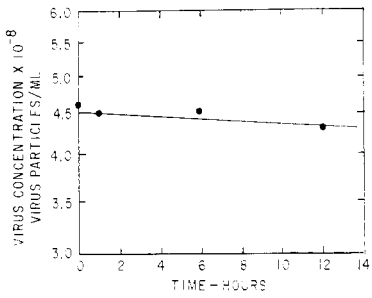

Figure 2. Natural inactivation of virus at pH 6.9 and ionic strength of 0.10
$C_t = 4.5 \times 10^8 e^{-0.004t}$

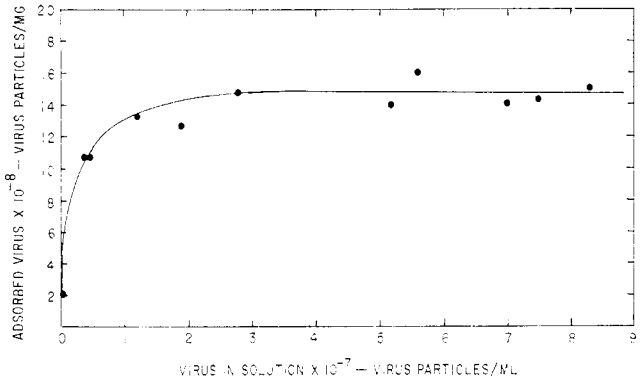

Figure 3. Relation between adsorbed virus per unit weight of carbon and virus concentration in solution at equilibrium

and will be discussed in a later communication on the role of ions in virus adsorption to activated carbon.

The temperature effect on adsorption was examined at 23° and 33°C. No significant difference was noted between the adsorption rates at these temperatures (Cookson, 1966). Physical adsorption processes are weakly dependent on temperature.

Effects of pH and ions on adsorption of bacteriophage T_4 to activated carbon were determined to clarify the mechanism of virus attachment to activated carbon, the role of ions in the primary reaction, and the physicochemical groups involved in the attachment. Detailed results will be presented elsewhere, but optimal adsorption occurred at pH 7.0 and ionic strength 0.08 (Cookson, 1966).

Adsorption Equilibrium

Equilibrium was established in the reaction solutions by both a desorption and an adsorption approach using the same carbon. After obtaining adsorption equilibrium, the carbon was removed by centrifugation. The supernatant was carefully removed with a calibrated hypodermic syringe and virus titer assayed. The volume of liquid remaining with the carbon and its titer thus were known. The carbon was then resuspended in a fresh reaction solution identical with that used for adsorption, but with no added virus.

The test solutions were placed on the Gyrotory shaker and assayed after equilibrium was established. The amount of virus carried over in solution when resuspending the carbon was computed and subtracted from the assay value at equilibrium, giving the net viruses desorbed from the carbon.

Tests were conducted with various initial concentrations of reactants at pH 7.0 and ionic strength 0.08 to determine virus adsorbed per unit of carbon and the virus concentration in solution at equilibrium. The time required to reach equilibrium was determined by periodically assaying adsorption tests over a 28.5-hour period. The amount of additional virus adsorbed after 12 hours (0.37%) was small compared to that adsorbed up to the 12-hour period. Tests run as long as 24 hours usually developed contamination. Equilibrium values, therefore, were presumed to exist after 12 hours of adsorption.

Equilibrium Results and Discussion

The adsorption isotherm for bacteriophage T_4 to activated carbon resembles a Langmuir curve (Figure 3). The Langmuir equation can be expressed by

$$c_e/q = \frac{1}{KZ} + \frac{1}{Z}c_e$$

where

c_e = virus concentration in solution at equilibrium, virus particles/ml.
q = virus adsorbed per unit weight of adsorbent at equilibrium, virus particles/mg.
Z = number of sites per unit weight of adsorbent, sites/mg. A site is defined as that which holds one virus particle
K = ratio of forward rate constant to reverse rate constant ($K = k_1/k_2$), ml./virus particle

From a least squares plot values of 1.6×10^9 sites per mg. and 4×10^{-7} ml. per virus particle, respectively, were obtained (Figure 4).

Tail fibers were the attaching unit during adsorption on host cells (Puck and Tolmach, 1954; Stent, 1963; Williams and Fraser, 1956). Tail fibers can also cause clumping of bacterial cells, undoubtedly have more than one site for attaching (Wildy and Anderson, 1964), and might also be the attaching units to activated carbon. As with long-chain polymers, a site might accommodate only segments of the chain or fiber. Such an adsorption system might be expected to deviate from the Langmuir isotherm.

Studies suggest that tail fibers are the attaching unit to activated carbon (Cookson, 1966). Tail fibers are not as flexible as long-chain polymers. Although the fibers can bend back and attach to the tail sheath of the virus, they have a fixed configuration when extended. Electron micrographs of extended fibers consistently reveal them as two straight segments with a bend about 900 A. from the end (see Figure 1). During adsorption different fibers probably occupy about the same number of attaching groups on the carbon. No significant deviation from the Langmuir isotherm would therefore be expected.

Multimolecular adsorption is not suggested by the equilibrium studies, because of agreement of the data with the Langmuir isotherm. Unilayer adsorption of T_4 phage to activated carbon is supported by comparing capacity of the carbon with the area occupied by one virus particle. Such a comparison can be made, since viruses do not agglomerate under the existing testing conditions. Equilibrium studies indi-

cate that carbon capacity was about 1.6×10^9 virus particles per mg. of carbon. A surface of 10 sq. cm per mg. was obtained for the 120/140 activated carbon. Dividing surface area by the carbon capacity yields 5.7×10^{-9} sq. cm. for the area of a virus site. The smallest and largest areas that one virus particle could occupy are 3.3×10^{-11} and 9.6×10^{-10} sq. cm. It appears that at maximum only 18% of the available carbon surface was utilized, supporting the model of unimolecular adsorption as predicted by the Langmuir isotherm.

If equilibrium was achieved in the above adsorption tests, it would also be obtained by desorption. T_4 phage was desorbed from carbon used in the previous tests and equilibrium values were determined. The data again corresponded to the Langmuir isotherm (Figure 5). A greater scattering of points, obtained for the isotherm plot, probably resulted from unavoidable experimental errors. Estimation of the amount of unadsorbed virus carried over with the carbon on resuspension reduces the accuracy. The 5-minute period of centrifugation used for removing carbon prior to resuspension later was found to have a small influence on the virus titer.

The method of least squares was used to fit a curve to the desorption equilibrium data (Figure 5). A value of 1.3×10^9 sites per mg. of carbon was obtained from the slope, compared to 1.6×10^9 when equilibrium was approached by adsorption for the same carbon. The closeness of these values indicates that essentially the same equilibrium was achieved by either adsorption or desorption.

The ability of virus particles to be desorbed from sites on activated carbon is very significant. A chemical reaction does not take place nor is the virus inactivated by adsorption. Electrostatic forces very similar to those involved in adsorption on host cells are responsible for adsorption on carbon (Cookson, 1966). Some investigators have speculated that the injection of viral DNA into host cells resulted from the electrostatic adsorption forces. Viral DNA was not ejected from the protein coat in present studies, however, because this would have created an irreversible process. Reversible adsorption was clearly demonstrated.

Equilibrium in Binary Solutions

In preliminary adsorption tests, tryptone broth caused a greater elution of viruses from carbon than pure buffer solutions of the same pH and ionic strength, suggesting that competition existed between tryptone and virus particles for sites. Viruses were adsorbed on carbon as above and then desorbed with tryptone added to the desorption solution at optimum pH and ionic strength. During adsorption 0.1-ml. samples of carbon plus reaction solution were withdrawn after 30, 60, 180, and 420 minutes and diluted in tryptone broth. Dilutions were 1 to 10, 1 to 100, 1 to 500, and 1 to 1000, yielding many different equilibrium points. Desorption solutions were assayed at various intervals over a 24-hour period to substantiate that equilibrium had been achieved. Total adsorbed and desorbed virus concentrations of each desorption series were known from the dilution factor, so that the amount of virus adsorbed per milligram of carbon could be computed at equilibrium after measuring virus concentration in solution.

Although the Langmuir equation is no longer valid, a qualitative approach can be taken to illustrate that competitive adsorption existed. From a Langmuir plot a value of 4.2×10^8 was obtained for the number of sites per milligram of carbon (Figure 6) reduced by tryptone from 16×10^8, explaining the greater elution ability of tryptone buffer compared to plain buffer solutions. The adsorption rate for viruses is probably also reduced.

Kinetics

In kinetic experiments various carbon and virus concentrations were buffered at pH 6.9 with potassium phosphate and at ionic strengths of 0.08 and 0.10 (Table III). Test solutions were continuously shaken at 23°C. and sampled periodically.

Adsorption of virus to carbon did not follow a first-order reaction. The expected relationship between adsorption rate and reactant concentrations would be first-order with each reactant and, therefore, second-order over all.

Equilibrium studies showed that adsorption is reversible. During the initial stages, however, the rate of the reverse re-

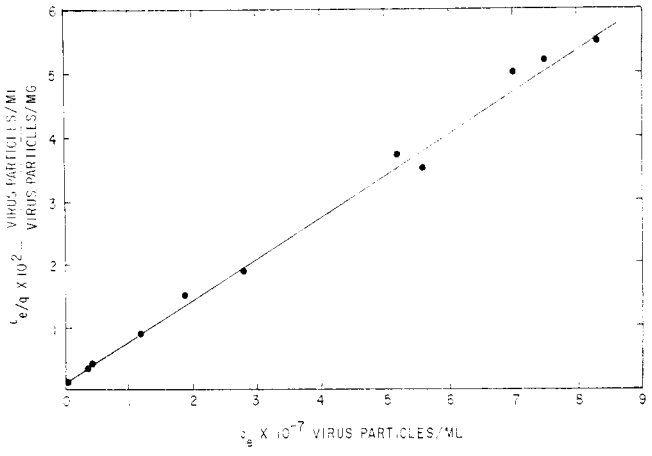

Figure 4. Langmuir plot for adsorption of T_4 phage on activated carbon by least squares analysis

$$c_e/q = 1/KZ + c_e/Z$$

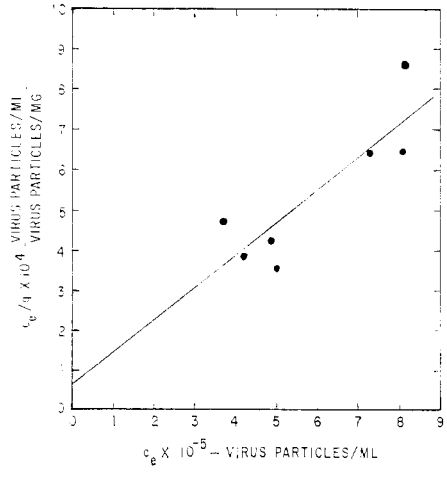

Figure 5. Langmuir plot for desorption of T_4 phage from activated carbon by least squares analysis

$$c_e/q = 1/ZK + c_e/Z$$

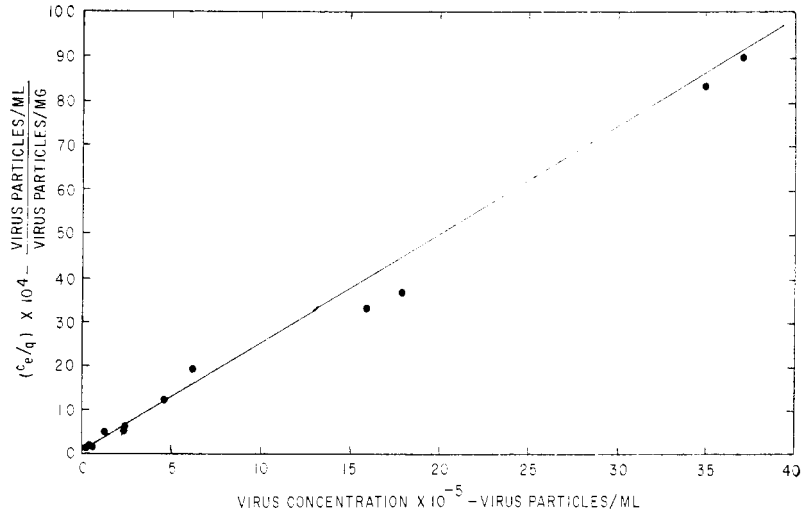

Figure 6. Langmuir plot for desorption equilibrium in tryptone broth by least squares analysis

$$c_e/q = 1/KZ + c_e/Z$$

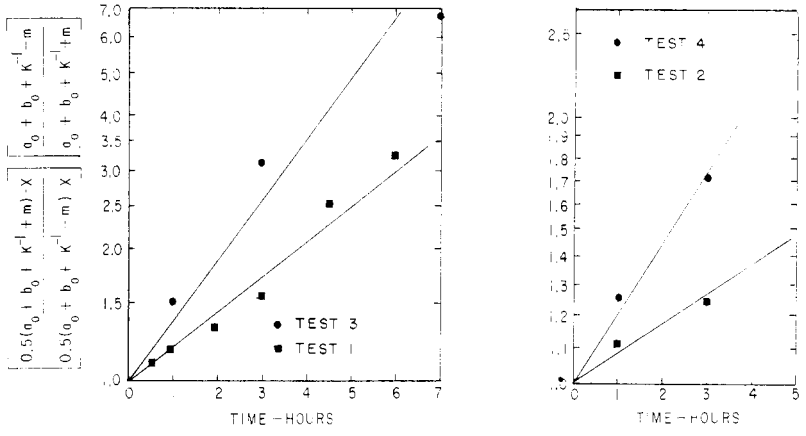

Figure 7. Reversible second-order kinetic plot of tests 1 to 4 for adsorption of T_4 phage on carbon

action is negligible and the data can be interpreted by irreversible kinetics. The mathematical representations of an irreversible and reversible system are given below.

IRREVERSIBLE

$$a + b \xrightarrow{k_1} x$$

$$\ln a/b = (a_0 - b_0)k_1 t + \ln a_0/b_0 \qquad (1)$$

REVERSIBLE

$$a + b \underset{k_2}{\overset{k_1}{\rightleftarrows}} x$$

$$tk_1 m = \ln\left[\frac{0.5(a_0 + b_0 + K^{-1} + m) - x}{0.5(a_0 + b_0 + K^{-1} - m) - x}\right] \times \left[\frac{a_0 + b_0 + K^{-1} - m}{a_0 + b_0 + K^{-1} + m}\right] \qquad (2)$$

where

a_0 = initial concentration of virus, viruses/ml.
b_0 = initial concentration of carbon sites, sites/ml.
a = virus concentration at time t, viruses/ml.
b = site concentration at time t, sites/ml.
t = time, sec.
k_1 = forward rate constant, ml./virus-sec.
k_2 = reverse rate constant, 1/sec.
K = equilibrium constant ($K = k_1/k_2$), ml./virus.
x = concentration of adsorbed virus at time t, virus/ml.
$m = [(a_0 - b_0)^2 + K^{-1}(2a_0 + 2b_0 + K^{-1})]^{1/2}$

Interpreting two adsorption tests by the irreversible equation provides a procedure for calculating the two unknowns of the system: initial concentration of carbon sites and forward rate constant. The rate constant is independent of site and virus concentrations, and the site concentrations of two

Table III. T_4 Phage Adsorption on Carbon Using Different Initial Concentrations of Reactants

Time, Hr.	Test No. 1	2	3	4	5	6	7	8
	Carbon, Mg./Liter: 250	50	100	25	250	250	250	Zero
	Virus Particles per Ml. Remaining in Solution							
0	4.8×10^8	1.0×10^8	1.0×10^8	1.05×10^8	1.2×10^8	1.5×10^7	4.7×10^5	4.6×10^8
0.5	...	...	...	...	3.8×10^7	5.4×10^6	2.7×10^5	4.3×10^8
0.6	2.4×10^8	...	...	...	...	...	...	..
1.0	2.2×10^8	7.0×10^7	4.7×10^7	9.1×10^7	2.4×10^7	3.6×10^6	2.2×10^5	4.5×10^8
1.8	1.5×10^8	...	...	...	...	...	...	...
2.0	...	...	...	...	1.6×10^7	1.3×10^6	...	...
3.0	1.1×10^8	5.7×10^7	1.7×10^7	8.0×10^7	7.6×10^6	4.6×10^5	8.6×10^7	...
4.0	...	...	...	...	9.0×10^6	2.9×10^5	...	...
4.5	6.3×10^7	...	...	...	...	...	7.9×10^7	...
5.5	...	...	...	...	6.7×10^6	2.0×10^5	...	...
6.0	4.3×10^7	...	...	...	...	...	5.5×10^7	4.5×10^8
7.0	...	5.8×10^7	6.0×10^6	9.2×10^7	3.2×10^6	8.0×10^4	...	...
12.0	...	...	...	...	...	...	...	4.3×10^8
13.5	...	5.1×10^7	4.1×10^6	7.9×10^7	...	...	...	...
23.5	...	...	3.9×10^6	7.3×10^7	...	...	...	...

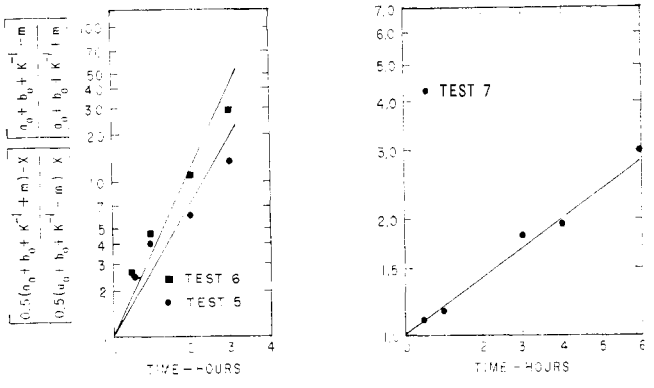

Figure 8. Reversible second-order kinetic plot of tests 5, 6, and 7 for adsorption of T_4 phage on carbon

Table IV. Rate Constants for Adsorption of Bacteriophage T_4 on Activated Carbon at pH 6.9 and Ionic Strengths of 0.08 and 0.10

Test No.	Ionic Strength	Figure	Slope, mk_1, Hr.$^{-1}$	Rate Constants: Forward $k_1 \times 10^{13}$ Ml. Virus Particle/sec.	Rate Constants: Reverse $k_2 \times 10^7$ sec.$^{-1}$
1	0.10	7	0.161	7.2	18.0
2	0.08	8	0.091	8.3	20.5
3	0.08	7	0.304	8.8	22.0
4	0.08	8	0.192	8.3	20.8
5	0.08	9	1.022	8.1	20.3
6	0.10	9	0.131	7.9	19.8
7	0.08	10	0.188	8.6	21.5

tests vary in proportion to their carbon concentrations. This procedure gave an average value of 1.9×10^9 sites per mg. of carbon and a forward rate constant of 6.5×10^{-13} per second, compared with 1.6×10^9 sites per mg. of carbon obtained from equilibrium studies.

A better representation would be reversible second-order kinetics. The adsorption data presented in Table III were linearized by plots of

$$\ln \left[\frac{0.5(a_0 + b_0 + K^{-1} + m) - x}{0.5(a_0 + b_0 + K^{-1} - m) - x}\right] \left[\frac{a_0 + b_0 + K^{-1} - m}{a_0 + b_0 + K^{-1} + m}\right]$$

vs. t (Figures 7 and 8). Values of 4.0×10^{-7} ml. per virus particle and 1.9×10^9 sites per milligram were used for the equilibrium constant, K, and the number of sites per milligram of carbon, b_0, respectively.

The forward rate constant, k_1, was obtained from the slopes of each plot (Figures 7 and 8 and Table IV). The reverse rate constant, k_2, was then calculated from the equilibrium constant. These rate constants should be identical for all tests of like pH, ionic strength, and temperature, given the proper values of K and b_0 used in Equation 2.

The rate constants varied little. At ionic strengths of 0.08 and 0.10, the forward rate constants ranged from 8.3×10^{-13} to 8.8×10^{-13} and 7.2×10^{-13} to 7.9×10^{-13} ml. per virus particle-second, respectively. This variance for a particular ionic strength is reasonable and indicates that reversible second-order kinetics can be used to represent the adsorption process.

Conclusions

The same equilibrium was obtained by either adsorption or desorption. The reversible nature of the adsorption process was also demonstrated by greater desorption in the presence of a competitive adsorbate, tryptone. Adsorption and desorption did not alter the infecting ability of the virus.

Adsorption of bacteriophage T_4 on activated carbon can be represented by the Langmuir isotherm. Such agreement suggests formation of a unimolecular layer. The maximum surface coverage of the adsorbent was estimated at only

18%. This small surface coverage not only confirms single layer adsorption, but also indicates that pore areas are probably not utilized. The size of phage T_4 probably completely excludes it from pores. All carbon sites must be approximately equal in their adsorbing ability. Application of the Langmuir isotherm yielded a carbon capacity of 1.6×10^9 sites per mg.

Escherichia coli bacteriophage T_4 adsorption on activated carbon can be described by reversible second-order kinetics. Adsorption was first-order with respect to both virus concentration and carbon sites. Equilibrium constant and carbon capacity as determined by the Langmuir isotherm gave satisfactory linearization of data when used in the reversible second order equation.

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Biophysical Limnology

Separation of Suspended and Colloidal Particles from Natural Water

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■ Many materials in the size range of suspended particles (10 mμ to 200 microns) contribute to water pollution. Such particles are heterogeneous with wide ranges of size, shape, density, and metabolic or surface activity. The distribution of many species is discontinuous and the concentration in a given volume of water is often too low for direct study. This laboratory has been developing biophysical methods, principally centrifugal, for investigating suspended particles in water. Continuous-flow, density-gradient, and zonal centrifugation are yielding most promising results for concentrating, isolating, and purifying suspended particles from large volumes of water. Centrifugal processes can yield information about density, size, and sedimentation rate. Monitoring the effluent stream from the centrifuge can yield information on chemical composition, concentration, and biological activity. Bioassay, x-ray diffraction, and electron microscopy can be used with the particles from the effluent. This laboratory is systematically investigating the physical and chemical characteristics of particles suspended in water. These data are useful in designing density gradients, centrifugal processes, and equipment, and helping to pinpoint the location of particular species of particles, such as viruses, in the effluent after centrifugation. Since large volumes of water may be used as the initial sample (up to 100 liters), the sensitivity of detection is significantly greater than by other methods.

Qualitative and quantitative fractionation of the various organic and inorganic species of particles occurring in natural water is a prerequisite to investigations of cycling of biologically or surface active contaminants in the aquatic biosphere. With the increasing release of these contaminants coinciding with increasing re-use of water, interest is developing in means of assessing the possible biohazard the contaminants may present. Evidence from this laboratory shows that contaminants such as radionuclides are not found in a single fraction, but are differentially distributed among the various fractions of the water mass (Lammers, 1964). The fraction with which the contaminant is associated determines to a large degree its importance as a biohazard, since it also determines how much of the contaminant enters the food chain and to some extent the degree of bioconcentration one can expect.

This paper deals chiefly with the suspended and colloidal-sized organic particles typically found in natural water, although the same theories and general methods apply to similar particles in other fluids and to inorganic particles. Generally, centrifugal methods are not practical for particles with a molecular weight of less than 10^6 and a sedimentation

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