KINETICS AND MECHANISMS OF ADSORPTION OF *ESCHERICHIA COLI* BACTERIOPHAGE T_4 TO ACTIVATED CARBON

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ABSTRACT

A study was conducted on the adsorption of Escherichia coli bacteriophage T_4 to activated carbon. Preliminary adsorption experiments were also made with poliovirus Type III. The effectiveness of such adsorbents as diatomaceous earth, Ottawa sand, and coconut charcoal was also tested for virus adsorption.

The kinetics of adsorption were studied in an agitated solution containing virus and carbon. The mechanism of attachment and site characteristics were investigated by varying pH and ionic strength and using site-blocking reagents.

Plaque assay procedures were developed for bacteriophage T_4 on Escherichia coli cells and poliovirus Type III on monkey kidney cells. Factors influencing the efficiency of plaque formation were investigated.

The kinetics of bacteriophage T_4 adsorption to activated carbon can be described by a reversible second-order equation. The reaction order was first order with respect to both virus and carbon concentration. This kinetic representation, however, is probably incorrect at optimum adsorption conditions, which occurred at a pH of 7.0 and ionic strength of 0.08. At optimum conditions the adsorption rate was satisfactorily described by a diffusion-limited process. Interpretation of adsorption data by a development of the diffusion equation for Langmuir adsorption yielded a diffusion coefficient of 12×10^{-8} cm^2/sec for bacteriophage T_4. This diffusion coefficient is in excellent agreement with the accepted value of 8×10^{-8} cm^2/sec. A diffusion-limited theory

may also represent adsorption at conditions other than the maximal. A clear conclusion on the limiting process cannot be made.

Adsorption of bacteriophage T_4 to activated carbon obeys the Langmuir isotherm and is thermodynamically reversible. Thus virus is not inactivated by adsorption. Adsorption is unimolecular with very inefficient use of the available carbon surface area. The virus is probably completely excluded from pores due to its size.

Adsorption is of a physical nature and independent of temperature. Attraction is due to electrostatic forces between the virus and carbon. Effects of pH and ionic strength indicated that carboxyl groups, amino groups, and the virus's tail fibers are involved in the attachment of virus to carbon. The active sites on activated carbon for adsorption of bacteriophage T_4 are carboxyl groups. Adsorption can be completely blocked by esterifying these groups.

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CHAPTER 1

INTRODUCTION

Viruses were first proposed as a special class of subcellular pathogenic agents at the turn of the century by M. W. Beijerinck (1). Viruses consist of a protein shell and an infectious core composed of deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). The shell serves as a protective jacket and in some instances for breaching cell walls during infection. The nucleic acid enters the cell and redirects cell metabolism to produce more virus.

Viruses vary in size and shape. Most viruses range from 10 to 200 millimicrons in the longest dimension. Smaller viruses are usually spherical and possess an extremely high degree of structural symmetry. Larger, more complex viruses can be elongate or even exhibit some structural flexibility.

Significant developments in animal virology date from 1925. Research effort on viruses has been directed primarily towards development of methodology and emergence of new concepts. Virus research received great impetus in 1948 when Enders and his colleagues showed that poliomyelitis virus could be cultivated *in vitro* by cell culture techniques (2). Definitive research on structure, reproduction, and infection mechanisms of viruses has been made by enhanced utilization of cell and tissue culture techniques.

1-1 Significance of Viruses in Human Illnesses

A significant character of a virus is its ability to produce disease. As a result much of the research on viruses is directed at the disease-producing process and preventing or curing diseases. In preventing disease, work has concentrated in the direction of immunization. Scant attention has been given to ridding man's environment of pathogenic viruses. Today many more viruses are known to be medically significant because increased knowledge of diseases has recently implicated many viruses. Of the few known viruses, 120 have been discovered in the past ten years (3). Additionally, recent investigations have demonstrated that many known viruses may produce diseases with which they are not yet associated (4). Previously viruses were thought to infect only specific host cells. It is now known that viruses may multiply in cells which apparently are not invaded normally (5)(6)(7). This phenomenon is not surprising when the virus particles are examined in detail.

For example, many viruses of human origin appear to be similar or identical to certain viruses of animal origin. They may be serologically unrelated but nonetheless share many characteristics. Adenoviruses contain a common complement-fixing antigen regardless of species specificity (with possible exceptions of the fowl strain). They produce similar cytopathic changes in susceptible cell cultures. Currently it is assumed that viruses of animal origin can enter human cells, regardless of serological relationships to human viruses. Newcastle disease, a highly contagious infection of chickens and turkeys, may be transmitted to poultry-plant workers by splash infection of the eye (4).

Viruses similar to Coxsackie viruses are found in swine, cattle, chickens, monkeys, and dogs (8). Some are pathogenic to man but their relationship to human disease is poorly understood (9). Other viruses of man and animals are serologically indistinguishable. The Myxovirus para-influenza, var. bovine (SF-4), associated with sleeping fever in cattle appears serologically similar to its human counterpart (10). Likewise strains of reoviruses, isolated from cattle and other animal species, are serologically indistinguishable from human strains. They have been associated with respiratory and enteric illnesses in humans (11).

The ability of non-human viruses to produce disease in man is poorly understood. Some animal viruses apparently produce only benign illnesses in their natural hosts but can produce malignant tumors in other species. Trentin (12), Huebner (13), and Girardi (14) found that two human viruses, adenovirus types 12 and 18, and simian virus SV_{40}, can induce cancer in newborn hamsters. Mouse polyoma virus also induces cancer tumors in other animals. These observations lead Trentin (12) to speculate that some viruses manifest themselves as cancer when infecting unnatural hosts. There is no knowledge of plant viruses infecting animal cells. Poliovirus, turnip yellow mosaic virus, and southern bean mosaic virus, however, have very similar characteristics (4). It is apparent that animal viruses as well as human viruses are of concern to human medicine. More investigations are desirable to understand clearly the role of viruses in human illnesses.

1-2 Aspects of Water-borne Viruses

Undisputably viruses occur in water (15)(16)(17). Many aquatic microorganisms are host cells for particular viruses, but at present only animal viruses are of major medical concern. Many are introduced into surface waters and municipal sewer systems through human and animal feces. All enteric viruses occur in excreted feces in considerable numbers. The Sabin poliovirus vaccine, reproduced in the immune human for six weeks or longer, yields 10^5 - 10^6 viruses per gram of feces (18)(19). Over 100 different viruses are excreted in human feces, including 3 types of poliomyelitis, 30 types of Coxsackie, 28 types of ECHO virus, infectious hepatitis, and various adenoviruses (20)(21)(22). The typical expected density of enteric viruses in sewage would average about 7000 viruses per liter of raw, untreated sewage (19).

1-2-1 Pathogenic Water-borne Viruses

Many types of enteric viruses are found in the gastrointestinal tract of man and higher animals. At present only a few groups of fecal viruses are of major interest. Many poorly understood viruses, however, constitute potential hazards to man. Viruses, for example, have been recently considered as possible etiological agents in the nervous and muscular degenerative diseases (4). Degenerative diseases of man for which no proven etiology exists may originate from viruses. Certainly, this has been indicated by research on animal cancer where viruses have been clearly implicated.

At present the major virus groups of interest in sewage include poliovirus, Coxsackie viruses, enteric-cytopathogenic-human-orphan (ECHO) viruses, reoviruses, adenoviruses, influenza viruses, and infectious hepatitis.

The poliovirus group consists of three primary types. Small spherical particles, 25-30 millimicrons in diameter, these viruses affect nerve tissue, causing aseptic meningitis and paralysis.

The Coxsackie viruses are divided into two groups: A, containing 28 serotypes, and B with 6 serotypes. They are also small viruses, 25-30 millimicrons in diameter. Coxsackie viruses are associated with aseptic meningitis, mild encephalitis, myocarditis, pharyngitis, pleurodynia, and obscure fever.

The ECHO viruses are also small spherical particles, 30 millimicrons in diameter. American virologists presently recognize 28 serological types (3). They cause diseases similar to the Coxsackie group.

The reoviruses, containing 4 serotypes, possess certain characteristics common to the ECHO and Coxsackie viruses, but are notably larger, being about 72 millimicrons in diameter. The virus is connected with enteric and respiratory infections in children.

The adenoviruses, 28 recognized serological types (3), are excreted in large numbers in stools of infected individuals. They are 60 to 80 millimicrons in diameter and are associated with some respiratory infections and conjunctivitis (23). Certain adenoviruses are capable of producing tumors in hamsters (3).

The influenza viruses consist of three groups, designated A, B, and C. They are spheres ranging from 90 to 120 millimicrons in diameter,

and are classified under the grouping Myxoviruses. These viruses typically have been isolated from the feces of infected individuals (24).

Infectious hepatitis has just recently been proven to be caused by a virus (25)(26). Investigations on this virus have been hindered by inability to detect and cultivate it. In spite of these difficulties, however, it has been isolated from human feces (26).

There are many other viruses in feces with some remaining undetected and unclassified. A virus-like agent has been isolated from bacteria-free filtrates of feces from diseased persons. It is considered responsible for epidemic gastroenteritis (27). Viruses of the adenoidal-pharyngeal-conjunctival (APC) group, responsible for upper respiratory disease and conjunctivitis, have also been found in feces (27). The common cold and certain other respiratory infections are due to viruses. These are yet to be cultivated and studied, but it is highly probable that they are also excreted in feces by infected individuals. It is easily seen that viruses are constantly introduced into sewage systems and their receiving streams. The fates of these viruses in water and the possibility of transmitting diseases from such sources will now be examined.

1-2-2 Virus Survival

For a virus species, a prime condition for continued survival is that the number of sources of virus shall never fall so low that probability of transfer to other susceptible hosts becomes unlikely. In the case of influenza A virus, many strains flourished for a year or two and then became extinct. At least two important infectious

diseases of the past have vanished completely ---- "English Sweats" of the Tudor period and encephalitis lethargica that flourished around 1923 (28). Presumably in both instances the epidemics were manifestations of some virus which had previously existed in a harmless form and had undergone mutation to a pathogenic type. Descendants of these viruses may yet exist in a mutated form as before. Thus the persistence of viruses in their pathogenic form is the first requirement in the proliferation of virus disease. The second requirement, which has been discussed regarding water, is that the virus must be transmitted through the environment to cause infections. We shall now consider the ability of viruses to survive in a water environment.

Viruses can survive for long periods of time in very adverse environments. Viruses such as that of human poliomyelitis have been reported to survive in feces suspended in river water for over 188 days at 4°C (29). Clarke and Chang (24) found that 99.9 percent destruction of a poliovirus suspension in water was obtained when heated to 60°C for 30 minutes. They also report that viruses suspended in proteinaceous fluids are more difficult to destroy by heat than as water suspensions. Gilcreas and Kelly (30) found that the survival time of Coxsackie virus in feces suspended in water was of the order of 200 days at 8-10°C and over 140 days at 20-30°C. These survivals are very long when compared to coliform organisms, used as indicators of water pollution. In sewage the coliform organisms drop to 1 percent of their original value in 3 weeks at 8°-10°C , but Coxsackie and Theiler virus concentrations remained unchanged for 4 months. Very slight loss of the viruses occurred after 10 months. The presence of phosphate and organic

material has been shown to aid virus survival (27). Clarke and Chang (24) also found virus survival periods to be longer in grossly polluted water than in moderately polluted water. They also reported, however, that clean water prolonged the survival period more than moderately polluted water. More fundamental knowledge is thus necessary to understand the survival mechanism of viruses in water. Clarke (19) gives the following survival times of four enteric viruses in sewage as compared to three bacterial indications of pollution. Note that survival times are shorter than indicated by the above-mentioned studies.

TABLE 1-1

Time in Days for 99.9 Percent Viral and Bacterial Reduction in Raw Sewage

	28°C	4°C
Poliovirus I	17 days	110 days
ECHO 7	28	130
ECHO 12	20	60
Coxsackie A9	6	12
Aerobacter aerogenes	10	56
Escherichia coli	12	48
Streptococcus fecalis	14	48

The discrepancies in reported survival times for viruses in sewage are probably due to the varying properties of sewage. This effect of environment was originally shown by Clarke and Chang (24).

According to the literature, survival studies have been performed mainly on Coxsackie virus and poliovirus. The survival of other sewage and water-borne viruses, such as adenoviruses, ECHO viruses, and infectious hepatitis is poorly documented. Infectious hepatitis survives at least 10 weeks in relatively clean water (24).

Season affects the type and amount of viruses present in sewage. During summer months viruses could be detected 80 percent of the time in sewage but only 10 percent of the time during winter months (31). Other investigators also found peak virus concentrations in sewage during summertime (19)(32). These findings represent only the viruses that infect mainly during the summer months and for which cultivation techniques have been well established, i.e. poliovirus, Coxsackie, some adenoviruses, and ECHO viruses. In contrast, cultivation techniques do not exist for the major portion of virus infections common during winter months. Seasonal variation in human virus infections is directly related to the virus content of sewage (33). Coxsackie Group B and ECHO virus, type 12, have been found in sewage mainly in June and July whereas poliovirus, Coxsackie Group A, and other types of ECHO viruses predominate in sewage during August and September (31). In Albany sewage, Kelly (32) has continuously found Coxsackie viruses between June and November but only sporadically during the remainder of the year.

The ability of viruses to survive in ground water and soil has been demonstrated directly by experiments and by finding viral-contaminated wells. Poliovirus and mouse encephalomyelitis viruses have extraordinary capacity to survive in soil. Murphy and coauthors (34) report that the mouse encephalomyelitis virus strain FA and the GD VII strain were actually protected by soil. The viruses were inactivated in 1 to 2 weeks in suspension, but survived 3 to 6 weeks in unsterilized soils. These workers also found an initial rapid decrease

in poliovirus, which then remained present for 6 to 7 weeks in sterile soil and 2 to 3 weeks in nonsterile soil. The initial decrease was probably due to adsorption to the soil and the inability of the assay procedure to desorb these viruses for detection. Both the mouse encephalomyelitis virus and the poliovirus were consistently resistant to microbiological degradation (34). This resistance is not surprising since viruses, although displaying essentially a protein surface, are not readily degraded by the very proteolytic enzyme, pepsin. Cereal mosaic virus has the astonishing ability to survive for years in contaminated field soils (35). Other investigations indicate that soil-borne viruses can preserve their infectivity while being harbored in soil-inhabiting microorganisms (36). For example, Grape fanleaf virus can be transmitted in soil by a nematode.

1-2-3 Transmission of Virus Disease

When enteric viruses contaminate water or air, the environment becomes a potent medium for infection. Inhaling droplet nuclei containing viruses, transferring blood, biting by insect, and direct ingestion can cause infections. The only important artificial mechanism of virus transfer is for serum hepatitis, which is transferred only by the physician's syringe, so far as known. Ingestion or inhalation of fecally contaminated material are considered the most important ways of transmitting infections (28).

Spread of virus disease by contaminated water has been conclusively demonstrated by an epidemic of infectious hepatitis in New Delhi, India. It resulted from sewage contamination of the city's water supply, which

was reported to be fully treated (37)(38). In New York, hepatitis outbreaks occurred from contamination of the municipal water supply (in this case a free chlorine residual of 0.35-0.60 ppm was insufficient to inactivate the hepatitis virus). In Kentucky infection was traced to wells contaminated by seepage from septic tanks (39). Other outbreaks of hepatitis occurred among students in Bathurst, Australia, drinking raw river water polluted by the effluent from the municipal sewage works. Similar infections developed in an Austrian mountain resort from drinking unchlorinated water contaminated by septic tanks (39). In recent years, epidemiologic evidence gathered during hepatitis outbreaks in Pennsylvania, New York, Kentucky and many foreign countries has strongly suggested that the outbreaks were caused from drinking contaminated water (40-44). Outbreaks of hepatitis resulted from consumption of raw oysters and clams harvested from sewage-contaminated beds (4)(45)(46).

Among the viral diseases, only infectious hepatitis has yielded proven epidemics from drinking water. Outbreaks of poliomyelitis, however, have been suspected from sewage-contaminated water supplies (47)(48)(49). Poliomyelitis virus has been isolated from well water supplies in both the United States and Sweden and from a creek in Ohio (24). Viruses can be present in the water distribution system of many cities. At least 6 different virus types have been isolated from four different locations in the water distribution system of Paris, France (50).

Outbreaks of sore throats and Pink eye with fever have occurred in Washington D.C. and Toronto from bathing in areas contaminated by

adenovirus types 3 and 7 (39)(47). Enteroviruses in swimming pools thus constitute a problem. These two occurrences are not the only incidents from bathing areas. On several occasions during the summer of 1959, enteroviruses were isolated from wading pools in Albany, New York (51). Para-influenza-1-virus was isolated from an indoor pool during the March, 1962 influenza epidemic (39). The spread of vesicular exanthema in Toronto, 1957, caused by Coxsackie A 16 virus was aided by backyard swimming pools (39). Incidences of gastrointestinal and ear and throat infections were high among swimmers at two beaches along Lake Michigan.

Although far from complete, occurrences listed here illustrate the need for improved methods of handling water for domestic use. A few investigations have attempted to evaluate virus removal by present methods of treating water. Such investigations have been hindered by lack of a quantitative method of assaying viruses in sewage. Assays have indicated removal of viruses in sewage only by decrease in the frequency of their isolation.

1-3 Procedures for Detecting Viruses in Sewage

As previously pointed out, the usual bacterial indications of sewage pollution are not suitable criteria for determining that sewage has been rendered non-infectious from viruses. The present methods of detecting viruses in sewage have a very low efficiency and are not quantitative.

All enteric viruses can be excreted in the feces in considerable numbers, but at present no detection method will account for all types

of enteric viruses in sewage. This problem is well illustrated by infectious hepatitis, which, until recently, could only be detected by the disease symptoms. Besides inability to cultivate all types of viruses present in sewage, assay is made more difficult by the diluting effect of sewage and entrapment and adsorption of viruses on larger solid matter. All assay methods are basically concentration mechanisms followed by infection of suitable host cells or animals.

Various concentration methods include precipitation, ion exchange, centrifugation, and combinations of these. Precipitation has involved calcium phosphate, protamine sulfate, methanol, and ammonium sulfate (52). Work has not been extensive however on this procedure. Ion-exchange resins have been investigated to a greater extent (53)(54)(55)(56). LoGrippo (53) found that a cation resin adsorbed the extraneous nitrogenous material leaving the virus in the effluent. Anion resins adsorbed both virus and nitrogenous material. The virus was eluted with 10 percent phosphate solution. Kelly (32) recommended beef-extract broth for elution of the virus from anion resins. Beef-extract broth can be injected directly into animals for testing whereas the phosphate elution is toxic to mice, and must be preceded by dialysis before injection.

A method used extensively collects the virus on cheese cloth swabs, suspended in flowing sewage. A cotton-filled maternity pad covered with a tubular surgical stockinet has also been used. The pad is removed and the fluid expressed after raising the pH to about 8. This increase in pH overcomes any adsorption of viruses to the pad at or near their isolectric point. The expressed fluid has been treated by three

methods. It can be assayed directly or the viruses can be further concentrated by centrifuging or by adsorbing and eluting from ion-exchange resins. Gravelle (55) has demonstrated that ultracentrifugation will recover almost twice as many viruses as the ion-exchange method.

Metcalf (57) reports the use of membrane filters for selectively separating bacterial and influenza virus. The virus was collected on a virus-retaining membrane and washed off. This technique may prove useful but no information on quantitative recovery is available. Another method of concentrating viruses involves centrifugation in an organic solvent but little information is available (58). The virus in phosphate buffer at pH 7.5 is mixed with 2 ethoxyethanol and 2 butoxyethanol, agitated, and centrifuged at 3000 rpm. The virus is then found in the gel-like interface between the organic solvent top layer and the aqueous bottom solution. Complete recovery of viruses has been reported. If further developed, the method might be ideal for monitoring sewage treatment plants since an expensive centrifuge is not necessary.

Regardless of the concentration procedure used, the presence of infectious particles can only be demonstrated by the infection of suitable hosts. A few viruses, such as poliomyelitis, Coxsackie, ECHO viruses, and bacteriophages can be assayed quantitatively by plaque formation. A plaque is a contiguous group of host cells infected and lysed by progeny from a single virus in the infecting solution (59). Bottle cultures of explants of monkey kidney epithelium, human amnion tissue, and HeLa cells are utilized for plaque assays. Tissue cells

can also be suspended in tubes and infected with various dilutions of the viral solution to be assayed. The "Most Probable Number" method for estimating virus concentration is then applied (60). Tissue cell cultures have not been developed, as yet, for many viruses. In these cases it is necessary to use animals and note the number infected to determine virus concentration for the solution.

All of these procedures fall short of a quantitative goal because viral particles adsorbed or entrapped on solid matter are not recovered in the assay procedure. Viruses are obviously attached to larger particles since gauze pads increase the number of viruses recovered. The pad cannot filter out the viruses and must therefore adsorb them or filter out the larger particles on which viruses are adsorbed. Kelly (61) has attempted to apply a theoretical pad concentration factor to the swab expressions. It was hoped that the method could be made quantitative. Her experiments, however, showed that the concentration factors for selected sewage parameters (E. coli, enteroviruses, COD and nitrogen) increased in the swab expressions with increasing exposure periods to sewage except for E. coli which decreased. The concentration factor, therefore, changes with exposure time, and is bound to be influenced by varying conditions in sewage affecting the rate viral particles attach to these pads. Viruses not attached initially to solid matter may not be collected on the pad at all. The procedure, like others that are presently proposed, is thus far from quantitative and makes the establishment of standards for virus pollution of water difficult.

1-4 Effects of Sewage Treatment Processes on Viruses

A review of the available investigations on efficiency of virus removal by sewage treatment processes gives very diverse results. This incongruity is apparently due to the inadequate means of assaying viruses, to experiments performed without adequate controls, and to lack of fundamental knowledge of the process.

Experiments on the destruction of viruses by sewage treatment processes have been limited to a few laboratory observations and to studies of gross effects in diversely operated field plants. Many authors attribute certain outbreaks of infectious hepatitis and other viral diseases to raw water sources that are grossly polluted by domestic sewage. In such case the concentration of viruses is so great that it cannot be adequately reduced by water purification procedures (62)(63)(64). If this situation is true, then operation of sewage treatment facilities should be modified to insure virus removal.

Investigations indicate that viruses are not necessarily removed even by secondary biological treatment. Kelly and others (19)(31) report that no decrease in viruses occurred as a result of primary treatment. Viruses were isolated in the Hudson River 400 feet from the Albany plant outfall. Secondary biological treatment processes reduce the viral content of sewage but they do not insure complete removal. Even chlorinated secondary effluents may contain viruses about 1/3 of the time (31).

1-4-1 Trickling Filters

Investigations on trickling filters indicate that viruses are not removed even after secondary settling and chlorination (19)(31). Although sewage thus treated contained viruses as frequently as raw sewage, plaque counts were reduced by approximately 40 percent. No viruses have been found on filter-stone growths. Kabler (65) reports 60 percent removal of Coxsackie A virus and 15-75 percent removal of E. coli bacteriophage. This is the total reported experimentation on virus removal by trickling filters. Hence this particular phase of water reclamation is poorly understood.

1-4-2 Activated Sludge Process

The activated sludge process has received more consideration but the knowledge is again very incomplete. In laboratory studies, Kelly (31) found that 99 percent virus inactivation occurred in 4 hours. Clarke (66) obtained 99 percent reduction in 45 minutes and 99.9 percent reduction in 6 hours. Other authors report reductions of 98 percent for Coxsackie virus and 90 percent for poliovirus (19)(67), hence a high level of removal can be obtained in the laboratory. These efficiencies are not achieved in the field as frequent isolations indicate. For example, the effluent of the Santee, California activated sludge plant consistently contains animals viruses (68).

Sludge plays an important role in the removal mechanism (19)(69). Without sludge only 60 percent removal is obtained compared with 98 percent with sludge. The removal is described as occurring in two steps, 1) aeration in the presence of sludge floc and nutrients and 2) the

floc's settling ability, which depends on its metabolic state. Aeration, redox potential, and mechanical stability of the floc were not significant parameters of the removal mechanism. It has been suggested that aerobic, mechanistic, metabolic and antagonistic characteristics of the sludge were involved in virus removal. This explanation incorporates a very complex array of removal mechanisms.

Suspended solids and other colloidal material in sewage contribute substantially to virus reduction. A 75 percent reduction in virus titer occurs within 5 minutes by simple mixing of sewage and viruses. This reduction is very rapid for a biological mechanism; hence it seems more likely that the reduction is due to physical or chemical processes such as adsorption to suspended solids and colloidal matter. The removal pattern has been shown to conform to the Freundlich adsorption isotherm, indicating adsorption (19)(66). Such a mechanism is also supported by studies of Mack and coauthors (70). They noted a higher recovery of virus from settled activated sludge than from the liquid phase of the sewage. Adsorption is the initial removal step of pathogenic bacteria. The reduction is merely a pseudo-decrease resulting from adsorption on sludge floc and not actual destruction of the organism (71)(72). After adsorption, the pathogenic bacteria are destroyed by much slower biological mechanisms. Attempts have been made to extract virus from activated sludge. The extraction methods utilize various solvents and buffer systems (66). Only a small percentage of virus has been recovered by extraction. These investigations and the data of Clarke and Kabler (19) indicate an adsorption process that forms a stable complex.

1-4-3 Anaerobic Digestion

Sedimentation in Imhoff tanks was unsuccessful in removing a significant percentage of viruses. Some investigators have not found any viruses in anaerobic digested sludge (31). Kelly and coauthors (32), however, found that Coxsackie virus survived anaerobic digestion and the virus was demonstrated more often in the sludge than in the liquid portion. As with trickling filters, the knowledge of virus destruction by anaerobic digestion is very limited.

1-4-4 Summary

In summary, the activated sludge process is the most consistent treatment method for removing viruses. Laboratory studies reveal a 90 percent removal but field results are much poorer. An adsorption mechanism has been proposed as the initial removal step, but the fate of the virus sludge complex and the infectious potential of such sludge is unknown. Little information is available on the other sewage treatment processes and no set conclusions can be drawn.

1-5 Effects of Water Purification Processes on Viruses

1-5-1 Flocculation

The effect of flocculation has been extensively studied by Chang and coauthors (73). Using aluminum sulfate and ferric chloride they found removals of 85 to 99.9 percent for Coxsackie and bacterial virus at pH 6.2 and 25°C (Table 1-2).

TABLE 1-2

Removal of Viruses by Flocculation

		Coxsackie	bacterial
$Al_2(SO_4)_3$	40ppm	86.3%	93.5%
$FeCl_3$	20ppm	96.6%	99.3%
	40ppm	98.1%	99.9%

Ferric chloride was the more efficient coagulant. In other studies by these workers, using Ohio River water, Coxsackie virus was removed to a slightly greater extent than the bacterial virus. Apparently chemical composition of the water affected removal, as demonstrated by the effect of different buffer systems. With phosphate buffer, virus removal decreased as pH increased from 5.2 to 8.2. With bicarbonate buffer, however, a maximum occurred with an optimum range between pH 6.2-7.2. Although rapid floc formed at pH 5.5 and 8.2, virus removal dropped to 80 percent. The period of floc formation is very important. Chang (74) found virus removal dropped to 85 percent if the floc was allowed to form for one minute before virus addition. After 5 minutes of floc formation with 25ppm of $Al_2(SO_4)_3$ no viruses were removed. Using 360ppm of $Al_2(SO_4)_3$ a floc 30 minutes old only removed 60 percent of the added virus. This reduction in efficiency is probably from the reduced contact time between virus and metal ion. The aluminum ions must form an aluminum-virus precipitate and under the right conditions the precipitate aggregates to form a floc.

The removal of viruses by flocculation follows a Freundlich adsorption isotherm. The virus could be reactivated if the settled

flocs were redispersed. The alum-virus precipitate, therefore, did not destroy the virus, but a high association constant exists. The dissociation is not complete at pH 7.6 (75). Sixty percent of the Coxsackie was recovered from alum floc, but attempts to separate the virus from the ferric chloride floc were unsuccessful. Using optimum floc formation, Gilcreas and Kelly (30) obtained only 40 percent removal of Coxsackie and Theiler virus and 84 percent of bacteriophage. They were able to recover 25 percent of the Coxsackie virus and 15 percent of phage from the floc. Incomplete removal of poliomyelitis by alum flocculation has also been reported (76).

1-5-2 Filtration

A few observations have been made on filtration of viruses through sand and garden soil. The data are too incomplete to yield explanations of the removal mechanism. Complete removal of poliovirus has been reported by using sand columns subjected to an upward flow. The flow rate, however, was very slow, i.e. only 3 ft/day (0.0156 gpm/ft^2) (77). At a higher flow rate of 55.8 ft/day (0.29 gpm/ft^2), Carlson found little removal of poliovirus when filtered through a 30-inch column of Ottawa sand with 0.508 mm effective size (76). Significant increases in removal could be obtained by impregnating the filter with alum floc, but this process reduced the flow rate considerably. Gilcreas and Kelly(30) found 58 percent removal of E. coli. bacteriophage and no removal of Coxsackie virus when a solution was passed through an 18-inch column of garden soil. A peculiarity exists in this latter filtration study since increasing the column length to 3 ft. removed less bacteriophage

(only 22 percent) while removing 48 percent Coxsackie virus. Penetration of viruses through 50 to 75 feet of soil has been considered established by infectious hepatitis outbreaks from well-water contamination (19).

Rapid sand filters and diatomaceous earth filters have a low efficiency in removing viruses (30)(78). Virus removals of 10 and 35 percent were obtained by rapid sand filtration of flocculated supernates (30). These same tests were capable of removing 75 percent of E. coli. This information again points out the inadequacy of assuming that bacterial indices are indicative of virus removal. Better virus removals were obtained with slow sand filtration where 98 percent reductions were obtained (30).

Using Staphylococcus albus bacteriophage, Dieterich and Ryckman are reported to have found that filtration removal of virus was due to adsorption (24). Poor adsorbents such as sand have a low efficiency and limited capacity for virus removal (79). Dieterich (79) gives additional support for adsorption mechanisms. Higher filtration rates in his studies did not affect the total number of viruses removed, but the rate of removal decreased. Twice the volume of sand removed twice as many viruses. Apparently there is only a limited number of sites on the sand for virus adsorption.

1-6 Effects of Disinfectants on Viruses

Viruses are much more resistant than bacteria to chlorination. Different types of enteric viruses vary widely in degree of resistance to free chlorine (80). This variation in resistance could be partly a result of poor chemical definition of the water used in the tests.

As with bacteria, it is advantageous to increase the contact time instead of raising the chlorine concentration to inactivate viruses (81). The viricidal efficiency is markedly affected by pH and temperature. Lowering pH from 7.0 to 6.0 reduces inactivation time by 50 percent and raising pH from 7.0 to 9.0 increases it sixfold (81). Higher dosages of chlorine are necessary for virus inactivation (10-20 mg/l) than for bacterial destruction (0.4-2.0 mg/l) (82)(83)(84).

Enteric viruses have varying resistance to iodine as well as to chlorine (85)(86). Iodine concentrations of 10 mg/l are necessary for 99 percent destruction of Coxsackie A 9 virus (87). The contact time for destruction of Coxsackie virus by iodine was 1,700 times longer than that required for E. coli. (87).

1-7 Necessity for Research in the Field

The need for removal or inactivation of viruses has been demonstrated beyond question by their transmission in drinking water and polluted streams. Animal viruses have been found in effluents and distribution systems that carry supposedly purified drinking water. Additionally viruses are much more resistant to disinfectants than coliform and most other bacteria. Potential health hazards definitely exist and are increasing rather than diminishing because of the decrease in available water resources. Treated sewage must be recirculated to the water supplies in certain areas. Industrial development has necessitated injection of treated sewage to the ground water for repeated use. There is a need to learn more about the survival and removal of viruses in sewage treatment and water purification processes.

Adsorption appears to play a major role in the removal of viruses. Where studied, virus adsorption occurs in all of the treatment processes, except disinfection. The mechanism of virus removal has been described as an adsorption process in the activated sludge, flocculation, and filtration processes. Except for studies on adsorption to host cells, little information is available on adsorption of viruses. Likewise, few kinetic data are available for virus adsorption because of assay difficulties and the lack of familiarity with virological techniques among researchers.

CHAPTER 2

ADSORPTION OF VIRUSES

2-1 The Nature of Adsorption

Adsorption is difficult to investigate. The phenomena are diverse and cannot be represented by simple relationships. A multitude of factors influence adsorption, including concentration of the material to be adsorbed, the type of dispersion medium, and any special characteristics of the adsorbent such as surface area and its chemical and physical properties. These variables are usually unknown and difficult to determine.

Adsorption can be physical or chemical. The extremes of these categories are easily distinguished, but no sharp classification can be made for intermediate cases. Physical adsorption usually takes place in seconds or minutes. Chemisorption may be rapid or very slow. Reversibility is one of the main distinguishing attributes of physical adsorption. In true chemisorption there is some degree of specific chemical interaction between the adsorbate and the adsorbent. This interaction leaves the adsorbate chemically altered and unrecoverable. The energies of adsorption may be relatively large, comparable to chemical bond formation. Physical adsorption involves van der Waals or other nonspecific forces of attraction between the solid and the adsorbate. No chemical bonding occurs. Secondary bonds, however, such as hydrogen bonding may be involved. Physical adsorption may sometimes occur first and a chemical reaction with the surface of the solid may occur more slowly.

Adsorption of nonelectrolytes from dilute solutions is similar to gas adsorption. Multilayer adsorption can occur for both but this is not the usual case. Although strong enough to compete favorably with solvent in the first adsorption layer, interactions are usually weaker in second and further layers.

Adsorption may occur at active centers on the adsorbent's surface. These centers may be corners, edges, and extra-lattice atoms which have a relatively high degree of unsaturation. The valency forces of the active centers are only partly satisfied by the lattice underneath. Adsorption thus confirms the assumption that the surface of colloidal particles is non-uniform. Adsorption of separate molecules does not necessarily occur on active centers. Molecules of high molecular weight may adsorb to crystal surfaces if some structural unit of the molecules corresponds to a lattice spacing on the crystal face. There must be active attractive forces at these points.

2-2 Colloidal Characteristics of Viruses

Viruses are colloidal in size and most range from 20 to 200 millimicrons. There are, however, noted exceptions such as members of the pox virus family, visible in the light microscope. Viruses have a shell of protein enclosing a core either of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA). The protein serves as a protective jacket for the infectious nucleic acid. The surface characteristics of colloidal particles largely determine their behavior in

solution. Hence viruses act primarily like proteins even though the internal structure is more complex.

Viruses can be called associated macromolecules. Primary and secondary bonds, as well as van der Waals forces, help link their subunits. The protein shell of viruses displays many amino acid radicals, joined by covalent bonds into long folded chains. Free carboxyl and amino groups exist at the ends of the chains and in side chains of some amino acids. Virus surfaces are contaminated with various adsorbed constituents from the host cell. The surface of a particular virus, however, is sufficiently reproducible for electrokinetic studies. In their electrokinetic properties viruses behave as typical proteins (88).

Proteins are amphoteric electrolytes, forming ionized salts with acids and alkalies. The net charge on viruses will depend on solvent pH. Carboxyl groups are almost completely dissociated at high pH while at low pH amino groups are charged. At the isolectric point the net charge is zero over a time average. Particles, however, may not behave as neutral colloids. Under certain conditions the net charge can behave like two individual charges.

The isolectric point may differ for each virus. It occurs below pH 4.2 for E. coli T_2 bacteriophage (89), at pH 3.9 for adenovirus M-2 (90), and at pH 6 for foot and mouth disease virus (91). Hydration usually stabilizes proteins at their isolectric point. Some viruses, however, precipitate at or near their isolectric point (89). This instability may be caused by the high molecular weight of viruses.

Most viruses maintain a negative charge through their pH stability range. There are usually more carboxyl than amino groups present in large proteins (92). This characteristic may account for a virus's net negative charge. Negative charges may also persist from the binding of chloride ions by ion pair formation (93). The amount of ion pair formation depends on the square of the activity of the chloride ion. The large number of charged sites on proteins tends to enhance ion pair formation. This effect produces an appreciable change in the net charge of the protein molecule.

Negative ions are usually less hydrated than positive ions and attach to hydrophobic surfaces easily. This attachment increases the abundance of negative particles in liquids. Certain areas on proteins may be sufficiently hydrophobic to become charged by this process. The major part of a protein's surface is highly hydrated because of the large number of carboxyl, amino, and other polar groups. In addition, all available evidence indicates that this mechanism is of minor importance. Charges are primarily determined by their acidic and basic groups.

2-3 Adsorption to Host Cells

Attachment of viruses to a host cell usually leads to infection. This adsorption process has been studied intensively to differentiate between adsorption of the virus and infection of the cell.

2-3-1 Bacteriophage Adsorption

Adsorption processes for bacteriophages involves at least two steps or states of adsorption. The second step is rate limiting at

high host cell concentrations and is more sensitive to temperature changes than predicted by collision frequency. Three possibilities have been proposed for the two step system. A virus might exist in two states. In one form it readily adsorbs. The other form must be converted to the first before adsorption takes place and is the limiting process. Alternatively the virus may attach to the cell in two different ways, only one of which initiates infection. The third explanation postulates that the virus adsorbs in two steps. The first step would be reversible, the second, an irreversible enzymatic process.

Puck, Garen, and Cline (94) found a reversible step virtually independent of temperature. This step is followed by a temperature-dependent reaction that is irreversible. The irreversible step is believed to be enzymatic and with an activation energy of 18,000 calories per mole (95). Garen (95) has blocked the irreversible step by 1) lowering the temperature to 0°C., 2) irradiation of the host cell, and 3) treating the host cell with zinc ions. By blocking the second step the first can be shown to be a thermodynamically reversible system (95)(96).

Fundamental investigations have been confined to bacteriophage research. Varying salt concentration invariably produces a maximum attachment rate by phage to bacteria. E. coli bacteriophages T_1, T_2, T_3, and T_4 do not adsorb to their host cells in distilled water (94) (96). Adsorption is only initiated after addition of ions. The adsorption rate is retarded at ion concentrations below and above the

optimum. Identical results are obtained with the same concentrations of LiCl, KCl, NaCl, and NH_4Cl. The salts $MgCl_2$, $BaCl_2$, $MnCl_2$, and $CaCl_2$ gave curves similar to those for univalent cations except that the maximum adsorption rate occurred at a lower salt molarity. The difference was attributed to the cations since the anions were the same (Puck, Garen, and Cline 94), but this explanation is not necessarily true. The shift in maximum adsorption rate may arise from changes in ionic strength of the solutions and not from the different cations. Garen (95) proposed that the slower rate of adsorption at ion concentrations higher than optimum resulted from competition between the ions and the virus for the same site. Alternatively cations may attach to two different kinds of sites (94). One site is thought to adsorb viruses at all ion concentrations. For cation concentrations higher than optimum the second site takes up cations and hinders adsorption.

This ionic effect on adsorption rate does not appear to be a biological phenomenon. Lag periods are usually associated with biological processes, but adsorption is immediately activated upon the addition of ions.

The viral loading capacity of bacteria cells corresponds approximately to a close-packed, single-layered array of phage on the bacterium surface (95). Under optimum conditions, adsorption rate is equivalent to collision frequency. as predicted by the von Smoluchowski equation (94)(97)(98).

Krueger (99) applied the Freundlich equation to adsorption of bacteriophage by living and dead bacteria. A saturation condition occurred, not predicted by the Freundlich equation. The Langmuir theory predicts saturation, but it could not be applied. Krueger was unable to determine the constants in the Langmuir equation because of lack of experimental data. He found no differences in reaction rates between shaken and stationary mixtures. A glycine medium produced the same rate as water. He concluded that diffusion through the medium was not limiting the adsorption process.

Adsorption of phage to bacterial cells was pH dependent as reported by Puck and Tolmach (100, 101). Adsorption was maximum at pH 7, but dropped to zero at 4.8 or at about 10.5. The low and high pHs are regions where ionization of carboxyl and amino groups respectively is suppressed. They selectively blocked carboxyl and amino groups on the virus and host cell to test whether these groups were the combining units in adsorption. Carboxyl groups on the host cell combined with amino groups on the virus, and adsorption did not result from cationic bridges between virus and host.

2-3-2 Animal Virus Adsorption

Most research investigating virus attachment to host has used bacteriophage and bacteria. Studies on animal viruses and their host cells reveal similar attachment characteristics. Influenza and some other myxoviruses, for example, attach electrostatically to specific receptor sites on the surface of host cells (28)(102).

Adsorption of influenza virus to red cells is completely inhibited at low salt concentrations and is virtually temperature

independent between 0° and $37^{\circ}C$. Only 2 percent of the cell area is used for virus adsorption (103). The adsorption rate is not diffusion limited, but it approaches the theoretical maximum for interaction between the virus and the fraction of cell area known to contain sites. Adsorption of influenza virus to host cells was reversible and an equilibrium between adsorbed and free virus appeared to exist (104).

Attachment of poliovirus to monkey kidney cells has been reported to be electrostatic, salt-dependent, and temperature independent (105). Adsorption of poliovirus to monkey kidney cells does not occur at low concentrations of $CaCl_2$ (10^{-5} M). At higher concentrations (10^{-1} M) again no adsorption was observed. Maximum rate of adsorption occurred at an intermediate concentration of $CaCl_2$ (10^{-3}M). Experiments with $MgCl_2$ yielded the same results (105). On addition of salt, immediate activation of the adsorption system occurred. Electrophoresis experiments on monkey kidney cells indicate that they are negatively charged and the adsorption of poliovirus is physical and electrostatic (105). Another similarity between bacteriophages and poliovirus and their adsorption to host cells is the inhibition of adsorption by antiserum. Possibly the antibody adsorbs on the cell surface and exerts steric hinderance to virus adsorption (106)(107).

Adsorption of Newcastle disease virus (NDV) to chick red cells also resembles bacteriophage adsorption to host cells (108)(102). Salt concentration effects, pH dependence, and temperature independence are also characteristic of NDV. The adsorption is by electrostatic bonding. The similar pH dependence suggests that binding between carboxyl and amino groups is also involved in the animal virus case.

Three strains of Coxsackie A 9 virus required optimum concentrations of salts for attachment to host cells (109). Attachment by pneumonia virus of mice (PVM) to mouse erythrocytes was likewise influenced by salt concentration (110). The virus-combining capacity was independent of ionic composition of the mixture. At least one of each ion pair tested was apparently able to cause the effect. This indicates that ionic strength may be more important than the particular ions present.

In summary, animal viruses and bacteriophage appear to adsorb to host cells by similar mechanisms. In both the attachment is exceedingly rapid, probably diffusion limited. Rates are essentially constant between 0° and $37^{\circ}C$ and are similarly affected by ions and pH. Both animal viruses and bacteriophage initially form a reversible attachment to host cells, followed by an irreversible reaction (94) (109). The irreversible reaction is temperature dependent and is believed by some investigators to be enzymatic. Other investigators do not accept the reversible and irreversible steps. It is hypothesized that the virus exists in two forms. It adsorbs reversibly as one form and converts to the second form by a temperature dependent reaction. The second form then adsorbs irreversibly.

2-4 Adsorption in Sewage

Most viruses in sewage are adsorbed or entrapped on larger particles. This phenomenon was discussed in Chapter 1 when describing the use of gauze pads for collecting viruses, the rapid initial reduction of virus titer in sewage, and removal of viruses by the activated

sludge process. Low centrifugation (6000 rpm) removes 36 to 65 percent of virus titer from sewage (111). This loss occurs from the adsorption of viruses on larger sedimenting particles.

2-5 Substances Used to Adsorb Viruses

2-5-1 Glass

Probably one of the first substances found to adsorb virus was glass. Analytical procedures for assaying viruses produced inconsistent results until virus adsorption to glassware was discovered. Soaking glassware in acid cleaning mixture leaves the surface positively charged. Electrostatic forces readily attracted the negative viruses (112). Sproul (112) found that the removal of E. coli bacteriophage T_1 by glass filters was true adsorption rather than destruction during filtration. Treating glassware with an alkali removes the positive charge and prevents adsorption. Purified virus solutions are unstable and require small amounts of protein. This "instability" could be the adsorbing activity of the glass surface of containers (113).

2-5-2 Sand

Dieterich (79) investigated the adsorption of Staphylococcus pyogenes var. albus bacteriophage to Ottawa sand. The virus and sand were brought into contact by hand shaking for about 5 minutes. The mixture was allowed to reach equilibrium for 20-30 minutes before samples were withdrawn for assay. Test results were poor and little information could be gained from Freundlich and Langmuir isotherm

plots. Very few tests were made and no proof was given that an equilibrium condition was attained in the short testing time. The experiments indicate adsorption and the existance of electrostatic forces between sand and virus. Virus adsorption could be prevented by pretreating the sand with negatively charged egg albumin and Haemosol. The virus-sand complex was very stable. Washing and vigorous shaking did not produce elution. The sand, however, could be regenerated by boiling in concentrated HCl. This unpublished work is probably the major investigation on the mechanism of virus adsorption to sand.

2-5-3 Carbon

Carbon has a high adsorption capacity and efficiency for many substances. As with sand, adsorption of viruses by carbon is essentially an unexplored field. In 1931 Pyl used carbon to adsorb foot and mouth disease virus (114). Dilutions of virus could be rendered non-infectious by treatment with carbon. After adsorption the carbon was infectious upon injection into guinea pigs. Pyl (114) reported an inconsistency in the adsorption process. He found adsorption was independent of pH, but the virus could be eluted by a change in pH. One should keep in mind that assay procedures were not quantitative at this time and only gross changes in virus concentrations could be detected. While this fact prevented assessing adsorption capacity accurately, an equilibrium point did appear to exist.

Poppe and Bush (115) mixed solutions of foot and mouth disease virus with animal charcoal. After adsorption they tested the carbon and solution separately by inoculation into guinea pigs. Results were

similar to the findings of Pyl. Except for the lowest carbon concentration the supernatant from all tests was virus-free. Inoculations of the carbon caused infection. Carbon apparently released the virus after inoculation in the animal.

More recently activated carbon has successfully adsorbed poliovirus and infectious hepatitis virus (24), although these experiments were limited in scope and have not been pursued.

2-5-4 Kaolin

Kaolin also adsorbs foot and mouth disease virus (115). Its capacity is not as great as carbon and the attachment is weaker since most of the virus can be eluted. Two cycles of adsorption and elution did not affect the infectivity of the virus.

2-5-5 Aluminum hydroxide

Adsorption to aluminum hydroxide has been studied as a concentration procedure for viruses (116)(117). Foot and mouth disease virus can be adsorbed on $Al(OH)_3$ and eluted with phosphate buffer (1/3 M at pH 7.5). By electron microscopy Matheka (118) found aluminum hydroxide aggregated foot and mouth disease virus. The centers of the aggregates were presumably small particles of aluminum hydroxide. Dissociation of the aggregates occurred upon elution with phosphate buffer at pH 7.5. A zone free of virus was formed around each particle of aluminum hydroxide. This substantiated the existence of electrostatic forces in adsorption and elution. Aluminum hydroxide normally exists in a positive state at pH below 9.2. Foot and mouth disease

virus possesses a negative charge throughout the pH range 7.0-9.2. Its isolectric point is about pH 6 (91). The elution process at pH 7.5 apparently would not reverse the negative charge of the virus. Elution may result from lowering the attracting forces to a critical point where repulsive forces are greater.

Poliovirus can also be adsorbed and eluted from Al $(OH)_3$ (116). Best adsorption occurred at pH 6.0. The most complete elution was obtained with 1/3 M phosphate buffer at pH 7.5 or with isotonic NaCl solution at pH 3.0 to 2.5. A/2/sing/1/57 influenza virus has been adsorbed on gamma-aluminumoxide. Adsorption obeys the Freundlich isotherm and is independent of pH in the range 6.3-7.8 (119).

2-5-6 Zinc hydroxide

Zinc hydroxide was used to concentrate viruses of vesicular stomatitis and infectious bovine rhinotracheitis. Complete recovery of the virus was obtained by elution with ethylenediamine-tetraacetic acid at pH 8.5 (120).

2-6 Adsorption to Ion Exchange Resins

Adsorption of virus on ion exchange resins has been mentioned above as a concentration method. Lo Grippo (53) used ion exchange resins for partial purification of viruses.

Attachment of bacteriophages T_1 and T_4 to ion exchange resins was similar to attachment to their host cells (Puck 121). He attributed the attachment to electrostatic bond formation between positive groups on the virus and negative sulfonate radicals. Salts are required to adsorb bacteriophage T_1 and T_2 to cationic exchanges, but

they are not necessary for anionic exchangers. Influenza virus action is similar, suggesting again that the same principles govern attachment of bacteriophages and mamalian viruses to host cells. Influenza virus and T_1 bacteriophage are readily eluted from cationic exchangers (121). T_2 bacteriophage, however, splits into its protein and DNA components almost immediately after attachment to the exchange resin. The splitting is probably a direct consequence of the ionic interaction.

2-7 Adsorption to Lipids

A few studies on virus adsorption have been performed using water-insoluable lipids with polar groups as adsorbents. Lipids with the polar groups--COOH,--OH,--NH_2--$CONH_2$ were tested for their adsorbing activities with influenza virus (122). The lipids included sterols as well as derivatives of long-chain alphatic hydrocarbons and all adsorbed the virus with 80-100 percent efficiency. The adsorption process is, therefore, not dependent upon specific sterochemical configurations of the adsorbent. Noll and Youngner (122) found that influenza, NDV, herper-B, herpes simplex, and vaccinia adsorbed strongly to one or more lipids, whereas poliovirus, Coxsackie, and ECHO failed to interact with any of the tested lipids. They proposed that the adsorption process in the first group of viruses is a lipid-to-lipid interaction. The second group of viruses contain no or very little lipid, so such an interaction cannot take place. They designate the first group as "lipophilic viruses" and the second as "hydrophilic viruses." Attachment of "hydrophilic viruses" to host cells could be

explained by ionic interactions. Other authors indicate that influenza and NDV, which are in the "lipophilic virus" category, also adsorb to host cells by ionic interactions. The actual mechanisms, thus, are not clearly understood.

Virus was eluted from saturated lipids (Noll and Youngner 122). As much as 30 percent of the virus was recovered in some cases while in others only 0.003 percent could be eluted. T_1 and T_2 bacteriophage have a high affinity for cholesterol (123). Neither washing in varying ionic strengths or in rabbit serum would elute the viruses. The viruses could be eluted, however, by ether-water partitioning.

CHAPTER 3

MATERIALS AND METHODS

3-1 Adsorbents and Preparation

Four commercially available materials were tested for use as adsorbents; diatomaceous earth, Ottawa sand, coconut charcoal, and activated carbon. The granular adsorbents were subjected to additional size fractionation and cleaning prior to use. From these adsorbents one (activated carbon) was selected for extensive use.

3-1-1 Diatomaceous Earth

Diatomaceous earth consists of diatom skeletons about 0.5 to 12 microns in size. It is a siliceous material and is often used for filtering.

The diatomaceous earth was cleaned by repeated washing in deionized water and dried in thin layers at 105°C for 24 hours. After drying, a stock solution of 2000 mg/l in deionized water was prepared, autoclaved, and stored at 4°C.

3-1-2 Ottawa Sand

Ottawa sand, a natural silica sand designated as Flint Shot, was obtained from Brumley-Donaldson Company. Specifications gave a fifty percent size of about 0.5 millimeters. The sand was washed three times in deionized water and dried at 105°C. It was weighed out as required and autoclaved.

3-1-3 Coconut Charcoal

Coconut charcoal of 4-16 mesh was tested for adsorption. It is a hard carbon and maintains its original particle size better than most activated carbons. The coconut carbon was not activated and is consequently not as porous as activated carbon. After washing three times in deionized water it was dried in a thin layer at 105°C. A stock solution of 20,000 mg/l in deionized water was prepared, autoclaved and stored at 4°C.

3-1-4 Activated Carbon

West Virginia Pulp and Paper Company's Nuchar C-190 activated carbon was used in a granular (30 mesh) and a powdered form. Nuchar C-190 is derived from a "black-ash" byproduct of a chemical pulping process (124). Activation selectively removes the hydrogen or hydrogen-rich fractions from the carbon to produce an open, porous residue. This effect is accomplished by exposing charcoal to oxidizing gases at high temperatures (400°-1000°C). The oxidizing gases react with a large part of the carbon, thus influencing its properties (125). Activated carbon can assume either a positive or a negative charge in water, depending on whether it was activated at 950°C with carbon dioxide or at 450°C with oxygen (126). Carbons activated in oxygen or steam are covered with carbon-oxygen complexes. The particular composition varies with the treatment of the carbon. Oxygen also adsorbs on charcoal at room temperature and is held very strongly. On heating, it comes off as carbon monoxide (127).

The matrix structure of carbon is changed very little by activation (126). Although carbon capillaries enlarge in size their shape remains

the same. X-ray studies provide strong evidence that the carbon in charcoal is arranged in platelets (126). Most charcoal is made up of platelets 10 Å thick and 20-60 Å wide. The platelet concept is supported by the expansion of charcoal by water vapor and density of carbon in the charcoal. Cylindrical capillaries have been used for convenience in calculating pore diameters for activated carbon. The conceptual structure of a cylindrical capillary honeycomb should be used with caution.

The specific surface area of Nuchar C-190 is 700-900 m^2/gm, based on nitrogen adsorption and application of the Brunauer-Emmett-Teller (Bet) isotherm (128). Total pore volume is 0.9 cc/gm. Pore diameters based on cylindrical capillaries have been determined for similar carbons (129-132). Different activated carbons of similar specific area and total pore volume also possess similar average pore diameters (Table 3-1). Very little pore volume results from pore diameters greater than 1,200 Å (132). Most pores, therefore, are less than 1,200 Å in diameter. Average pore diameters of 20 Å for activated carbon are expected from the concept of platelet structure.

The pore-size distribution for Nuchar C-190 is not available. It is expected, however, to have a distribution similar to the typical activated carbons listed (Table 3-1).

In the experiments below, additional size fractionation was performed on both types of Nuchar C-190. The carbon was separated into uniform particle sizes by sieving. From granular carbon, size 26/35 was obtained (particles passed a U. S. standard sieve number 26 but were retained on number 35). Size 120/140 was obtained from Nuchar powdered carbon. The sieved materials were washed in deionized water to remove

any remaining dust and were dried in thin layers at 150°C for 24 hours. Stock solutions of 200, 2500, and 20,000 mg/l in deionized water were autoclaved and stored at 4°C.

TABLE 3-1

Pore Characteristics of Activated Carbons

Carbon	Bet Surface area m^2/g	Total pore vol. cc/g	Pore vol. for Radius < 300 Å cc/g	Ave. Pore dia. Å
Darco	612	0.96	0.73	---
Columbia xA	1178	1.03	0.54	---
Columbia 4ACW	774	---	---	22
Columbia 8-14G	1027	---	---	18
Nuchar C-190	700-900	0.9	---	---

--- Data not available

- -

3-2 Particle Size Determination

Additional particle-size distributions were desired on the Ottawa sand and activated carbon size 120/140. The particle-size distribution for Ottawa sand was determined by sieve analysis. The sand was dried for 30 minutes at 105°C and sieved for 10 minutes on a Rototap shaker.

The particle-size distribution for the 120/140 carbon was obtained by a microscopic technique using a Porton's Graticule. Carbon particles were suspended in water to give uniform dispersions for counting. The graticule consisted of a series of circles with diameters increasing in a $\sqrt{2}$ progression. The graticule was calibrated for the objective with a stage micrometer. All carbon particles corresponding to a particular

circle on the graticule were counted within a fixed field. This procedure was repeated for all circles on the graticule. The technique gives particle-size distributions that correspond closely with sedimentation measurement methods (133).

3-2-1 Ottawa Sand

The plot of cumulative percent finer versus sieve mesh opening gives a fifty percent diameter of 0.55 mm and a geometric standard deviation of 1.21 (Table 3-2 and Figure 3-1).

TABLE 3-2

Particle Size Distribution for Ottawa Sand

Mesh Opening mm	Weight Retained gm	Cumulative Percent
pan	0.4	
0.295	0.9	0.55
0.351	2.9	1.78
0.417	14.2	5.73
0.495	31.8	25.1
0.589	17.7	68.4
0.701	4.7	92.5
0.833	0.7	98.9
0.991	0.1	99.9

3-2-2 Activated Carbon

A Porton's Graticule was used to size a total of 923 carbon particles (Table 3-3). Cumulative percent frequency versus diameter was plotted on log probability paper (Figure 3-2). The 120/140 activated carbon had a geometric mean diameter of 2.50 microns and a standard deviation of 2.00. The average surface area was obtained by assuming spherical particles. Areas for each size were determined and the total

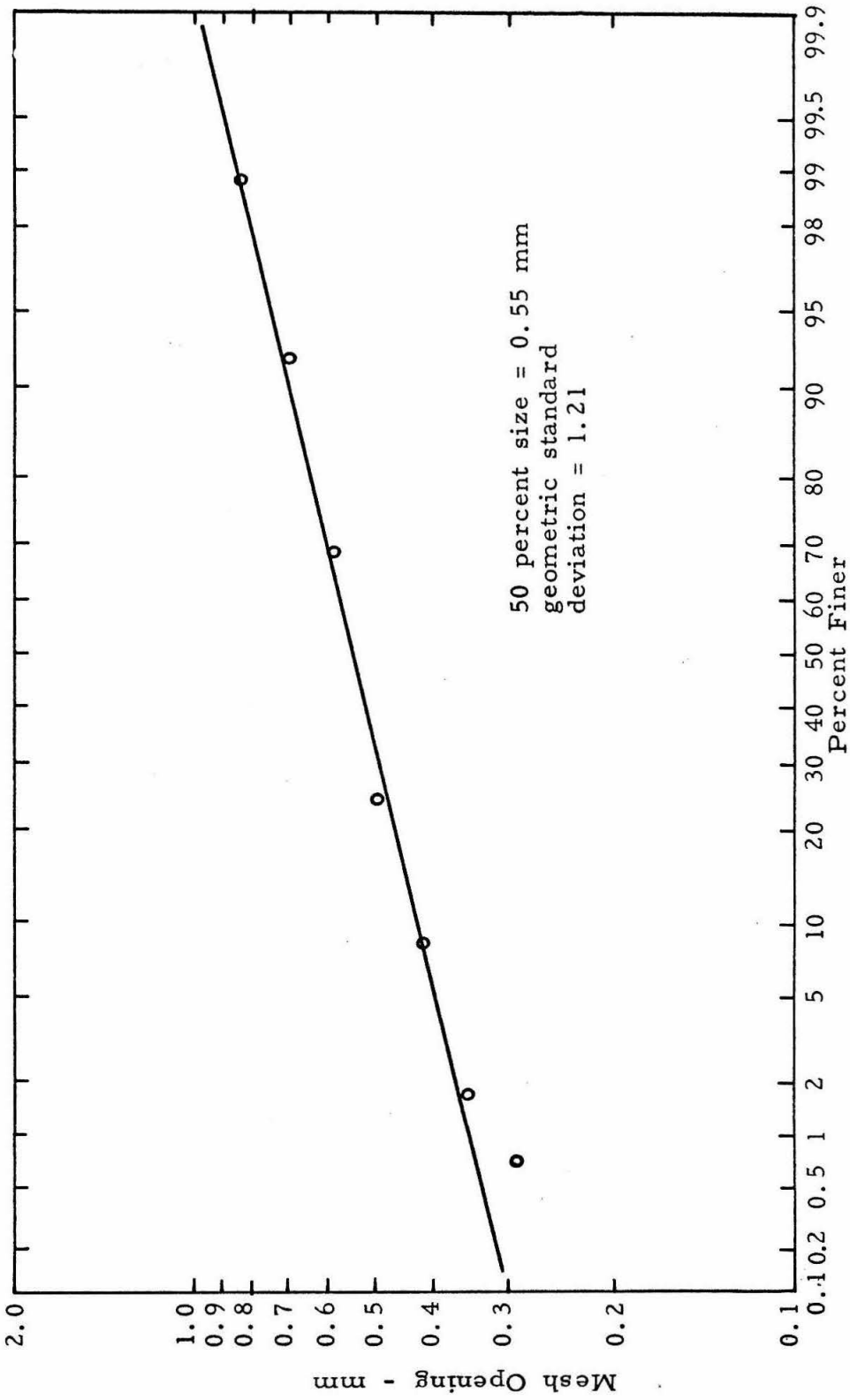

Figure 3-1. Grain-size Accumulation Curve for Ottawa Sand

sum was 88,300 square microns for 923 particles. The average area per particle was 96 square microns. The number of particles per ml of stock solution was determined microscopically with a counting cell, yielding 1.03×10^7 carbon particles per milligram. These values gave a surface area of $98.5 \times 10^7 \mu^2$/mg of carbon, or approximately 1 m^2/gm.

3-3 Viruses and Preparation

One of the main criteria in selecting a virus is feasibility of assay. Only a few pathogenic viruses can be assayed with the precision required in this study. The virus also had to be stable under adverse conditions and survive agitation. The availability of both virus and host cell are other important considerations. Some host cells require extensive methods and specialized equipment for growth and preparation.

3-3-1 Escherichia coli bacteriophage T_4

Assay techniques for bacteriophages are better developed than for animal viruses. Bacteriophage assays require about 24 hours compared with five to ten days for animal viruses, and culture procedures are simpler for bacterial hosts.

Properties of Escherichia coli bacteriophages are well established and the host, E. coli, is easily maintained. E. coli bacteriophages designated by even numbers are indistinguishable in the electron microscope and have many similar properties (97). Of this group, E. coli bacteriophage T_4 has the greatest stability to agitation and temperature changes. It was selected for the present study because of its

TABLE 3-3

Particle Size Distribution and
Surface Area for 120/140 Activated Carbon

Diameter microns	Number of Particles	Cumulative Percent	Surface Area $(\text{microns})^2$
0.9	81	8.8	225
1.3	106	20.3	590
1.9	124	33.8	1,377
2.7	183	53.6	4,040
3.8	181	73.2	8,000
5.3	111	85.3	9,800
7.5	77	93.6	13,600
10.6	38	97.7	13,400
15.0	13	99.1	9,180
24.8	6	99.7	1,170
35.0	1	99.8	3,850
49.5	1	99.9	7,700
70.0	1	100.0	15,400
Total	923		88,332

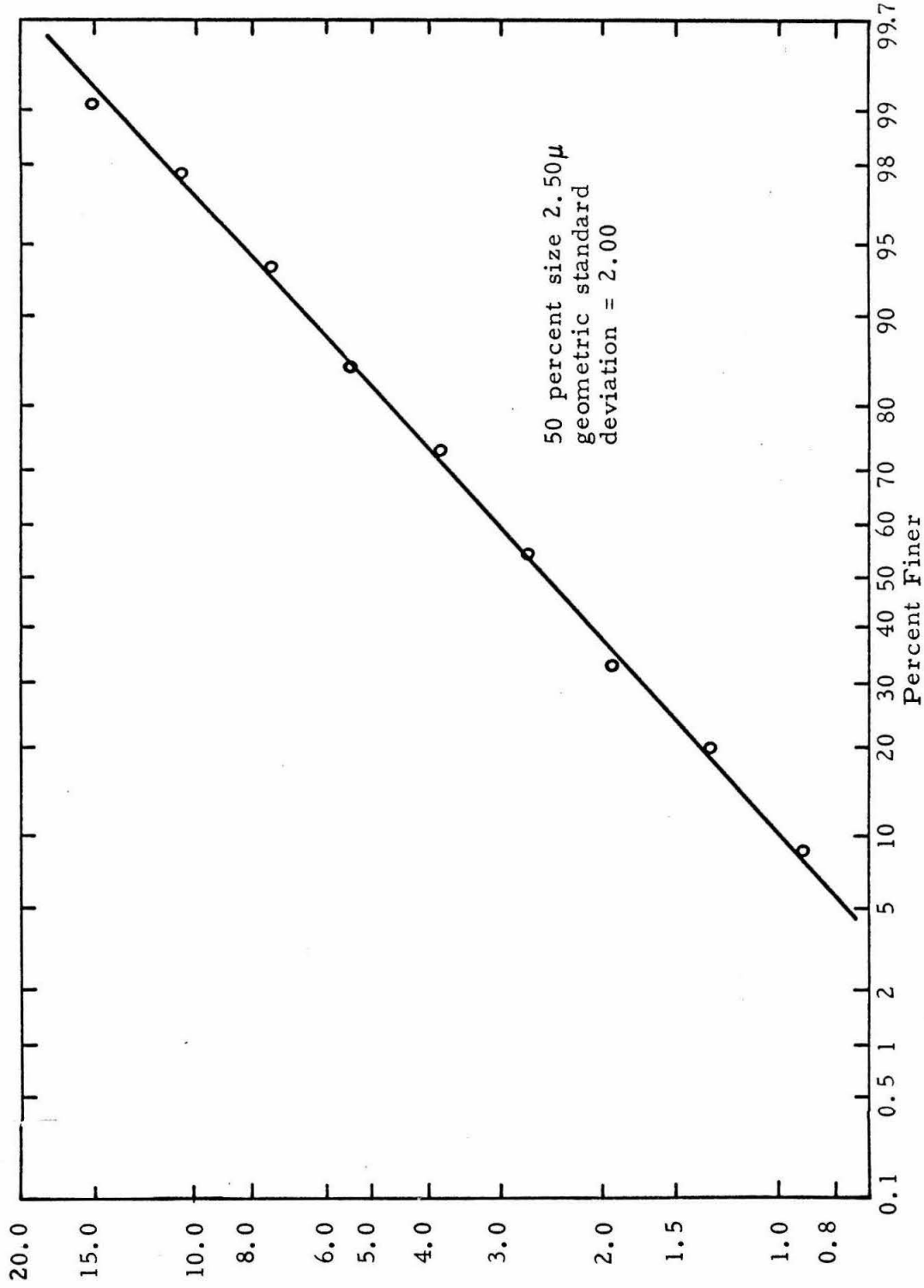

Figure 3-2. Grain-size Accumulation Curve for 120/140 Activated Carbon

stability and availability. The T_4 phage, (Figure 3-3) was obtained from Dr. Robert S. Edgar of the California Institute of Technology and has the following properties (Table 3-4) (89) (134-137).

TABLE 3-4

Properties of Escherichia coli bacteriophage T_4

Size	
head	65 X 80 millimicrons
tail	100 X 20 millimicrons
Diffusion constant at 20°C	0.8×10^{-7} cm^2/sec
Specific weight	3.3×10^{-16} gm per particle

A very similar phage, T_6, has a pH stability range between 4.6 and 9.5 (138). Putnam, Kosloft, and Neil (139) found T_6 phage to be negative over the entire pH stability range. Mobility towards the anode decreases from -7.3×10^{-5} cm^2 volt^{-1} sec^{-1} at pH 8.6 to -3.6×10^{-5} cm^2 volt^{-1} sec^{-1} at pH 5.1. The net negative charge, therefore, is decreased. The diffusion rate of T_3 does not change significantly with electrolyte concentration, but does vary with phage concentration (134). At 22°C the diffusion constant changed from 22×10^{-7} cm^2/sec to 3×10^{-7} cm^2/sec over the concentration range 10^5 to 10^9 virus particles per ml.

Stock solutions of T_4 bacteriophage were prepared by mixing virus with E. coli cells in a tryptone agar solution. This mixture was poured on a previously prepared agar plate and allowed to solidify. The plate

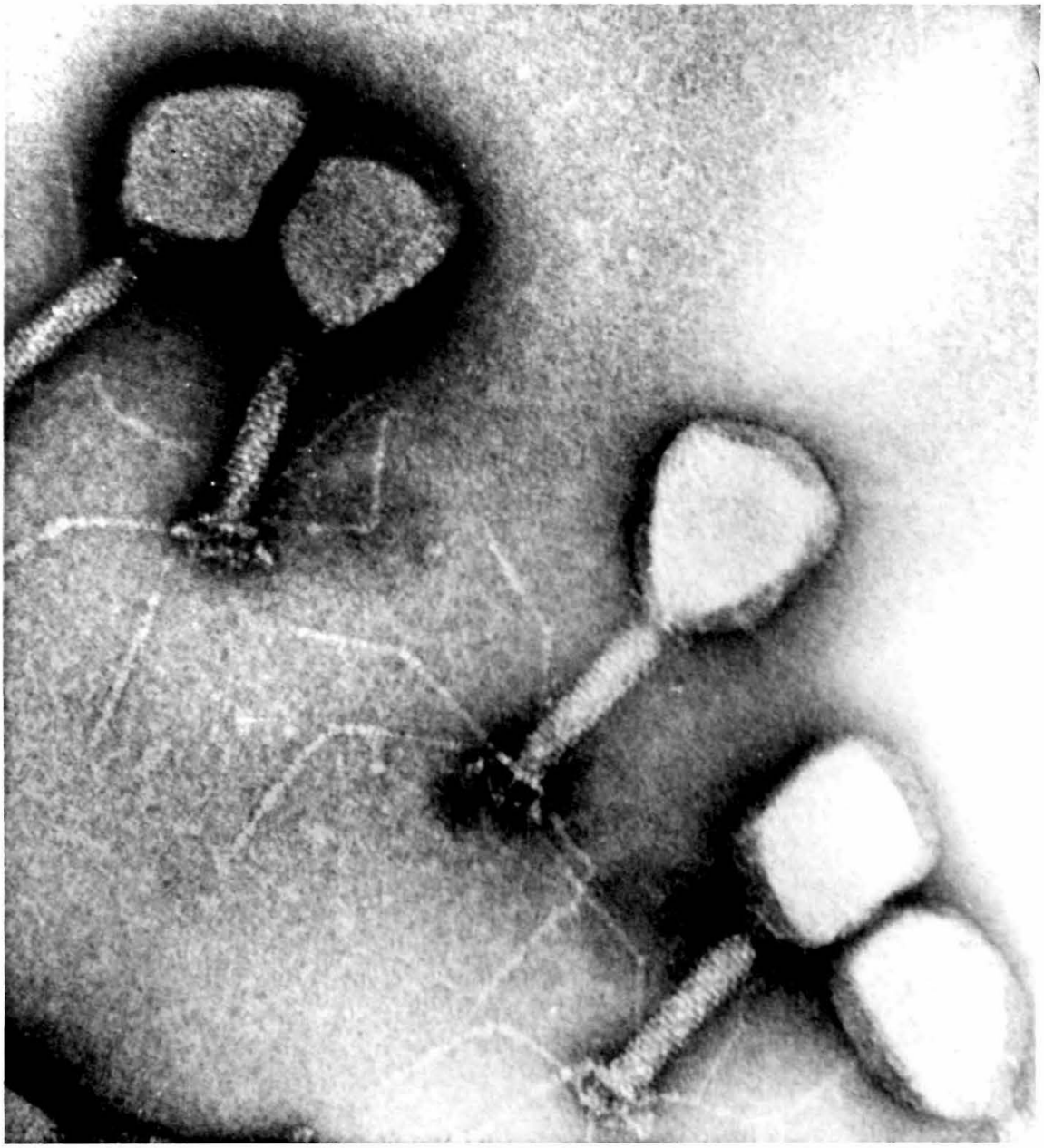

Figure 3-3. Electron micrograph of Escherichia coli bacteriophage T_4
(Approximate magnification of this electron micrograph: 3.4×10^5; negative stain; provided by Robert S. Edgar, California Institute of Technology)

was incubated at 37°C for 24 hours. The bacteriophage was harvested by pouring 5 ml of tryptone broth on the plate. After the virus diffused into the broth it was poured off and centrifuged to remove bacterial cells. This virus stock was stored at 4°C for short periods (3 months) and at -40°C over long storage intervals (6 months). Virus stock prepared in this manner usually titered 10^8 - 10^9 virus particles per ml. Preparation details for the tryptone agar and broth are given in Appendix 1.

3-3-2 Poliovirus Type III

Sabin Type III poliovirus was selected as the animal virus for the studies. Poliovirus can be assayed by the plaque method. This method has a great advantage over the use of live animals in determining the most probable dose to give 50 percent infection ($MPID_{50}$) assay. The $MPID_{50}$ does not give the necessary precision for kinetic studies (113). Plaque assays for animal viruses have a plaque-forming efficiency of about 50 percent compared with electron microscope counts.

Poliovirus will form plaques on commercially prepared host cells, eliminating the need for complex tissue-culture facilities. Sabin Type III poliovirus was used since it is attenuated, reducing the danger of pathogenic infections. The virus is spherical, 25 millimicrons in diameter, and composed mainly of nucleoproteins.

Sabin type III poliovirus was obtained from Pfizer Laboratories. The purchased virus solution was used as the stock and was stored at -40°C. The assay technique used Rhesus monkey kidney cells for hosts.

3-4 Assay Procedure for E. coli bacteriophage T_4

The assay procedure for T_4 bacteriophage was obtained from Dr. Robert S. Edgar. Before assaying, the virus solution was diluted in tryptone broth to yield about 300 plaques per plate. A liquid tryptone top-agar mixture was prepared from 2 ml of tryptone top-agar, 0.15 ml of a log-growth-phase solution of E. coli B cells, 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 a readily distinguishable clear spot, or "plaque".

After incubation the plaques could be counted with the aid of a colony counter. Duplicate plates were prepared from each sample to increase the accuracy. Samples and dilutions were stored at 4°C for reassaying if necessary. No change in sample titer could be detected over 4 weeks of storage. Preparation of all media is given in Appendix 1.

The accuracy and reproducibility of the assay procedure for E. coli bacteriophage T_4 was found to vary significantly with the growth phase of the host cells. Two separate assays of the same solution were made with log phase and endogenous phase E. coli cells.

Host cells in the log growth phase yielded three times as many plaques as cells in the endogenous growth phase (Table 3-5).

TABLE 3-5

Effects of Cell Growth Phase on Assay of Identical Solutions of *E. coli* bacteriophage T_4

Growth Phase	Assay virus particles/ml
log	2.0×10^{11}
log	1.7×10^{11}
endogenous	5.8×10^{10}
endogenous	5.4×10^{10}

The reproducibility of the assay also varied with incubation time of the plates. The maximum number of plaques was found after 10-14 hours of incubation. At earlier periods plaque formation was not complete, and at later periods some plaques were destroyed by antagonistic properties of the host cell.

3-5 Assay Procedure for Poliovirus Type III

The assay procedure was developed with the help of Dr. Robert M. McAllister of the Children's Hospital of Los Angeles. Plaques were developed on a monolayer of Rhesus monkey kidney cells which acted as host cells for the virus. The monolayer of monkey kidney cells was grown on the flat side of 2 oz plastic bottles with screw caps. Prescription bottles were also tried but they gave unsatisfactory results. The flat side of prescription bottles is slightly curved, resulting in poor cell distribution, and the optical quality of the bottles was poor for plaque counting. The plastic bottles gave better cell growth and

more distinguishable plaques. Sealed bottles were used to eliminate the need for a humidified incubator and a carbon dioxide-air mixture for cell growth.

Serial dilutions of virus samples were made in Hank's Balanced Salt solution (Hank's BSS) to obtain proper dilutions for plating. Before assaying, the growth medium and calf serum were removed from the monkey kidney cells by suction. The cells were washed three times with Hank's BSS. This was accomplished by adding 2 ml of Hank's BSS to the cells, rotating them, and drawing the solution off by suction. All such operations were performed under ultraviolet light in a hood to eliminate bacterial contamination.

Next, cells were exposed to the viruses. The diluted virus sample (0.1-0.3 ml) was transferred to each 2 oz bottle of cells and incubated for 1/2 hour at 37°C. The bottles were tilted in a slow rotating motion every 10 minutes to aid adsorption.

During the adsorption process white agar was melted and cooled to 43-44°C in a hot water bath. 2x LaYe medium was also brought to 44°C and equal parts of agar and 2x LaYe medium were mixed. After 1/2 hour of adsorption the cells were washed with 2 ml of Hank's BSS to remove unadsorbed virus. Five milliliters of the agar-LaYe medium were added to each 2 oz cell bottle and allowed to solidify. The cells were then incubated at 37°C and stained with neutral red on the third day. The staining solution consisted of equal parts of neutral red agar and 2x LaYe medium at 43-44°C. Three milliliters of this mixture were added to each cell bottle for staining and allowed to solidify. The cells were again incubated at 37°C and observed for plaques on the fourth through the seventh

day. Living cells take up the neutral red stain. This action aids in distinguishing plaques. Some of the plaques were distinguishable to the naked eye, but in most cases a low-power microscope was necessary. Poor plaques resulted when the neutral red stain was added before the third day. This condition probably resulted from a toxic affect of the stain.

As a precaution, all virus samples were first tested for bacterial contamination in thioglycollate medium. Inoculated thioglycollate medium was incubated at 37°C for 48 hours. When contamination was present the samples were treated with 100 units/ml of penicillin and 100 µg/ml of streptomycin at room temperature for 1 hour. Preparation techniques for the media and salt solution are given in Appendix 2.

3-6 Host Cells

Escherichia coli, strain B, was used as host for T_4 bacteriophage. Stock cultures of E. coli were maintained on tryptone agar plates at 4°C. New cultures were grown usually every 4 months. E. coli broth cultures were prepared by inoculating tryptone broth from the stock culture plates. The broth was incubated overnight in a Gyrotary incubator shaker at 37°C. This broth culture was used for preparing the log-growth-phase cells. It could be stored for one week at 4°C.

The log-growth-phase cells were prepared by inoculating 50 ml of tryptone broth with E. coli from 1 ml of the broth culture. This inoculation was incubated for 2.5 hours at 37°C in the Gyrotary shaker. At 2.5 hours of incubation most cells are in the log growth phase. After incubation the E. coli cells were collected by centrifuging at 3000 rpm for 20 minutes. They were then resuspended in 10 ml of fresh

tryptone broth and stored at 4°C. Log-growth-phase cells could only be stored for two days and retain their plaque-forming efficiency. After two days of storage, fresh cells were prepared.

Primary Rhesus monkey kidney cells were purchased from Microbiological Associates, Bethesda, Maryland, and from Tissue Culture Laboratory, Oakland, California. The monkey kidney cells were purchased as a monolayer growth in 2 oz bottles containing SV_5 antiserum, penicillin, streptomycin, and growth medium. The cells could be stored for 4 days at 37°C. The monolayer cultures were shipped by air in insulated containers. In many cases cells were not adequate for use because of changes during shipping and poor growth. Cell lines vary in longevity and some cannot survive the agar overlay that is necessary for plaque formation. In such cases assays were repeated with new cells.

3-7 Handling of Glassware

All glassware was washed in a detergent-alkali mixture to remove positive charges. This technique prevented adsorption of virus to the glass. After washing, all glassware was successively rinsed in tapwater, distilled water, and deionized water. Sterilization was accomplished in a hot air oven at 200°C for 1 hour or longer.

CHAPTER 4

EXPERIMENTAL PROCEDURES

4-1 Adsorption Systems

Two adsorption systems were studied. In the batch system, adsorbent and virus were continuously mixed, while the percolation system involved passing virus solution through a column of adsorbent.

4-1-1 Batch Operation

In the batch operation adsorbent and virus were continuously mixed by a Gyrotory incubator shaker at constant temperature. The reaction vessels were 125 ml Pyrex-glass flasks. Reaction solutions of known chemical composition were made by adding stock solutions to deionized water. All stock chemical solutions and deionized water were autoclaved and stored at 4°C.

The reaction solution was sampled for virus titer by one-tenth milliliter pipettes graduated in hundredths. Initial samples of 0.1 ml were directly withdrawn before the addition of adsorbent. In the presence of adsorbent two methods of sampling were used. The adsorbent was either allowed to settle or it was centrifuged for 3 minutes and 0.1 ml of the supernatant drawn off.

Samples were immediately diluted in 9.9 ml of tryptone broth. The broth was stored at 4°C and assayed as soon as possible. Duplicates without adsorbent were run at the same temperature, virus concentration, and chemical composition and served as controls. They were assayed in conjunction with the other tests.

The possibility that centrifugation influenced virus titer was detected in experiments intended for desorption studies. These desorption experiments demonstrate the necessity for investigating the centrifugation technique and will be described below. The experiments are of no value as desorption studies.

A total of five tests was made. At first it was suspected that an error occurred in the experimental procedure. In the first three tests, T_4 bacteriophage was adsorbed to 250 mg/l of 120/140 activated carbon. The pH was buffered at 7.0 with mono-and dipotassium phosphate. The buffer concentration gave an ionic strength of 0.08. Viruses were adsorbed on the carbon for 6 to 7 hours by mixing on the Gyrotory shaker at 23°C. The first three tests (4-1.1, 4-1.2, and 4-1.3) contained 1.1×10^8, 3.6×10^8, and 1.1×10^8 virus particles/ml respectively.*

Adsorption was stopped in test 4-1.1 after 7.25 hours. The carbon was removed by centrifugation at 3000 rpm and resuspended in a solution identical to that used for adsorption except that no virus was added. The mixture was placed on the shaker and assayed periodically for desorption. The supernatant from the centrifuged carbon was also assayed. The same procedure was used for tests 4-1.2 and 4-1.3 except adsorption was stopped after 6 hours and the supernatants were assayed by two procedures. In one case the carbon was allowed to settle and in the other it was centrifuged for 10 minutes at 3000 rpm.

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*Tests have been numbered according to the chapter in which they were first described. The first number designates the chapter, the second the chapter subdivision, and the third the test sequence.

Two other tests (4-1.4 and 4-1.5) were made in a similar manner. Virus was first adsorbed to 120/140 carbon and then the carbon was centrifuged and resuspended for desorption. The adsorption solution consisted of 250 mg/l of carbon and 1.4 X 10^8 virus particles/ml. Mono- and dipotassium phosphate buffer was used at pH 7.0 and adjusted to an ionic strength of 0.08. The total volume of the adsorption solution was 40 ml. This solution was periodically assayed through 7 hours of adsorption.

Ten milliliters of the carbon-virus solution were withdrawn after adsorption for one and seven hours. Each 10-ml sample was immediately centrifuged. The carbon was resuspended in 10 ml of identical solution as before except that no virus was added. These solutions were placed on the shaker and assayed over 7.5 hours.

As a result of the above tests, the need to evaluate the centrifugation technique was apparent. Centrifugation was studied with regards to its effect on virus titer of the centrifuged solution. The time of centrifugation was varied and compared by the corresponding assays. Sampling procedures of settling and centrifuging were also evaluated by comparing their results with that of no adsorbent removal before assaying. The adsorbent was removed by settling or centrifuging since desorption of virus was expected in the serial dilutions for assaying.

Effects of centrifugation were first tested by adsorbing T_4 phage to 120/140 activated carbon for 12 hours. After adsorption the reaction solution was divided. One portion was centrifuged 10 minutes and the other 20 minutes. Samples were withdrawn from the supernatant

and assayed. After the one portion was centrifuged for 20 minutes, it was shaken for 10 minutes to see if virus could be resuspended in the supernatant. After shaking, the solution was again centrifuged for 10 minutes and assayed.

Results of the above study required additional support before the effect of centrifugation on virus titer was certain. Samples were taken from an adsorption test by five different techniques. T_4 bacteriophage was adsorbed to 120/140 carbon. Initial virus and carbon concentrations were 4.5×10^7 virus particles/ml and 250 mg/l respectively. An ionic strength of 0.08 and pH of 7.0 were established in the test solution with mono- and dipotassium phosphate.

The five samples were collected after 5.5 hours of adsorption. One tenth of a milliliter of each sample was diluted in 9.9 ml of deionized water and assayed. The first 0.1 ml sample consisted of the reaction solution with virus and 250 mg/l of carbon. In the other four samples the carbon was first removed; 1) by settling, 2) by centrifuging for 3 minutes, 3) by centrifuging for 10 minutes, and 4) by centrifuging for 10 minutes, reshaking for 2 minutes, and centrifuging again for 3 minutes. If virus was centrifuged down with the carbon, the samples should give different assays. In the four cases where the carbon was removed the very top portion of the supernatant was sampled. Each 0.1 ml sample was observed in the pipette to detect if any carbon was carried over with the sample. In no case were any carbon particles noted. If some were present they were in very low concentrations.

4-1-2 Column Operation

Columns of 1/2, 3/8, and 1/4 inch diameters were packed with 0.5 gm of 120/140 activated carbon. Virus solutions of known composition were passed through the columns and the amount of removal determined by assaying the filtrate.

The 1/2 and 1/4 inch columns were glass with porous glass supports at one end. The 3/8 inch column was made from glass tubing with both ends openable. One could extrude the carbon column as a whole unit for sectional study. The carbon was supported by glass wool and the end sealed with wax. A small hole was placed in the wax seal to give a flow rate between 8 and 11 ft/day. A thin layer of diatomaceous earth was placed on the carbon to prevent the floatable portion from rising. Each column and its contents was autoclaved before use.

4-2 Preliminary Studies

Preliminary tests were made to determine the effectiveness of each adsorbent and to develop satisfactory techniques. Adsorbents were compared on the bases of their resistance to attrition, adsorption capacity, and ease of separating from test solutions. Technique development was made in conjunction with these observations. The batch and column systems were evaluated for adaptability to virus work, ease of varying adsorption conditions, and simplicity.

4-2-1 Batch Operation

Seven preliminary tests were made to evaluate the adsorbents and the batch system. Two tests each were conducted with diatomaceous earth and Ottawa sand. One test evaluated each carbon adsorbent. For

carbon and diatomaceous earth, test solutions contained 0.05 to 0.20 ml of stock T_4 phage solution, and 0.05 to 4 gm of adsorbent. The total volume was brought to 50 ml with deionized water. The test solutions using sand consisted of 1.0 ml of T_4 phage solution, 4-20 gm of Ottawa sand, and 24 ml of deionized water. These compositions gave a pH of 6.8 - 6.9 for the reaction solution. The exact quantities of adsorbent and virus in each preliminary test are given in Table 4-1. Each test was run for 10 hours or longer at 23°C. At the end of a test run the adsorbent was removed by settling and the supernatant assayed.

Activated carbon was an effective adsorbent for viruses. Two size fractions, 120/140 and 26/35, were further tested before one was selected for use. Two tests were made by the batch technique at 23°C (Table 4-2). Test solutions consisted of 20,000 mg/l of carbon and 2×10^8 and 3×10^8 T_4 bacteriophages per ml in 50 ml of deionized water at pH 6.9. One test solution contained the 120/140 size fraction and the other the 26/35. Samples were taken periodically during the complete run of 5 hours.

To compare the utility of the two viruses a preliminary test was made with poliovirus (Table 4-2). The test solution contained 250 mg/l of 120/140 carbon and 570 virus particles per ml. The pH was buffered at 6.9 with mono- and dipotassium phosphate, and the ionic strength was adjusted to 0.10 with 1.0 M NaCl. Samples were taken over a test run of 3 hours.

Five tests were made to determine a satisfactory concentration of carbon for additional testing (Table 4-3). The previous tests had

TABLE 4-1

Composition of Preliminary Testing Solutions
4-2.1 through 4-2.12
for Evaluating Adsorbents

Test No.	Adsorbent	Vol. of test solution ml	Virus concentration per ml	Adsorbent concentration mg/l	pH	Ionic strength
4-2.1	Diatomaceous earth	50	1.0×10^8	20,000	6.8	0.00017
4-2.2	Diatomaceous earth	50	3.1×10^7	80,000	6.8	0.00009
4-2.3	Ottawa sand	25	2.0×10^9	160,000	6.8	0.0034
4-2.4	Ottawa sand	25	1.6×10^9	800,000	6.8	0.0034
4-2.5	26/35 Activated Carbon	50	1.2×10^8	20,000	6.9	0.00035
4-2.6	120/140 Activated Carbon	50	2.1×10^8	20,000	6.9	0.00035
4-2.7	Coconut Carbon	50	2.2×10^8	20,000	6.9	0.00035
4-2.8	Control	50	3.5×10^7	Zero	6.8	0.00035
4-2.9	Control	25	2.0×10^9	Zero	6.8	0.0034
4-2.10	Control	25	1.6×10^9	Zero	6.8	0.00035
4-2.11	Control	50	1.3×10^8	Zero	6.8	0.00035
4-2.12	Control	50	2.1×10^8	Zero	6.8	0.00035

TABLE 4-2

Composition of Preliminary Testing Solutions
4-2.13 through 4-2.18
for Evaluating Adsorbents

Test No.	Adsorbent	Vol. of test solution ml	Virus concentration per ml	Adsorbent concentration mg/l	pH	Ionic strength
4-2.13	120/140 Carbon	50	3×10^8	20,000	6.9	0.00035
4-2.14	26/35 Carbon	50	2×10^8	20,000	6.9	0.00035
4-2.15	120/140 Carbon	20	570	250	6.9	0.1000
4-2.16	Control	50	3×10^8	Zero	6.9	0.00035
4-2.17	Control	50	2×10^8	Zero	6.9	0.00035
4-2.18	Control	20	550	Zero	6.9	0.1000

Tests 4-2.15 and 4-2.18 used poliovirus.

TABLE 4-3

Composition of Solutions for Preliminary Testing with Different Concentrations of 120/140 Activated Carbon

Test No.	Vol. of test solution ml	Virus concentration per ml	Adsorbent concentration mg/l	pH	Ionic strength
4-2.19	50	1.9×10^8	10,000	6.9	0.00035
4-2.20	50	1.8×10^8	5,000	6.9	0.00035
4-2.21	50	1.8×10^8	2,000	6.9	0.00035
4-2.22	50	1.8×10^8	1,000	6.9	0.00035
4-2.23	50	1.8×10^8	Zero	6.8	0.00035

used very high concentrations of adsorbents. The 120/140 activated carbon was used in concentrations from 1000 to 10,000 mg/l with bacteriophage T_4. The batch system was used and samples were periodically taken over 5 hours of mixing.

4-2-2 Column Operation

Three preliminary tests were made with the activated carbon columns. In the first test, five 10-ml solutions of bacteriophage T_4 were filtered through the 1/2-inch column. The column was packed with 0.5 gm of 120/140 carbon. The virus titers of the five solutions were 3.8×10^8, 6.3×10^8, 2.0×10^9, 2.0×10^9, and 6.0×10^8 virus particles per ml. The pH and ionic strength were 6.9 and 0.0003. The filtrate from each dosage was collected and individually assayed. In the second test, seven 5-ml solutions containing 2.0×10^9 T_4 phages per ml were filtered through the 1/4-inch diameter column. A pH of 6.9 and the low ionic strength of 0.0003 were used. The filtrates were assayed separately to determine the amount of virus removed.

The first two tests were conducted to gain technical experience for adsorption and desorption with columns. Desorption was attempted by passing 1.0 M NaCl and tryptone broth through the column. Previous tests showed that a 1.0 M NaCl solution had no harmful effect on the assay procedure for bacteriophage T_4. Elution was also attempted by suspending the carbon in tryptone broth. The suspension was agitated for 1 hour and assayed for viruses.

The 3/8-inch column was used in the third test. Three 5-ml solutions of T_4 virus (1.2×10^{10} virus particles per ml) were filtered through the column. After filtering, the carbon was extruded and

sectionally analyzed. The carbon column, 3.5 inches long, was divided into six sections. The first portion consisted of the diatomaceous earth top (approximately 1/8 inch). The carbon was divided into 1/4, 1/2, 1 , and 1 1/2 inch segments from top to bottom. The glass wool support constituted the sixth portion. Desorption techniques were applied to each of these sections. They were designed to determine where most of the virus was accumulating and if the virus was collecting on the diatomaceous earth layer. For desorption, the column segments were suspended in tryptone broth. The diatomaceous earth and glass wool portions were suspended in 5 ml of broth while 10 ml of broth were used for the carbon segments. The pH of the elution broth was changed from 7.4 to 8.5. The suspension was centrifuged and the supernatant assayed for viruses. The carbon was resuspended in fresh tryptone broth and once more centrifuged and assayed. The amount of virus carried over in solution with the carbon was calculated and deducted from the assayed value to obtain the amount of virus eluted. The desorption solutions were assayed three times over a 24-hour period. This procedure was used to determine if viruses were eluted from saturated sections of the carbon column only to be adsorbed again by available sites in another section.

4-3 Effects of Virus Age on Adsorption

The following experiment was set up to determine if adsorption is influenced by the age of the stock virus solution. A characteristic of some colloidal dispersions, particularly proteins, is coagulation, often called "aging". A similar effect might take place with the stock virus solutions.

It was necessary to make new virus solutions during the research period. Any difference that might exist in the virus preparations that affects adsorption must be known.

A new cultivation and stock solution of E. coli bacteriophage T_4 were prepared. Identical adsorption tests were made with this virus solution and virus from a 6-month-old stock solution. A carbon concentration of 250 mg/l was used in each test. The test solution with the old virus (4-3.1) contained initially 4.2×10^8 virus particles/ml and the one (4-3.2) with new virus assayed 1.5×10^8 virus particles/ml. The reaction solutions had an ionic strength of 0.063 and a pH 6.9 (Table 4-4). The batch system, which was selected for further studies, was used, and assay samples were taken periodically during the test run.

4-4 Effects of Temperature on Adsorption

Temperature was controlled with the Gyrotory incubator shaker. Preliminary tests described above were made at 23°C. To note temperature influences, adsorption to activated carbon was repeated at 33°C. The test solution (4-4.1) for the 33°C experiment was identical to test solution 4-2.14, tested at 23°C. Twenty thousand milligrams per liter of 26/35 carbon and 2×10^8 T_4 phages per ml were used in the test. The testing solution was unbuffered and consisted of 50 ml of deionized water at a pH 6.9. Samples were taken periodically during a 5 hour reaction time. A control flask without adsorbent (4-4.2) was also set up.

TABLE 4-4

Reaction Solutions Using T_4 Phage of Different Ages

Test No	Initial Viruses per ml	120/140 carbon mg/l	Buffer Vol. ml	Buffer Make-up	pH	Ionic strength
4-3.1	4.2×10^8	250	40	1 part KH_2PO_4 2 parts K_2HPO_4	6.9	0.063
4-3.2	1.5×10^8	250	40	1 part KH_2PO_4 2 parts K_2HPO_4	6.9	0.063
4-3.3	1.5×10^8	Zero	40	1 part KH_2PO_4 2 parts K_2HOP_4	6.9	0.063

test 4-3.1 - adsorbate was T_4 phage from a 6-month-old stock solution

test 4-3.2 - adsorbate was T_4 phage from a recently prepared stock solution

4-5 Effects of Virus and Carbon Concentration

A kinetic system can be mathematically characterized if the reaction rate depends directly on the concentration of adsorbent and solute in a form:

$$\text{rate} = KA^{x}B^{y}.$$

By varying the concentration of starting reactants it is possible to get information concerning the reaction order. The time required to consume a given fraction of one of the starting materials will depend on the initial concentration of the reactants in a way that is fixed by the order of reaction.

A group of experiments was conducted employing the batch process. The 120/140 carbon was used from stock solutions. Both carbon and virus concentrations were varied in the test solutions while the other parameters were held constant (Table 4-5). The reaction solutions were buffered with potassium phosphate at pH 6.9 and the ionic strength was raised to 0.08-0.10. As will be discussed in Chapter 5, these values gave an optimum rate of adsorption. The buffer system used a two-to-one ratio of K_2HPO_4 to KH_2PO_4. Stock buffer solutions were made 0.1 M, autoclaved, and stored at 4°C. The optimum ionic strength was obtained by adding a sterilized solution of 1.0 M NaCl. Sterile deionized water was added to give a final volume of 40 ml. The flasks containing the test solutions were placed on the Gyrotory shaker at 23°C. Samples were taken periodically during the test run.

To characterize a kinetic system the initial concentrations of both reactants must be known. Although the initial concentration of viruses can be measured, the number of available carbon sites has not been

TABLE 4-5

Initial Concentrations of Reactants for Testing Effect of Different Reactant Concentrations on Adsorption

Test No.	Ionic strength	Carbon mg/l	Virus particles per ml
4-5.1	0.10	250	4.8×10^8
4-5.2	0.10	50	1.0×10^8
4-5.3	0.08	100	1.0×10^8
4-5.4	0.08	25	1.05×10^8
4-5.5	0.10	250	1.2×10^8
4-5.6	0.08	250	1.5×10^7
4-5.7	0.08	250	4.7×10^8
4-5.8	0.10	Zero	4.6×10^8

established. One method of determining the number of sites is by equilibrium studies (to be discussed in section 4-6). The problem can also be approached by using a mathematical representation of the system plus results from two adsorption tests. The difference in the initial site concentrations of the two tests, however, must be known, as well as the changes in virus concentration. The difference in available sites between two test solutions can be easily established by using fresh carbon and carbon that has adsorbed a known amount of virus. If the mathematical representation of the reaction is correct, and the proper number of initial carbon sites are assumed, the reaction rate will be the same for both the new and the used carbon. This state will be true only if the adsorption conditions are identical for the two tests.

The two experiments with new and used carbon were made in one continuous test. In the first step bacteriophage T_4 was adsorbed on the 120/140 carbon for 7 hours. In the second step a new dose of virus was added to the reaction solution and adsorbed for 7 more hours. The virus concentration was continuously monitored in both steps of the experiment.

Many of the tests were multipurpose. This is true of the first step of this experiment which was also used to compare the effect of virus age on adsorption (Table 4-4, test number 4-3.1). The second step of this experiment was initiated after 7 hours of adsorption, by raising the virus concentration of the reacting solution (4-3.1) to 1.05×10^8 virus particles/ml. The chemical composition of the reaction solution remained unaltered.

4-6 Equilibrium Condition

Adsorption is ideally represented by isotherms. They express the variation in quantity of material adsorbed with concentration and in many cases have theoretical significance. Fundamental knowledge on adsorption can be obtained from such isotherms. Characterization of adsorption by isotherms requires an equilibrium condition between adsorption and desorption. In this set of tests the amount of virus adsorbed per unit of adsorbent at equilibrium was determined for various concentrations of reactants. Most of the previous experiments were not carried to an equilibrium condition.

The time required to reach equilibrium was determined by periodically sampling a long-term adsorption test. Other tests were then made with various carbon concentrations. Samples were taken at the start of each test and after equilibrium was established.

Bacteriophage T_4 and 120/140 carbon were used as the reactants. An optimum pH of 7.0 and ionic strength of 0.08 - 0.10 were maintained with phosphate buffer and sodium chloride. The temperature of each test was held at 23°C. Carbon and virus concentrations for each test are given in Table 4-6.

4-7 Desorption from Carbon

Establishment of an equilibrium between adsorption and desorption is not sufficient to prove that a state of thermodynamic equilibrium has been achieved. For thermodynamic equilibrium the same equilibrium is achieved whether the approach is by desorption or by adsorption. In the presence of a competitive adsorbate the equilibrium point should be

TABLE 4-6

Initial Concentrations of Reactants
in Equilibrium Tests
of T_4 Phage Adsorption to Carbon

Test No.	Carbon mg/l	Virus particles per ml
4-6.1	50.0	5.8×10^7
4-6.2	50.0	1.1×10^7
4-6.3	31.25	1.2×10^8
4-6.4	40.0	1.2×10^8
4-6.5	62.5	1.2×10^8
4-6.6	71.8	1.2×10^8
4-6.7	81.3	1.2×10^8
4-6.8	50.0	1.2×10^8
4-6.9	31.25	1.3×10^8

shifted. This shift would favor a greater desorption of viruses. With a competitive absorbate the same isotherm should apply only with different constants. To test for thermodynamic equilibrium, desorption studies were made without and with a competitive adsorbate.

4-7-1 Desorption Without a Competitive Adsorbate

Seven of the tests (numbers 4-6.3 through 4-6.9) described in section 4-6 were utilized to determine desorption equilibria. After obtaining adsorption equilibrium, the carbon was removed by centrifugation for 5 minutes. The supernatant was removed with a calibrated hypodermic syringe and assayed. The volume of liquid remaining with the carbon and its titer thus was known. The carbon was then resuspended in a fresh reaction solution. The reaction solution was identical with that used for adsorption except that no virus was added. Likewise, pH was buffered at 7.0 and ionic strength was adjusted to 0.08.

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 determined and substracted from the assay value at equilibrium. This procedure gave the net viruses desorbed from the carbon. Since the original amount adsorbed was known the amount remaining adsorbed could be calculated.

4-7-2 Desorption With Competitive Adsorbate

Viruses were adsorbed to carbon and then desorbed as before. In these tests tryptone was added to the desorption solution. Preliminary experiments described before (4-2) indicated that tryptone broth

caused a greater elution of viruses. This greater elution probably resulted from competition between tryptone and viruses for the same site on a carbon particle.

E. coli bacteriophage T_4 was adsorbed on 120/140 activated carbon at optimum pH and ionic strength. Carbon and virus concentrations were 250 mg/l and 1.4×10^8 virus particles/ml, respectively. During adsorption, samples of carbon were withdrawn with the reaction solution and diluted in tryptone broth. These samples were taken after 30, 60, 180, and 420 minutes of adsorption. The carbon samples, therefore, had adsorbed increasing amounts of virus. Each sample was diluted by factors of 1/10, 1/100, 1/500, and 1/1000. From these dilutions many different equilibrium points were obtained.

Each sample was withdrawn from the adsorption solution with an 0.1 ml pipette. The total 0.1 ml sample was placed in the desorption solution to give the proper dilution. The desorption solutions were assayed at various intervals over a 24-hour period to substantiate an equilibrium condition. From the dilution factor, the total virus concentration (adsorbed and in solution) of each desorption series was known. With this information and the virus concentration in solution at equilibrium, the amount of virus adsorbed per milligram of carbon was determined for each equilibrium condition.

4-8 Effects of pH on Adsorption

Adsorption of bacteriophage T_4 to 120/140 activated carbon was studied under different hydrogen-ion concentrations. Net charges on

viruses are greatly influenced by the acidity of the surrounding solution. This charge may influence adsorption and display an optimum value.

Constant concentrations of carbon and virus were used in the tests. Various pH values were maintained with phosphate buffer. The stability of bacteriophage T_4 was examined at different pH values. Virus was suspended in solutions ranging from pH 3.2 to 11.4 and assayed after 8.5 hours. The results were compared with a control at pH 7.0.

Four preliminary tests were then made at different pH values. High concentrations of 26/35 activated carbon were used (10,000 mg/l). By using a high concentration a pseudo-first order reaction occurs. Reaction rates from these were compared with those of preliminary adsorption tests described before (4-2). The four test solutions consisted of 23 ml of buffered water, 2 ml of stock virus, and 0.25 gm of carbon. The solutions were buffered at pH values of 10.4, 8.6, 7.0, and 5.0 for tests 4-8.1 through 4-8.4 respectively. The buffers were made from stock solutions of 0.1 N NaOH, 0.1 M $KHC_8H_4O_4$, 0.1 M KH_2PO_4, and 0.1 N HCl. No attempt was made to control the ionic strength of these solutions. As will be discussed in Chapter 5, the ionic strength also has a marked influence on the adsorption rate.

Other tests were made with a constant ionic strength. The interrelationship of the experiments makes it necessary to incorporate various phases of the study in each test. As will be shown in Chapter 5, optimum ionic strength was 0.08 - 0.10. Six tests were made at different pH values. The ionic strength of each test solution was raised to 0.10 with sodium chloride. In each test the

buffer system was mono- and dipotassium phosphate. The required ratios of mono- and dipotassium phosphate for a particular pH were determined in the presence of 1 ml of stock virus solution and 250 mg/l of carbon (Appendix 4). The extreme pH values were achieved with stock solutions of 0.1 N HCl and 0.1 N NaOH. The composition and pH of each test solution is given in Table 4-7. Controls were also run at each pH (Table 4-8). Samples were taken periodically as described above.

4-9 Effects of Ionic Strength on Adsorption

The preliminary studies at various pH levels suggested that the buffer solution influenced the adsorption rate. Three preliminary tests were made with different buffer concentrations. The buffer system consisted of mono- and dipotassium phosphate at pH 6.9. Buffer concentrations of 5×10^{-1}M, 5×10^{-2}M, and 5×10^{-3}M were tested (Table 4-9). Each test utilized 26/35 carbon. Controls without carbon were set up for the 5×10^{-1}M and 5×10^{-3}M buffer solutions. The usual sampling and assay procedures followed.

A series of experiments were made at different ionic strengths (Table 4-10). Bacteriophage T_4 and 250 mg/l of 120/140 carbon were used in all the tests except two. These two (test numbers 4-9.8 and 4-9.9) were conducted with 240 mg/l of carbon. In each test the pH was buffered at 6.9. Two buffer systems were used to determine if adsorption was similarly affected by both or if the influence was solely a property of the buffer. These buffers were mono- and dipotassium phosphate and sodium bicarbonate systems. A pH of 6.9 was

TABLE 4-7

Testing Solutions for Studying pH Effects on Adsorption of T_4 Phage to Activated Carbon

Test No.	Virus concentration per ml	pH	Buffer Vol. ml	Ratio of $\frac{KH_2PO_4}{K_2HPO_4}$	0.1M NaCl ml	0.1N HCl ml	0.1N NaOH ml
4-8.5	4.5×10^8	4.6	2.0	KH_2PO_4	3.66	1.00	0.0
4-8.6	4.3×10^8	5.7	2.0	17/1	3.71	0.0	0.0
4-8.7	4.5×10^8	6.5	20.0	15/1	1.16	0.0	5.00
4-8.8	4.6×10^8	7.85	2.0	1/50	3.43	0.0	0.0
4-8.9	4.35×10^8	8.8	16.0	K_2HPO_4	0.0	0.0	0.50
4-8.10	4.6×10^8	9.7	2.0	K_2HPO_4	3.39	0.0	0.50

Note: 250 mg/l of 120/140 was used in each test.

TABLE 4-8

Control Solutions for Studying pH Effects on Adsorption of T_4 Phage to Activated Carbon

Test No.	Virus concentration per ml	pH	Composition of test solution
4-8.11	4.4×10^8	4.6	Same as test No. 4-8.5
4-8.12	4.6×10^8	5.7	" " " " 4-8.6
4-8.13	4.6×10^8	6.5	" " " " 4-8.7
4-8.14	4.6×10^8	7.85	" " " " 4-8.8
4-8.15	4.3×10^8	8.8	" " " " 4-8.9
4-8.16	4.5×10^8	9.7	" " " " 4-8.10

TABLE 4-9

Test Solutions of Various Buffer Concentrations for Studying T_4 Phage Adsorption to Carbon

Test No.	Buffer molarity	Ionic strength	Carbon concentration mg/l	Virus concentration per ml
4-9.1	5×10^{-1}	0.83	10,000	2.6×10^{8}
4-9.2	5×10^{-2}	0.09	10,000	2.4×10^{8}
4-9.3	5×10^{-3}	0.02	10,000	2.4×10^{8}
4-9.4	5×10^{-1}	0.83	Zero	2.5×10^{8}
4-9.5	5×10^{-3}	0.02	Zero	2.3×10^{8}

TABLE 4-10

Test Solutions of Various Ionic Strengths for Studying T_4 Phage Adsorption to Carbon

Test No.	Ionic Strength			Virus concentration per ml	Buffer system
	buffer	NaCl	Total		
4-9.6	0.005	0.002	0.007	4.8×10^{8}	bicarbonate
4-9.7	Zero	0.003	0.003	4.7×10^{8}	none
4-9.8	0.001	0.003	0.004	7.3×10^{8}	phosphate
4-9.9	0.087	0.003	0.090	6.0×10^{8}	phosphate
4-9.10	0.036	0.004	0.040	4.0×10^{8}	phosphate
4-9.11	0.014	0.006	0.020	4.2×10^{8}	phosphate
4-9.12	0.076	0.044	0.120	4.9×10^{8}	phosphate
4-9.13	0.071	0.129	0.200	4.2×10^{8}	phosphate
4-9.14	0.007	0.003	0.010	4.0×10^{8}	phosphate
4-9.15	0.012	0.003	0.015	7.2×10^{8}	phosphate
4-9.16	0.014	0.006	0.020	1.1×10^{8}	phosphate
4-9.17	0.071	0.129	0.200	1.1×10^{8}	phosphate

maintained in the bicarbonate buffer solution by continuously bubbling a mixture of 5 percent CO_2 through the 125-ml reaction flask. Controls without carbon were monitored in conjunction with the ionic strength ranges being tested (Table 4-11).

The equilibrium tests described above (4-6) were made at optimum ionic strength. Four adsorption tests were conducted at an ionic strength of 0.02 to investigate its effect on the equilibrium state. Three additional tests were made at a high ionic strength of 0.20. The tests were made with T_4 phage, 120/140 carbon, and a phosphate buffer system, pH 6.9 (Table 4-12). The ionic composition of the tests were the same as tests 4-9.11 and 4-9.13 (Table 4-10). The tests were terminated after 22 hours. Within 28 hours bacterial contamination had developed in the reaction solution.

4-10 Treatment of Activated Carbon with Carboxyl-Blocking Reagents

The pH studies indicated that adsorption may involve amino and carboxyl groups. The presence of carboxyl groups on carbon would explain the similarity of virus adsorption on carbon with that of host cells. Carboxyl groups can be esterified to prevent the binding of amino groups.

4-10-1 Treatment with Acid-Alcohol

Esters can be formed by the reaction of a carboxylic acid with an alcohol in the presence of a strong mineral acid. The acid catalyzes ester formation from alcohol provided that the acid is not present in large amounts. The function of the acid catalyst is to protonate the carbonyl oxygen. The carbonyl carbon thereby becomes more susceptible to attack by the alcohol molecule (140). Usually an excess of alcohol

TABLE 4-11

Controls at Various Ionic Strengths
for Studying T_4 Phage Inactivation

Test No.	Ionic strength			Virus concentration per ml	Buffer system
	buffer	NaCl	total		
4-9.18	0.071	0.129	0.200	4.5×10^8	phosphate
4-9.19	0.014	0.006	0.020	4.5×10^8	phosphate
4-9.20	0.005	0.002	0.007	4.9×10^8	bicarbonate
4-9.21	0.087	0.003	0.090	6.4×10^8	phosphate

TABLE 4-12

Initial Concentration of Reactants
in Equilibrium Experiments at
Ionic Strengths of 0.02 and 0.20

Test No.	Carbon mg/l	Virus per ml
Ionic strength of 0.02		
4-9.22	71.8	1.35×10^8
4-9.23	50.0	1.1×10^8
4-9.24	31.25	1.1×10^8
4-9.25	100.0	1.1×10^8
Ionic strength of 0.20		
4-9.26	31.25	1.2×10^8
4-9.27	50.0	1.2×10^8
4-9.28	100.0	1.0×10^8

must be employed to favor ester formation. The following equation shows the overall reaction.

$$R\text{-}\overset{O}{\overset{\|}{C}}\text{-}OH + R\text{-}OH \xrightarrow{H^+} R\text{-}\overset{O}{\overset{\|}{C}}\text{-}O\text{-}R + H_3O^+$$

This same procedure of ester formation with alcohol was applied to activated carbon. Olcott and Conrat (141) report that carboxyl groups on proteins are almost quantitatively esterified with 0.02 to 0.1 N HCl in methanol. A similar acid-alcohol solution of 0.1 N HCl in absolute methanol was used to treat activated carbon. A carbon suspension of 1000 mg/l was made in the acid-alcohol. The solution was allowed to react for 2 days at 25°C.

After two days of treatment the carbon was washed three times and suspended in deionized water to give 1000 mg/l. The deionized water suspension was used as a stock solution. Washing involved repeated centrifugation, resuspension in deionized water, and shaking.

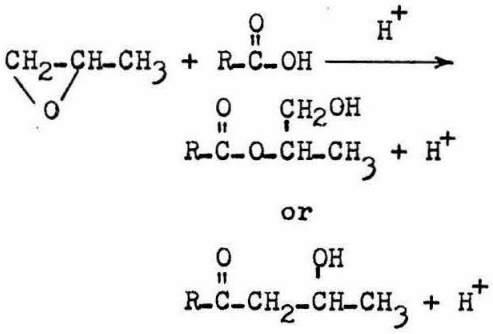
Bacteriophage T_4 was mixed with the acid-alcohol treated carbon (test 4-10.1). The reaction solution contained 50 mg/l of treated carbon, and 3.6×10^7 virus particles per ml. A phosphate buffer was used to obtain the optimum pH of 7.0 and ionic strength of 0.08.

The reaction solution was mixed on the shaker for 7 hours. Adsorption was measured by periodically sampling the reaction solution. Each sample of the supernatant was taken after the carbon settled.

4-10-2 Treatment with Propylene Oxide

Tomach and Puck (100)(101) used propylene oxide to block the attachment of T_1 and T_2 bacteriophage to E. coli host cells. Although they did not report the use of an acid catalyst, a proton is necessary

for the reaction (140). The epoxide ring of propylene oxide is cleaved under acid conditions. A carbonium ion intermediate is formed which causes ester formation with carboxyl groups. The overall reaction can give two products.



or

Activated carbon was treated for 2 days at 25°C with concentrated propylene oxide. The carbon was washed three times. The washing technique was identical as that used for the acid-alcohol treated carbon. A stock solution of 1000 mg/l of the treated carbon was prepared in deionized water.

Reaction solution 4-10.2 contained 4.7×10^7 virus particles per ml and 50 mg/l of carbon treated with propylene oxide. The solution was mixed on the shaker for 7 hours. Optimum pH and ionic strength were maintained with phosphate buffer. The reaction solution was periodically assayed to measure adsorption. In each case samples of the supernatant were taken after the carbon settled.

A second procedure was used to treat activated carbon with propylene oxide. In this case carbon was treated with an 80-percent solution of propylene oxide containing 0.08 N HCl. The first procedure did not use an acid catalyst. The carbon was again treated for 2 days at 25°C. Washing and stock solution preparations were the same.

A reaction solution (test 4-10.3) containing 3.6×10^7 virus particles per ml and 50 mg/l of treated carbon was tested for adsorption as before. Optimum pH and ionic strength were obtained with phosphate buffer.

CHAPTER 5

RESULTS AND DISCUSSION

5-1 Preliminary Experimentation with the Batch Technique

Preliminary experiments were made to develop techniques and evaluate the experimental procedures. The batch system and sampling procedures were evaluated. In conjunction with these studies the effectiveness and adequacy of the adsorbents and viruses were noted.

5-1-1 Evaluation of the Sampling Procedure

It was suspected that settling and centrifuging yielded different assays for identical solutions. This problem was noted in desorption experiments. Samples were treated by settling except when noted as otherwise. Centrifugation was tried in the desorption studies to enable complete recovery and resuspension of carbon for desorption. Chance of contamination was lower with the settling technique, since centrifugation required transferring the reaction solution to a centrifuge tube and then back to the 125-ml reaction flask for each sample collected. Another potential complication of centrifugation was carbon agglomeration during the sampling period. The masses, however, separated on additional shaking and any effect that agglomeration had on decreasing the available surface area for adsorption was of short duration and probably negligible.

In test 4-1.1, 1.1×10^8 virus particles/ml were mixed with carbon for 7.25 hours (Table 5-1), after which the supernatant of the centrifuged test solution assayed 4.0×10^6 virus particles/ml. Resuspending the carbon in 40 ml fresh solution with a maximum carryover of 1 ml of supernatant should yield 1×10^5 virus particles/ml. A sample of

TABLE 5-1

Adsorption of T_4 Phage to 120/140 Carbon and Resuspension of Carbon for Desorption

Time Hr.	Virus Concentration in Solution Virus particles/ml				
	test 4-1.1	test 4-1.2	test 4-1.3	test 4-1.4	test 4-1.5
Adsorption:					
0.00	1.1×10^8	3.6×10^8	1.1×10^8	1.4×10^8	
0.50	----	----	----	1.4×10^7 *	same as
1.00	----	----	----	3.5×10^6 *	test 4-1.4
3.00	----	----	----	2.3×10^6 *	
6.00	----	4.8×10^7	1.2×10^7	----	
6.00	----	1.0×10^7*	2.1×10^6*	----	
7.00	----	----	----	7.3×10^5*	
7.25	4.0×10^6 *	----	----	----	

*Supernatant samples were obtained by centrifuging.
All other supernatant samples were obtained by settling.

TABLE 5-1 (cont'd)

Time Hr.	Virus Concentration in Solution Virus particles/ml				
	test 4-1.1	test 4-1.2	test 4-1.3	test 4-1.4	test 4-1.5
Resuspended Carbon for Desorption:					
0.00	1.0×10^6	2.0×10^7	4.9×10^6	2.7×10^6	8.2×10^5
0.25	----	2.9×10^7	9.4×10^6	6.7×10^6	----
0.50	3.7×10^6	----	----	----	4.7×10^6
1.00	3.3×10^6	2.7×10^7	6.7×10^6	----	1.5×10^6
1.25	----	----	----	4.2×10^6	----
2.00	3.1×10^6	----	----	2.4×10^6	1.4×10^6
3.50	2.3×10^6	1.7×10^7	5.0×10^6	----	----
4.00	----	----	----	1.1×10^6	9.1×10^5
5.00	2.4×10^6	----	----	----	----
6.50	----	8.8×10^6	3.3×10^6	----	----
7.00	----	----	----	----	5.7×10^5
7.25	2.2×10^6	----	----	----	----
7.50	----	----	----	5.0×10^5	----

All supernatant samples were obtained by settling.

the desorption solution taken directly after the carbon was resuspended in this manner yielded 1.0×10^6 virus particles/ml (Table 5-1). If this ten-fold increase in virus titer resulted from rapid desorption from the carbon, presumably the amount desorbed would increase until an equilibrium point was reached. The desorption equilibrium, however, did not occur (Table 5-1 and Figure 5-1). Virus titer increased rapidly during the first 30 minutes and then was reduced as adsorption proceeded. It should be noted that an adsorption equilibrium was not established before the carbon was resuspended. Resuspension of the carbon, therefore, does not have to result in desorption.

The same results were obtained in tests 4-1.2 and 4-1.3, and the two methods of sampling gave different assays (Table 5-1 and Figure 5-1). After 6 hours of adsorption, samples treated by settling gave assays of 4.8×10^7 and 1.2×10^7 virus particles/ml for tests 4-1.2 and 4-1.3 respectively. The centrifugation procedure gave 1.0×10^7 and 2.1×10^6 virus particles/ml. From these results the centrifugation procedure yielded one-fifth the values obtained by settling.

Initial assays of the desorption solutions for tests 4-1.2 and 4-1.3 were 2.0×10^7 and 4.9×10^6 virus particles/ml instead of the expected values of 1.2×10^6 and 3.0×10^5 virus particles/ml. Samples were taken after 15 minutes of carbon resuspension. These samples gave the highest virus titer. Later samples showed a steady decrease in virus concentration (Figure 5-1).

Tests 4-1.4 and 4-1.5 were made from carbon resuspensions obtained from one adsorption test. Test 4-1.4 was run from carbon resuspended in fresh solution after one hour of adsorption; test 4-1.5 was after

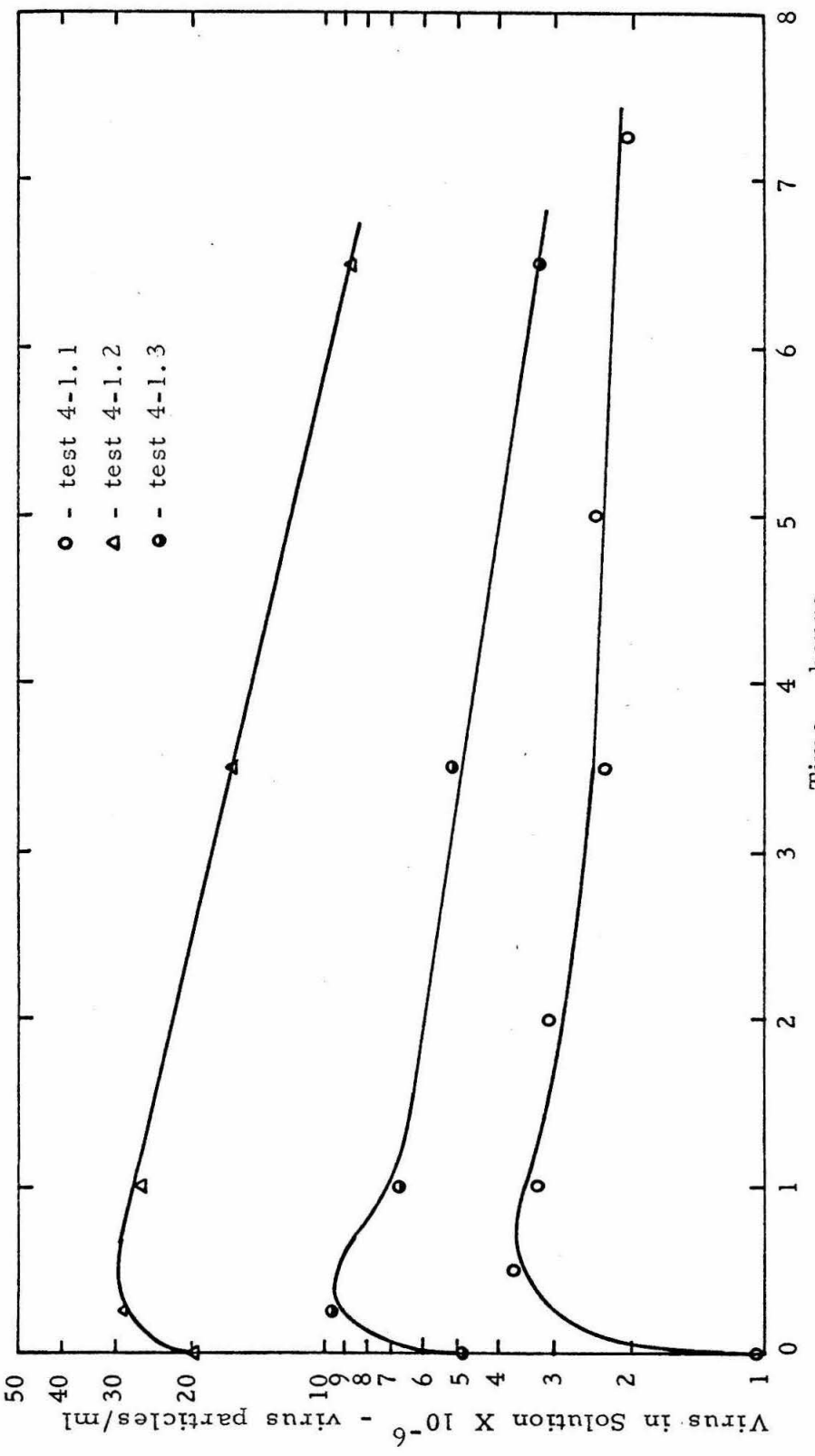


Figure 5-1. Resuspension of 120/140 Carbon Containing Adsorbed T_4 Phage for Desorption Studies (Tests 4-1.1, 4-1.2, and 4-1.3)

seven hours of adsorption. After one and seven hours of adsorption the supernatant of the centrifuged reaction solution assayed 3.5×10^6 and 7.3×10^5 virus particles/ml (Table 5-1). For desorption runs, the calculated amount of virus carried over in 1 ml of supernatant upon resuspension of the carbon was 3.5×10^5 and 7.3×10^4 virus particles/ml for the one and seven hour samples respectively. The solutions of resuspended carbon actually assayed 2.7×10^6 and 8.2×10^5 virus particles/ml for tests 4-1.4 and 4-1.5 respectively (Table 5-1). The rapid increase in virus titer followed by a slow decrease was again experienced (Figure 5-2).

Section 5-6 will show that an equilibrium condition does exist between adsorption and desorption. The above results of a rapid increase in virus titer followed by a decrease is not typical of an equilibrium process. The decrease in virus titer appears to be adsorption by the carbon. At a particular virus concentration the slopes of the curves for the resuspended carbon, tests 4-1.4 and 4-1.5, correspond very well with the slopes of the adsorption curve at that concentration (Figure 5-2). This relation indicates that after the rapid release of virus had taken place, the test became a repetition of the original adsorption test. An explanation of the high initial virus titer is that virus may be centrifuged down with the carbon. The difference in the two assays for tests 4-1.2 and 4-1.3 by settling and centrifugation definitely supports this idea.

To investigate the effect of centrifugation, two identical virus solutions were exposed to carbon for 12 hours and then centrifuged 10 and 20 minutes at 5000 rpm. Supernatant from the sample centrifuged

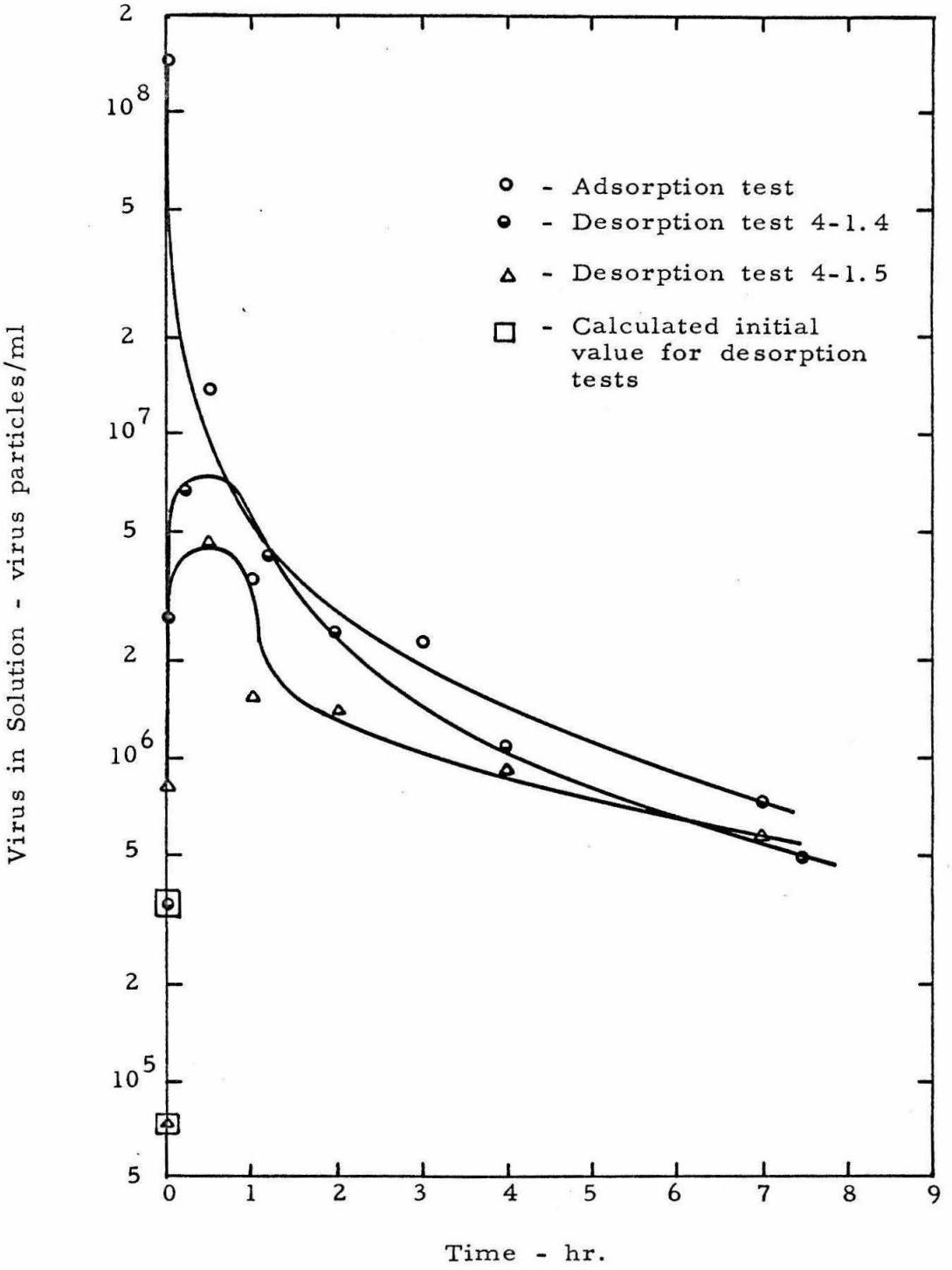


Figure 5-2. Adsorption of T_4 Phage to 120/140 Carbon and Resuspension of Carbon for Desorption (Tests 4-1.4 and 4-1.5)

10 minutes assayed 1.1 X 10^8 virus particles/ml. The sample centrifuged 20 minutes assayed 8.3 X 10^7 virus particles/ml in the supernate. After being centrifuged for 20 minutes the second sample was shaken for 5 minutes to resuspend carbon and virus. Centrifuging again for 10 minutes and assaying gave 9.0 X 10^7 virus particles/ml in the supernate. These results support the hypothesis that removal of virus occurs by centrifugation as used above. Twenty minutes of centrifuging yielded a much lower value than 10 minutes and some of the virus was restored by shaking.

By using five different sampling techniques as described in section 4-1.1, the removal of virus by centrifugation was demonstrated (Table 5-2). When a sample (1) was withdrawn without removing carbon a virus titer of 6.0 X 10^6 virus particles per ml was obtained. Sampling by centrifuging for 10 minutes (4) resulted in a virus titer of 1.5 X 10^6 virus particles per ml. If the total difference between these two assays is due to desorption of virus from the carbon in sample one (4.5 X 10^6 virus particles/ml), then 93 and 78 percent of the carbon had to be carried over in samples 2 and 3 (Table 5-2). Sample 2 was taken after settling and sample 3 after centrifuging for 3 minutes. No carbon particles were observed in samples 2 and 3. It was not possible for any significant amounts of carbon to be carried over with the sample, and the difference in assay value is a result of the centrifugation procedure and not desorption. Three minutes of centrifugation removed carbon as adequately as 10 minutes. The difference in assays from the two centrifuging periods is considered significant. In sample 5 an attempt was made to resuspend the virus. The assay value

was higher than that of sample 4 but more shaking was apparently necessary for complete resuspension (Table 5-2).

The experiments indicate that T_4 phage was removed by centrifugation. During experimentation virus removal was not expected at centrifugation speeds of 3000 to 5000 rpm. Subsequent theoretical analysis, however, indicates that the centrifugal force developed at 5000 rpm may cause significant removal. The problem was particularly important since samples were withdrawn from the very top of the supernatant.

The sedimentation coefficient for bacteriophage T_4 varies from 7×10^{-11} to 10×10^{-11} sec (135). Sedimentation coefficient is defined by the following expression,

$$S = \frac{v}{\omega^2 r} \qquad (5\text{-}1)$$

where S is the sedimentation coefficient, and v the particle velocity achieved at distance r from the center of rotation at an angular velocity ω .

The centrifuge was operated at 3000 to 5000 rpm and had a mean radius of 19 cm from the center of rotation to the centrifuge tubes. Equation (5-1) gives a velocity of 5.2×10^{-4} cm/sec for bacteriophage T_4 under the centrifugal force developed at 5000 rpm. At this velocity T_4 phage would descend 0.63 cm during 20 minutes of centrifuging.

These calculations indicate that some virus removal should have been expected from centrifuging. The removed virus was probably entrapped by the agglomeration of carbon at the bottom of the centrifuge tube. A gradient of increasing concentration would be established along the length of the tube and concentrations would be most dilute at the top, where samples were withdrawn.

TABLE 5-2

Evaluation of the Sampling Procedure Used in the Batch Operation

Sample No.	Sampling technique	Virus Assay virus particles/ml
(1)	No removal of carbon	6.0×10^6
(2)	Carbon removal by settling	5.7×10^6
(3)	Carbon removal by centrifuging for 3 min.	5.0×10^6
(4)	Carbon removal by centrifuging for 10 min.	1.5×10^6
(5)	Carbon removal by centrifuging for 10 min., reshaking for 2 min., and centrifuging again for 3 min.	1.7×10^6

5-1-2 Evaluation of Adsorbents

Diatomaceous earth, Ottawa sand, activated carbon, and coconut carbon were tested for adsorption of E. coli bacteriophage T_4. Adsorption varied considerably with each adsorbent. Diatomaceous earth did not adsorb T_4 phage (Table 5-3). Exposure to 20,000 mg/l of diatomaceous earth (test 4-2.1) gave no change in virus titer of the reaction solution after 13 hours. A second test (4-2-2) with 80,000 mg/l of diatomaceous earth resulted in a 19.4 percent drop of virus titer after 48 hours of adsorption. Virus titer in the control flask (4-2.8) without diatomaceous earth, however, dropped 68.7 percent from agitation, temperature, and other environmental factors causing virus inactivation. Since more virus was inactivated in the control flask than in the test flask with diatomaceous earth, the 19.4 percent decrease was probably not significant.

It appears that the diatomaceous earth stabilized the virus, preventing natural inactivation from the testing operation. This stabilizing effect on viruses is also exhibited by proteins. The mechanism of the stabilization is not clearly understood.

Adsorption may have occurred on Ottawa sand (Table 5-4), but the capacity of the sand was very low. Only 10 percent reduction in virus was noted during seven hours exposure to 160,000 mg/l of sand (test 4-2.3). The control and test solutions were assayed again after 20 hours, and during this period inactivation of virus by the testing system (test 4-2.9) was much greater than the amount adsorbed. A second test (4-2.4) was made with 800,000 mg/l of sand. No net adsorption occurred after 28 hours. The virus reduction corresponded to that

TABLE 5-3

Preliminary Testing of T_4 Virus Adsorption by Diatomaceous Earth

Test No.	Diatomaceous earth concentration mg/l	Time Hrs.	Virus in solution virus particles/ml	Percent titer drop	Percent Adsorbed (corrected for control)
4-2.1	20,000	0	1.0×10^8		
		13	1.0×10^8	0	0
4-2.2	80,000	0	3.1×10^7		
		48	2.5×10^7	19.4	0
4-2.8	Control	0	3.5×10^7		
		48	1.1×10^7	68.7	--

TABLE 5-4

Preliminary Testing of T_4 Virus Adsorption by Ottawa Sand

Test No.	Sand concentration mg/l	Time Hrs.	Virus in solution virus particles/ml	Percent titer drop	Percent Adsorbed (corrected for control)
4-2.3	160,000	0	2.0×10^9		
		3	2.0×10^9	0	0
		7	1.8×10^9	10	10
		20	1.6×10^9	20	
4-2.9	Control	0	2.0×10^9		
		7	2.0×10^9	0	--
		20	1.4×10^9	30	--
4-2.4	800,000	0	1.6×10^9		
		28	1.2×10^9	25	0
4-2.10	Control	0	1.6×10^9		
		28	1.2×10^9	25	--

of inactivation as monitored in the control test (4-2.10).

All three types of carbon adsorbed a significant amount of T_4 bacteriophage (Table 5-5). The virus loss clearly resulted from adsorption and was easily distinguished from natural inactivation. Granular activated carbon (26/35) adsorbed 97.5 percent of the viruses in 10 hours (test 4-2.5). Powdered activated carbon (120/140) adsorbed 95 percent of the viruses in 4 hours (test 4-2.6), corrected with respect to the control (test 4-2.12).

The control series gave a first-order inactivation of virus. The equation

$$C_t = C_o e^{-kt} \qquad (5\text{-}2)$$

can be used to calculate the natural inactivation rate for the particular testing conditions existing in this experiment. The inactivation rate is k, t is time, C_o is the concentration at time zero, and C_t is the concentration at time t. A plot of the results from the control test, 4-2.12, gave an inactivation rate of 0.011 per hour (Figure 5-3).

Coconut charcoal also adsorbed virus but with lower capacity than the activated carbons (test 4-2.7). Fifty percent of the virus was adsorbed in eight hours, but very little adsorption occurred thereafter. Presumably an equilibrium condition was approached even though a high concentration of coconut charcoal (20,000 mg/l) was used.

Only the carbon materials adsorbed appreciable virus, hence diatomaceous earth and Ottawa sand were rejected for future adsorption tests. Coconut charcoal was also rejected because of low capacity, and concentrations of 20,000 mg/l are considered high for practical use. The activated carbons achieved almost 100 percent removal at this con-

TABLE 5-5

Preliminary Testing of T_4 Virus Adsorption by Carbon

Test No.	Carbon Type and concentration mg/l	Time Hrs.	Virus in solution virus particles/ml	Percent titer drop	Percent Adsorbed (corrected for control)
4-2.5	26/35	0	1.2×10^8		
	20,000	10	3.1×10^6	97.5	97.5
4-2.11	Control	0	1.3×10^8		
		10	1.3×10^8	0	--
4-2.6	120/140	0	2.1×10^8		
	20,000	4	3.2×10^4	100	95
		20	1.3×10^2	100	100
4-2.12	Control	0	2.1×10^8		
		4	2.0×10^8	4.7	
		8	1.8×10^8	14.3	
		20	1.7×10^8	19.0	
4-2.7	Coconut	0	2.2×10^8		
	20,000	4	1.2×10^8	45.5	41.3
		8	9.6×10^7	56.5	50.0
		20	7.6×10^7	65.5	54.0

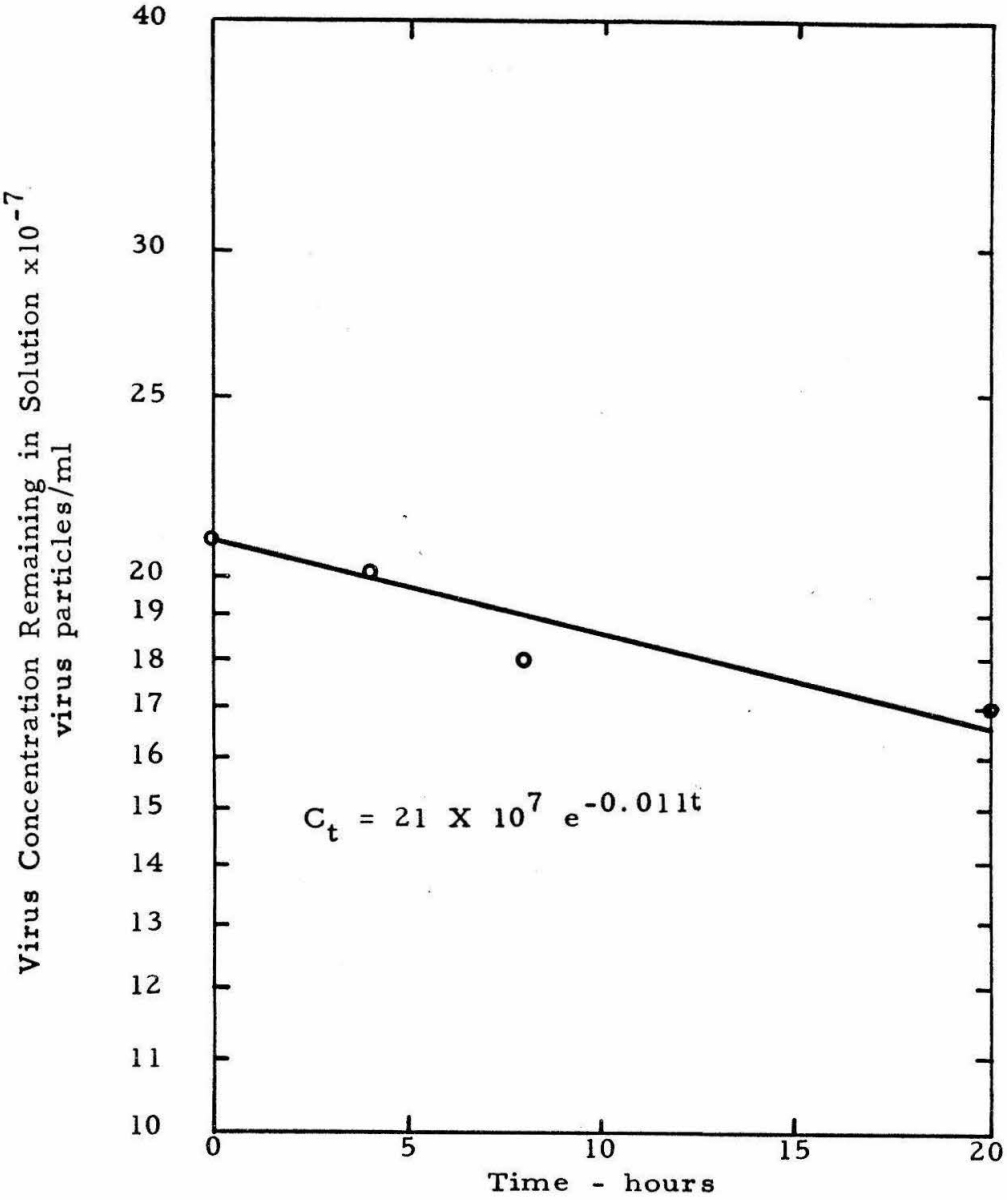


Figure 5-3. Natural Inactivation of T_4 Phage at pH 6.8 and Ionic Strength 0.004 (Test 4-2.12)

centration. Additional tests were made on both sizes of activated carbon before one was selected for extensive studies. The reaction solutions for these tests were presented in Table 4-2. In two tests bacteriophage T_4 was adsorbed on the powdered (120/140) and granular (26/35) size carbons. Powdered carbon adsorbed essentially 100 percent of the virus in two hours, and in eight hours the granular carbon adsorbed 95 percent (Table 5-6). The control series (4-2.16 and 4-2.17) showed that the quantity of virus inactivated by the testing system was insignificant compared to the amount adsorbed.

The results of adsorption by the two carbon sizes were plotted on semi-logarithmic paper (Figures 5-4 and 5-5). A first-order reaction is indicated by the straight-line representation of the data in these plots. As will be shown later, the reaction order in this case is pseudo-first order, since the concentration of carbon sites was much greater than the concentration of virus.

A pseudo-first-order reaction is adequately described by the first-order equation,

$$C_t = C_o \, e^{-kt} \qquad (5\text{-}2)$$

where k is the adsorption rate. For the powdered and granular carbons the adsorption rate k is 6.24/hr and 0.26/hr respectively. The powdered carbon, which has a much greater surface area per unit weight, had the higher adsorption rate, as expected.

Powdered carbon was selected for the more extensive adsorption studies, because of its high adsorption capacity for bacteriophage T_4, enabling lower carbon concentrations. The powdered carbon also withstood agitation much better than the granular carbon. Fracturing was

TABLE 5-6

Adsorption of T_4 Phage to 120/140 and 26/35 Activated Carbon

Time	Viruses in Solution virus particles/ml		Virus particles adsorbed per ml	Percent adsorbed
120/140 carbon:				
	test 4-2.13	Control 4-2.16		
0	3.0×10^8	3.0×10^8	0	0
5 min	1.2×10^8	--	1.8×10^8	60
20 min	3.1×10^7	--	2.7×10^8	90
40 min	6.3×10^6	--	2.9×10^8	98
1 hr	4.6×10^5	2.9×10^8	3.0×10^8	100
2 hr	1.5×10^3	--	3.0×10^8	100
3 hr	5.0×10^2	--	3.0×10^8	100
26/35 carbon:				
	test 4-2.14	Control 4-2.17		
0	2.0×10^8	2.0×10^8	0	0
5 min	1.9×10^8	2.0×10^8	1.0×10^7	5
20 min	1.6×10^8	1.9×10^8	4.0×10^7	14
50 min	1.4×10^8	1.9×10^8	6.0×10^7	20
2 hr	1.2×10^8	1.8×10^8	8.0×10^7	31
3 hr	8.9×10^7	1.8×10^8	11.0×10^7	49
5 hr	4.5×10^7	1.7×10^8	15.5×10^7	74
8 hr	8.8×10^6	1.7×10^8	19.2×10^7	95

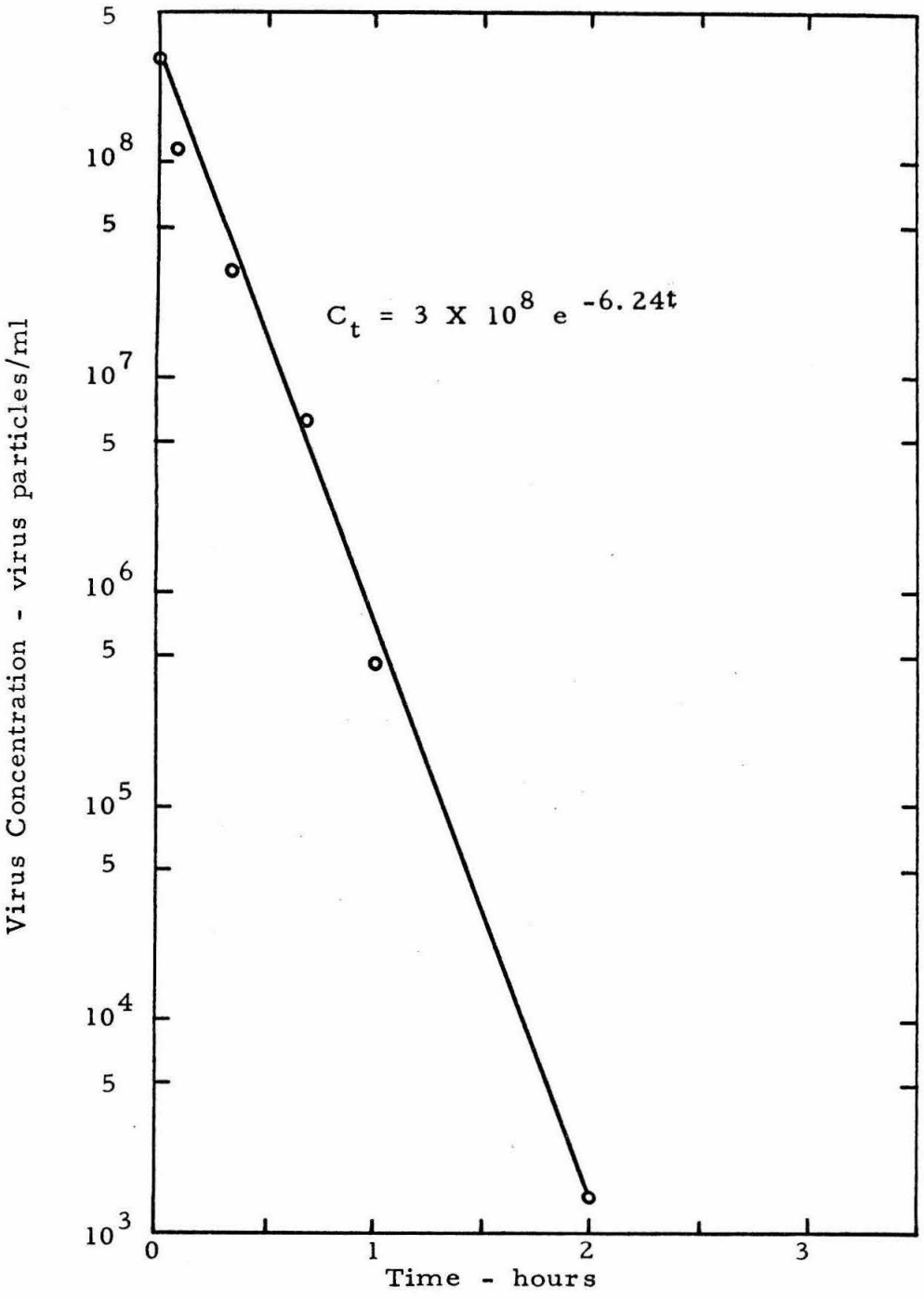


Figure 5-4. Viruses Remaining in Solution vs. Time of Contact with 120/140 Carbon

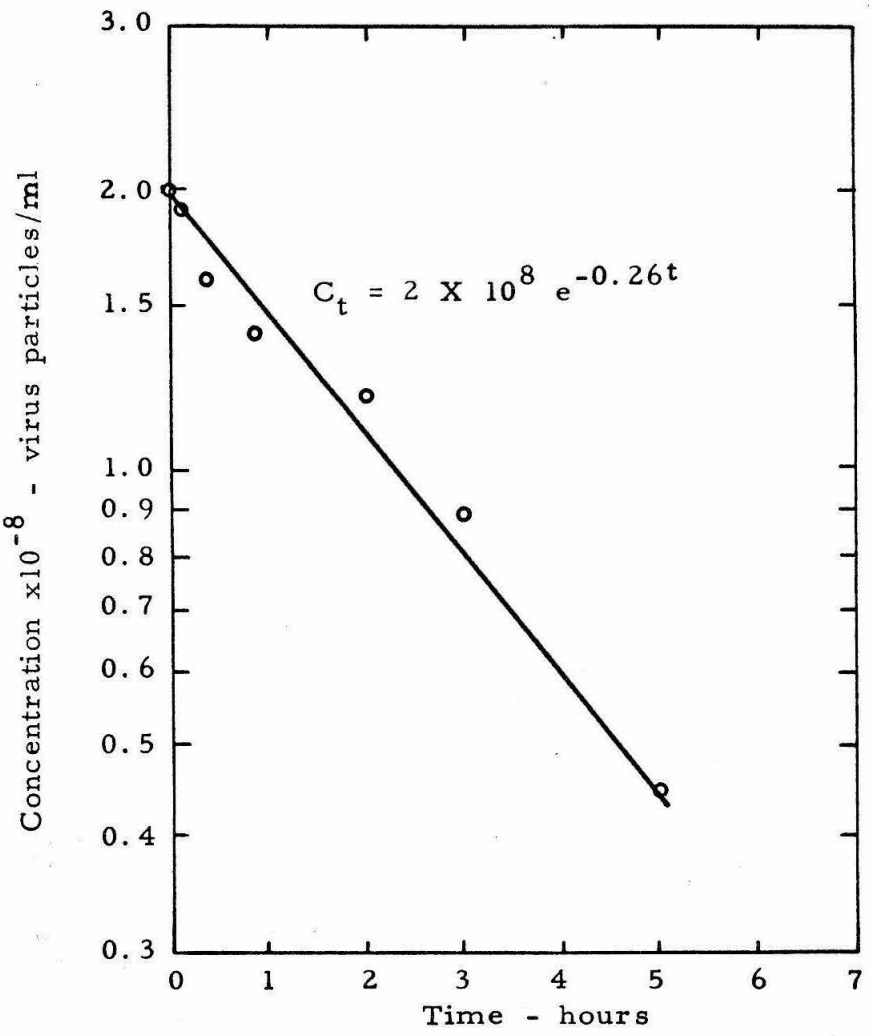


Figure 5-5. Viruses Remaining in Solution vs. Time of Contact with 26/35 Carbon.

very noticeable in the granular carbon after 10 hours of agitation. This condition exposed new surface areas, complicating interpretation of the adsorption process. No noticeable fracturing of the powdered carbon was observed.

5-1-3 Poliovirus Adsorption

To expand the scope of this study, poliovirus Type III was tested for adsorption by powdered carbon (test 4-2.15). The difficulties and expense encountered soon forced abandonment of this line of investigation and only preliminary results were obtained. Descriptions of the test and composition of the reaction solution were given in Chapter 4 (Table 4-2). A total virus reduction of 91 percent was obtained in 3.75 hours of adsorption (Table 5-7). This reduction, however, cannot be attributed completely to adsorption. Some inactivation of the virus probably occurred from the testing system. The inactivation rate could not be determined from the control test 4-2.18 as intended. Assays of the control flask resulted in poor plaque formation and unusable counts. Samples were exhausted before the condition could be corrected. The cause of poor plaques was not found. The monkey kidney cells were either bad or contamination in the samples affected plaque formation.

The reduction of poliovirus can also be expressed as a pseudo-first-order reaction (Figure 5-6). A removal rate of 0.62/hr was obtained.

Poliovirus presented many additional problems. The first resulted from monkey kidney cells flown weekly from Bethesda, Maryland, or Oakland, California. In most cases cells gave best plaque formation

TABLE 5-7

Adsorption of Poliovirus to 120/140 Activated Carbon

Time min	Virus Remaining in Solution virus particles/ml		Percent Reduction
	test 4-2.15	Control 4-2.18	
0	480	430	
	540	550	
	640	600	
	590	470	
	570	580	
	640	650	
	Ave. 570	Ave. 550	--
15	310		
	300		
	120*		
	440		
	440		
	500		
	Ave. 398		30
45	240		
	320		
	460		
	240		
	Ave. 315		45
105	170		
	110		
	130		
	140		
	Ave. 138		76
225	80		
	50		
	60		
	40		
	40		
	Ave. 54		91

* This value was not included in calculating the average. Plaque development was poor.

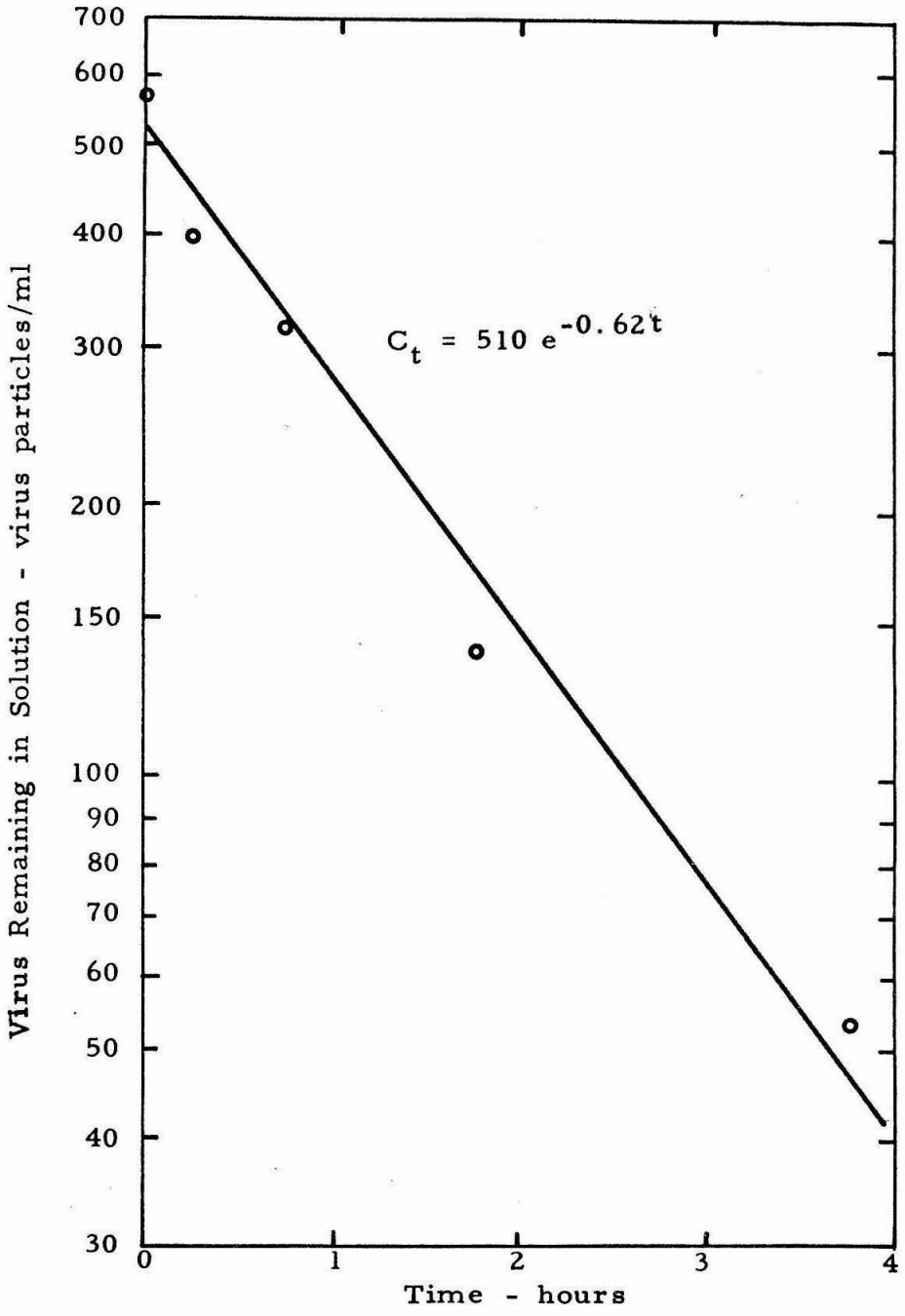


Figure 5-6. Adsorption of Poliovirus to 120/140 Activated Carbon (Test 4-2.15)

when used one to two days after arrival. If used immediately, poor plaques resulted from the inadequate metabolic state of the cells caused by lower than optimum temperature during shipment.

Incubation at 37°C for one day enhanced good plaque formation. Cells kept longer than two days would break loose from the plastic bottle, destroying the monolayer growth. Separation of cells from the bottle surface resulted from an overgrowth of cells. Cells beginning to grow up the sides of the bottle would curl back causing the separation.

The short time interval during which monkey kidney cells were suitable necessitated prior preparation of samples. This procedure would have been satisfactory if cells always arrived in a usable condition. Over one-third of the time poor cells were obtained due to contamination, poor growth, or damage in shipping. In some cases poor quality was not discerned until after the agar overlay was applied. In these instances the utilized samples were not recoverable. When cells were noted to be poor before use, samples were stored at -40°C until new cells were obtained. Some titer loss, however, was bound to occur from storage.

In addition to delays caused by poor cells, the procedure for poliovirus assay required seven days for completion. The bacteriophage required only 24 hours. This long delay before assays were obtained made it difficult to go back to the original testing solution if additional information was necessary. Changes in virus titer and bacterial contamination in testing solutions were very probable after storage for a week.

Monkey kidney cells were very susceptible to bacterial contamination. An absolutely sterile testing solution was difficult to maintain throughout an experiment. This problem necessitated treating the samples with antibiotics and testing for contamination. Such procedures required 2 to 5 additional days before assays could be obtained.

With the bacteriophage assay, contamination was not as critical. The bacterium, E. coli, host cell for bacteriophage T_4, was used in high concentrations. Such high concentration enabled E. coli to reproduce rapidly and destroy any contamination that entered the testing solution.

Another disadvantage of poliovirus was the large number of cell bottles that had to be used for a single experiment. One test (4-2.15) and its control (4-2.18), required 54 two-oz bottles of cells for assaying 10 samples. A maximum of 30 plaques can be distinguished in a two-oz bottle, whereas 400 plaques can be distinguished on the agar plates used in assaying T_4 phage. More bottles of monkey kidney cells must be used to obtain the accuracies comparable to the bacteriophage assay.

For these reasons E. coli bacteriophage T_4 was selected for the adsorption studies. The frequent occurrence of inadequate monkey kidney cells, the time required to obtain experimental results, and the cost of the cells precluded the use of poliovirus where large numbers of experiments were required.

5-1-4 Determining Optimum Carbon Concentration

Five tests, described in Table 4-3, were made to determine a satisfactory concentration of 120/140 carbon to use in additional experiments. Carbon concentrations were varied from 1000 to 10,000 mg/l.

A concentration of 20,000 mg/l had been used previously in test 4-2.13. The Control (4-2.23) revealed no significant amount of virus decrease resulting from natural inactivation, but adsorption occurred in the other solutions (Table 5-8).

TABLE 5-8

Results of Preliminary Tests Made with Different Concentrations of 120/140 Activated Carbon

Test No.	Carbon concentration mg/l	Time Hr.	Virus Remaining in Solution virus particles/ml
4-2.19	10,000	0.0	1.9×10^8
		1.0	1.0×10^8
		2.0	1.0×10^6
		4.5	2.0×10^6
4-2.20	5,000	0.0	1.8×10^8
		1.0	1.5×10^8
		2.0	5.1×10^7
		4.5	2.7×10^7
4-2.21	2,000	0.0	1.8×10^8
		1.0	1.4×10^8
		2.0	7.6×10^7
		4.5	7.4×10^7
4-2.22	1,000	0.0	1.8×10^8
		1.0	1.7×10^8
		2.0	1.1×10^8
		4.5	1.0×10^8
4-2.23	(Control) Zero	0.0	1.8×10^8
		1.0	1.8×10^8
		2.0	1.7×10^8
		4.5	1.9×10^8

The change in virus concentration followed a first-order reaction for carbon concentrations of 10,000 and 5,000 mg/l (Figure 5-7). The results for 1000 and 2000 mg/l of carbon did not plot as a straight

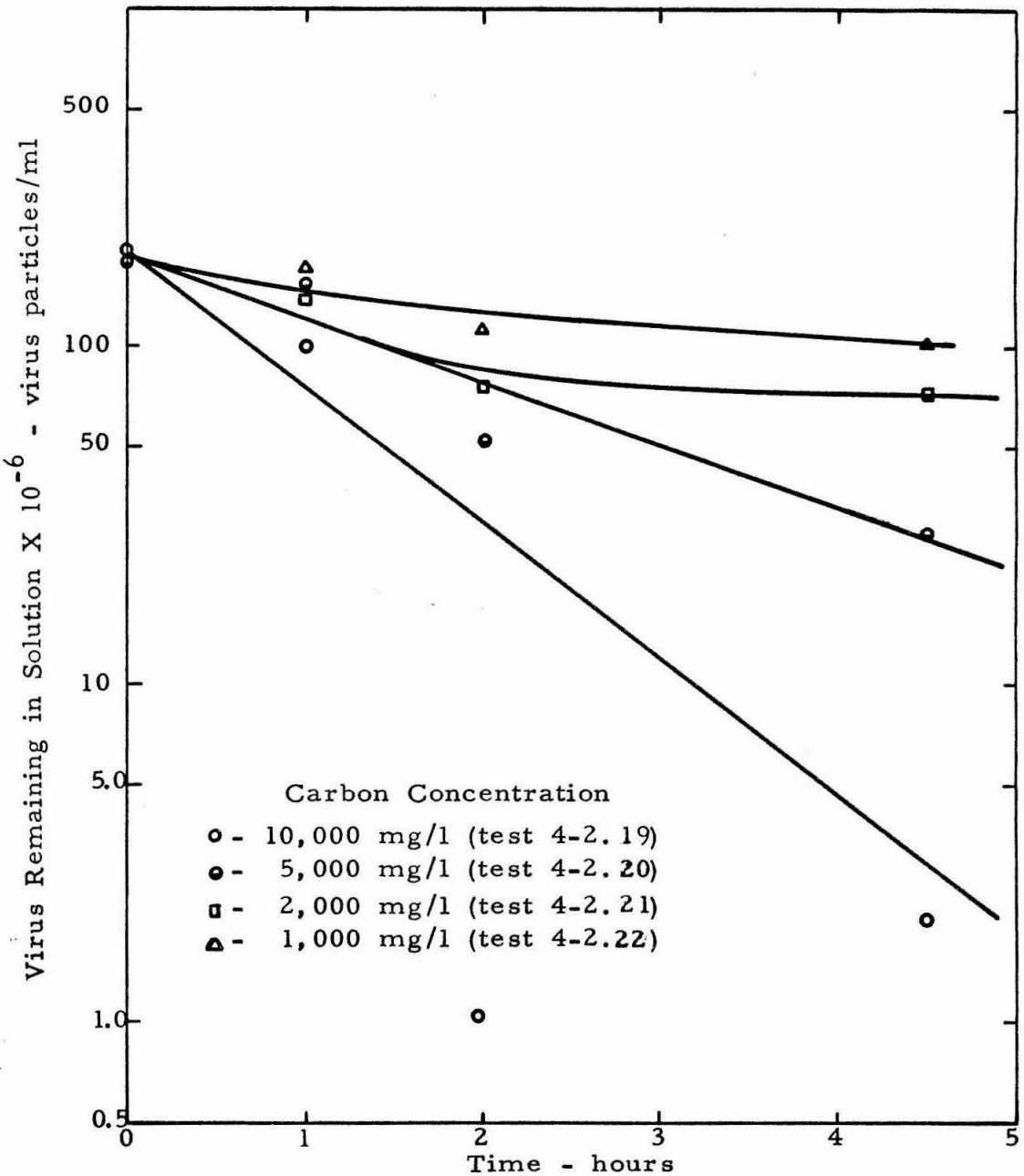


Figure 5-7. Adsorption of T_4 Phage to Different Concentrations of 120/140 Carbon

line on a semi-logarithmic scale. Decreasing rates occurred with decreasing carbon concentrations. This indicated that the adsorption process was not a true first order reaction. Adsorption rate varied with both the carbon and virus concentrations.

Test 4-2.22, containing 1000 mg/l of carbon, gave 50 percent virus removal after 4.5 hours of adsorption and appeared to approach an equilibrium condition (Figure 5-7). Studying the kinetic system required removals of this order. Other preliminary experiments at various pH values and ionic strengths (discussed in sections 5-9 and 5-11) indicated that greater removals per unit of carbon could be obtained. From these considerations a carbon concentration of 250 mg/l was adopted for future tests.

5-2 Preliminary Experimentation With the Column Technique

Trial adsorption runs were first made with a $\frac{1}{2}$-inch diameter column. Five solutions, described in section 4-2-2, containing bacteriophage T_4 were passed through the column. The filtrates from the five solutions were assayed without success. Plaque formation was inhibited by contamination in the column. Two other problems were noted in using the column; first, the depth of column or contact time was not sufficient, and second, some of the carbon floated on the virus sample undergoing filtration. To overcome these problems the column diameter was reduced to $\frac{1}{4}$-inch, and a thin layer of diatomaceous earth was placed on the carbon to keep the floatable portion from rising.

A total of 7×10^{10} virus particles were filtered through the $\frac{1}{4}$-inch column. No virus was detected in any of the filtrates from the seven virus solutions filtered. It was apparent that less carbon should

have been used if virus was desired in the filtrates. Virus concentrations higher than 10^{10} are impracticable to prepare in large quantities such as 100 ml.

Before repeating the test with a smaller portion of carbon an attempt was made to recover adsorbed viruses from the column. The first procedure consisted of passing a 1.0 M NaCl solution through the column. Assays of the effluent revealed no viruses. Next, a solution of tryptone broth was passed through the column. No virus was recovered in the broth. A total of 1×10^6 virus particles was eluted from the column when the carbon was removed and suspended in tryptone broth. The eluted viruses, however, only accounted for 0.001 percent of those added to the column.

The fact that virus was eluted by suspending the carbon and not by filtration may be explained if the top portion of the column was saturated but the bottom portion was not. Virus eluted in the top portion would only be adsorbed again in the bottom portion. This possibility was tested in a 3/8-inch column. The carbon column was extruded and analyzed sectionally.

A total of 1.8×10^{11} T_4 phages was added to the 3/8-inch column. The effluent revealed no viruses. The carbon was extruded and divided into six sections. A total of 9.6×10^{10} virus particles were recovered (Table 5-9). This value accounts for 53.3 percent of the adsorbed viruses, but 88.5 percent of these came from the diatomaceous earth layer. Previous experiments (tests 4-2.1 and 4-2.2) showed that diatomaceous earth would not adsorb T_4 phage. This condition indicates that

other mechanisms in filtration may be the major factors in retaining the virus in the column.

These other mechanisms of filtration, however, cannot be the complete explanation, because some adsorption undoubtedly took place. Virus was released from the upper section of the column and not from the lower portion (Table 5-9). The top 0.25 inch of carbon released virus from saturated layers of carbon. The virus was then readsorbed by unsaturated layers from this section after suspension in broth. This phenomenon is indicated by the drop in virus titer from 11 X 10^9 to 7.5 X 10^9 virus particles in 24 hours. This condition would also prevent elution of virus by passing broth through the column unless the whole column was saturated. Only 0.5 percent of the total added viruses was eluted from the top 0.25 inch of carbon. Changing the pH of the elution broth from 7.4 to 8.5 failed to release any additional viruses.

The batch operation for studying adsorption was selected over the column procedure. Many difficulties as described above were encountered with the column arrangement. One was contamination, which was hard to control in the column. The batch procedure utilized 125-ml flasks that could be sealed from the atmosphere with rubber stoppers or cotton. This technique kept contamination at a minimum.

The floatable portion of carbon as experienced in the column was not a problem in the batch system. Carbon utilized in the batch operation was obtained from stock solutions. The carbon, therefore, was prewetted and did not float as the carbon packed in columns.

TABLE 5-9

Adsorption of T_4 Virus to Activated Carbon in a Column

Column Portion	Content of Column Portion	Virus Particles Recovered from Each Portion after Suspension in Broth for Designated Time		
		0 Min.	30 Min.	24 Hours
#1	0.125 inch diatomaceous earth	44×10^9	--	85×10^9
#2	0.25 inch activated carbon	11×10^9	8.5×10^9	7.5×10^9
#3	0.5 inch activated carbon	9×10^4	10×10^4	7×10^4
#4	1.0 inch activated carbon	0	--	1×10^5
#5	1.5 inches activated carbon	0	0	0
#6	0.125 inch glass wool	0	0	0

Total virus added 1.8×10^{11}

Total virus recovered 9.6×10^{10}

Carbon Size 120/140

A major disadvantage of the column was the large quantity of virus needed to saturate the carbon. Smaller columns would present additional problems in uneven distribution of flow and controlling flow rates. The column was also difficult to prepare and use by comparison with the batch technique.

5-3 Effects of Virus Age on Adsorption

Identical tests made with virus from a recently prepared and a 6-month-old culture gave similar results (Table 5-10). The results cannot be easily compared in the particular form that they are given in Table 5-10. As will be shown in section 5-8 the adsorption system can be mathematically represented by the equation for a second-order reaction given below.

$$tk_1m = \ln \left[\frac{0.5\ (a_o+b_o+K^{-1}+m)-X}{0.5\ (a_o+b_o+K^{-1}-m)-X} \right] \left[\frac{a_o+b_o+K^{-1}-m}{a_o+b_o+K^{-1}+m} \right] \qquad (5\text{-}3)$$

where: t = time

k_1 = forward rate constant (ml/virus particles-sec)

a_o = initial concentration of virus (virus particles/ml)

b_o = initial concentration of carbon sites (sites/ml)

K = equilibrium constant (ml/virus particle)

X = concentration of adsorbed virus at time t (virus particles/ml)

$m = \left[(a_o-b_o)^2+K^{-1}(2a_o+2b_o+K^{-1}) \right]^{\frac{1}{2}}$

By applying reversible second-order kinetics, rate constants for the forward reaction were obtained (Table 5-11, Figures 5-8 and 5-9). Tests 4-3.1 and 4-3.2 yielded rate constants of 7.2×10^{-13} and 7.8×10^{-13} ml/virus particle-sec respectively. These similar values

TABLE 5-10

Comparison of Tests Made with T_4 Phage Solutions of Different Ages

Time Hr.	Virus Concentration Remaining in Solution Virus particles/ml		
	test 4-3.1	test 4-3.2	Control 4-3.3
0	4.2×10^8	1.5×10^8	1.5×10^8
0.25	2.7×10^8	8.8×10^7	--
1.0	1.6×10^8	5.6×10^7	--
2.0	1.2×10^8	2.2×10^7	--
3.7	6.2×10^7	--	--
5.0	4.1×10^7	--	--
7.5	3.3×10^7	--	1.3×10^8

test 4-3.1 - 6-month-old stock virus solution.

test 4-3.2 - Recently prepared stock virus solution

TABLE 5-11

Representation of Results for Adsorption with Different Age Virus Solutions by Reversible Second-order Kinetics

Test No.	Time Hr. t	Virus Adsorbed per ml	$\frac{0.5\ (a_o+b_o+K^{-1}+m)-X}{0.5\ (a_o+b_o+K^{-1}-m)-X}$	$\frac{a_o+b_o+K^{-1}-m}{a_o+b_o+K^{-1}+m}$
4-3.1	0			
	0.25	1.5×10^8		1.10
	1.0	2.6×10^8		1.31
	2.0	3.0×10^8		1.50
	3.7	3.58×10^8		2.34
	5.0	3.7×10^8		3.15
	7.5	3.87×10^8		4.92
4-3.2	0			
	0.25	1.02×10^7		1.53
	1.0	9.4×10^7		2.20
	2.0	12.8×10^7		5.40

test 4-3.1 - 6-month-old stock virus solution

test 4-3.2 - Recently prepared stock virus solution

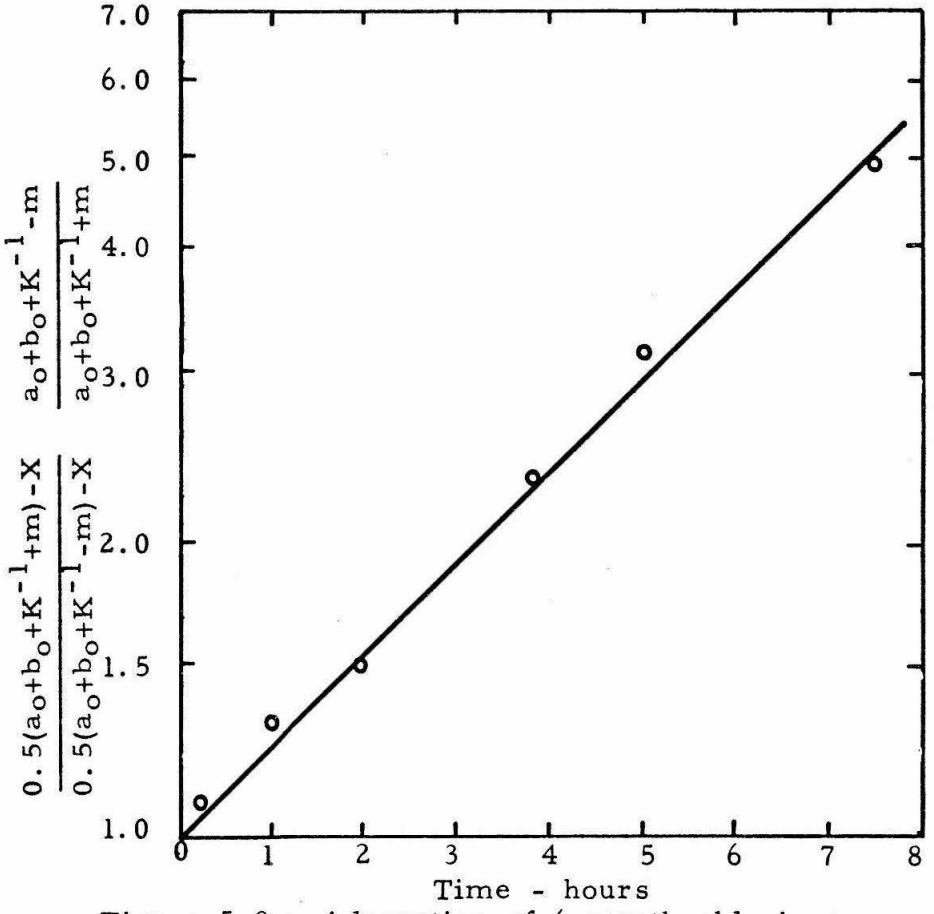


Figure 5-8. Adsorption of 6-month-old virus Culture as Analyzed by Reversible Second-order Kinetics (test 4-3.1).

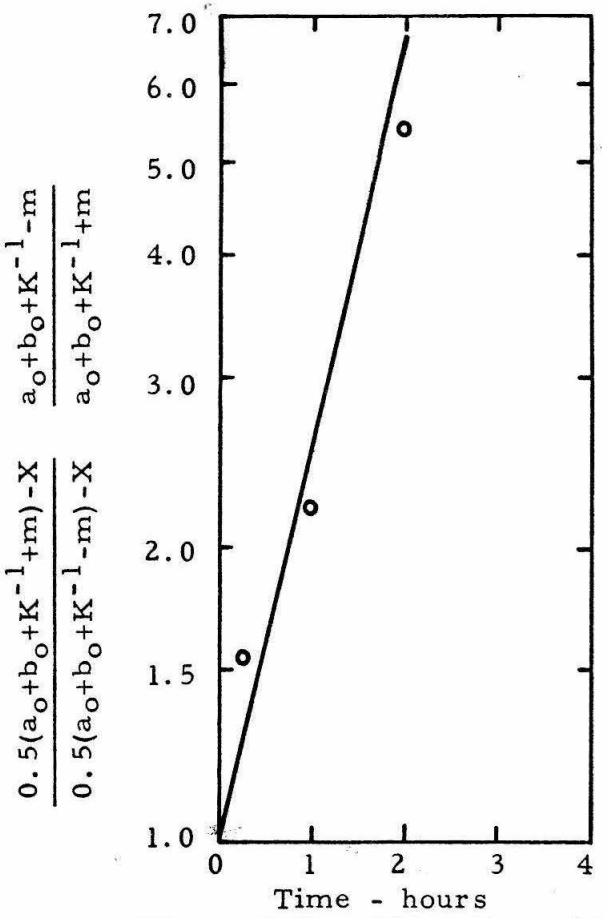


Figure 5-9. Adsorption of New Virus Culture as Analyzed by Reversible Second-order Kinetics (test 4-3.2).

indicate that there is no noticeable difference in adsorption of virus from old or new cultures. The control flask indicated that no significant amount of virus was inactivated by the testing procedure. In the presence of ionic strengths of 0.02 to 0,2 the stability of the virus was much greater. This greater stability was experienced in many of the following tests. As a result most control tests indicated very little natural inactivation.

5-4 Effects of Temperature on Adsorption

T_4 phage was adsorbed to granular carbon (26/35) at 33°C. The results (Table 5-12) can be directly compared with those of test 4-2.14 (Table 5-6 and Figure 5-5). The same concentration of carbon was used in each test , but test 4-2.14 was made at 23°C. A high concentration of carbon was used to give a pseudo-first-order reaction (Figure 5-10). Comparison of rate constants of the two tests at 23° and 33°C shows no change in adsorption. The adsorption rate was 0.26/hr at 23°C and 0.25/hr at 33°C. Small dependence on temperature is expected for physical adsorption.

The degree to which the adsorption rate is affected by temperature aids in describing the type of process. The effect of temperature on pure chemisorption is frequently very noticeable. Little effect is noted with physical adsorption. The initial attachment of viruses to their host cells also appears to be physical adsorption, since it is independent of temperature for animal and bacterial viruses (94)(105).

TABLE 5-12

Adsorption of T_4 Phage to 26/35 Activated Carbon at 33°C

Time Hr.	Virus Remaining in Solution - virus particles/ml		Percent Adsorbed
	test 4-4.1	Control 4-4.2	
0	1.4×10^8	1.2×10^8	0
0.25	1.5×10^8	1.5×10^8	0
0.5	1.2×10^8	1.4×10^8	18
2.0	8.6×10^7	1.5×10^8	40
3.0	7.9×10^7	1.5×10^8	45
4.0	5.1×10^7	1.4×10^8	65
5.0	4.5×10^7	1.5×10^8	69

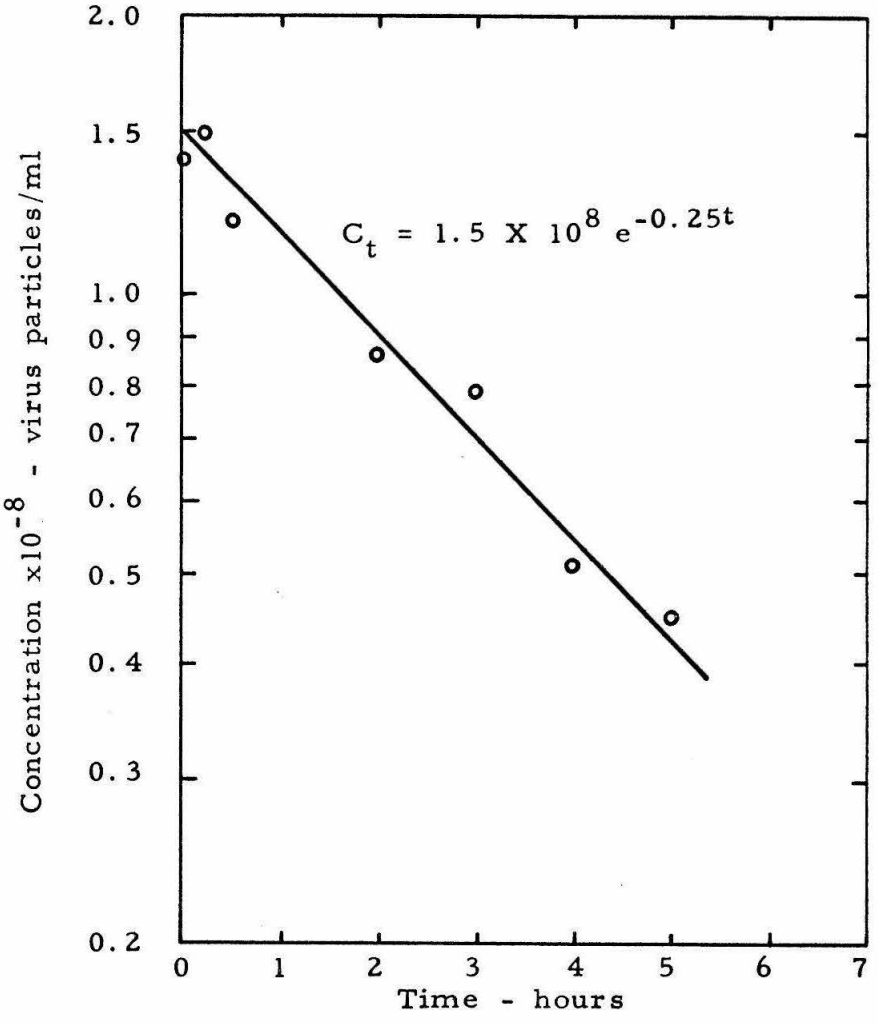


Figure 5-10. Viruses Remaining in Solution vs. Time of Contact With 26/35 Carbon at 33°C.

5-5 Effects of Virus and Carbon Concentration on Adsorption

Eight experiments were conducted with various concentrations of carbon and virus. The initial concentrations of each reactant and the change of virus concentration with time are given in Table 5-13. The total decrease in virus concentration can be attributed to adsorption. As indicated by the control (test 4-5.8) no significant loss of virus resulted from natural inactivation. The rate of virus inactivation in the control was first order, as can be seen from the straight line obtained for virus concentration versus time on a semi-logarithmic plot (Figure 5-11). Calculations showed that test 4-5.8, at an ionic strength of 0.10 and pH of 6.9 without carbon, gave an inactivation-rate constant of 0.004/hr.

Inactivation of virus by environmental factors was not significant in test solutions of pH 7.0 and ionic strengths of 0.02 to 0.20. In the preliminary tests, which used low ionic strengths (0.004), noticeable inactivation occurred (Figure 5-3). Variation of virus stability with ionic strength has been reported before. Sproul (112) found that the inactivation of Micrococcus pyogenes var. albus bacteriophage decreased as $CaCl_2$ molarity was increased from 0.001 M to 0.004 M. This effect, however, is more complicated than a simple ionic strength relationship. Sproul (112) also found that inactivation increased with increasing concentrations of NaCl from 0.001 M to 0.003 M. Ions are known to bring about some subtle structure changes in the virus. This phenomenon will be further discussed in relation to the effects of pH and ionic strength on adsorption (sections 5-9 and 5-11).

TABLE 5-13

Results of T_4 Phage Adsorption to Carbon Using Different Initial Concentrations of Reactants

Time Hr.	test no. Carbon mg/l	Virus Remaining in Solution - Virus particles/ml							
		4-5.1 250	4-5.2 50	4-5.3 100	4-5.4 25	4-5.5 250	4-5.6 250	4-5.7 250	4-5.8 Zero
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	4.7×10^8	4.6×10^8
0.5		--	--	--	--	3.8×10^7	5.4×10^6	2.7×10^8	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	2.2×10^8	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

TABLE 5-13 (Cont'd)

Time Hr	Carbon mg/l	Virus Remaining in Solution - Virus particles/ml							
		4-5.1 250	4-5.2 50	4-5.3 100	4-5.4 25	4-5.5 250	4-5.6 250	4-5.7 250	4-5.8 Zero
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	--	--	--	--

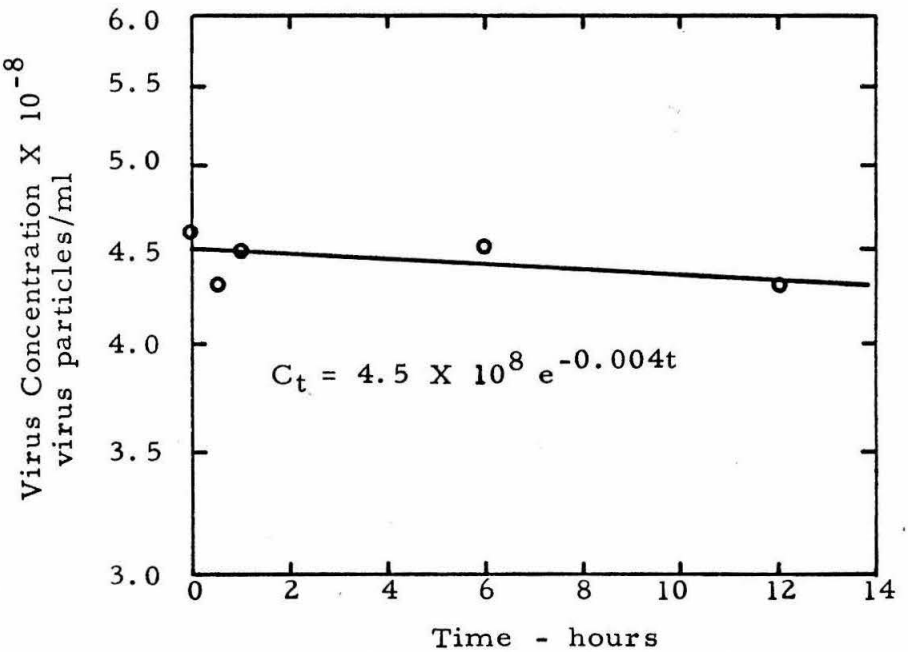


Figure 5-11. Inactivation of Virus in Control Test 4-5.8 at pH 6.9 and Ionic Strength of 0.10

Adsorption of virus to carbon did not follow a first-order reaction. This phenomenon is apparent in Figures 5-12 and 5-13. The rate of virus adsorption changed with time and appeared to diminish to zero. In two tests (4-5.2 and 4-5.4) adsorption reached an equilibrium or utilized the entire capacity of the carbon. In a third test (4-5.3), this condition was being approached. The expected relationship between adsorption rate and reactant concentrations would be first order with each reactant and, therefore, second order overall. This relationship does appear to exist.

When the rate is dependent on only one reactant concentration, the reaction order can be easily estimated by the relation between partial reaction times and reaction orders. It becomes fairly complicated, however, when applied to systems in which there is more than one reactant. Although the adsorption rate appeared to depend on both reactants, the method was applied to test if a second-order reaction with respect to virus concentration could exist (Table 5-14). The difference in times between one-half and three-quarters completion of the reaction divided by its half- life will be 0.50 for a zero-order reaction, 1.00 for first-order, 2.00 for second-order, and 3.00 for third-order.

The ratios of the reaction times in these experiments varied from 1.4 to 3.8 (Table 5-14). This spread of values indicates that the reaction rate does not depend solely on the concentration of virus. The other reactant, carbon, is also involved. If a carbon site is defined as the unit that accepts one virus particle, then the reaction can be

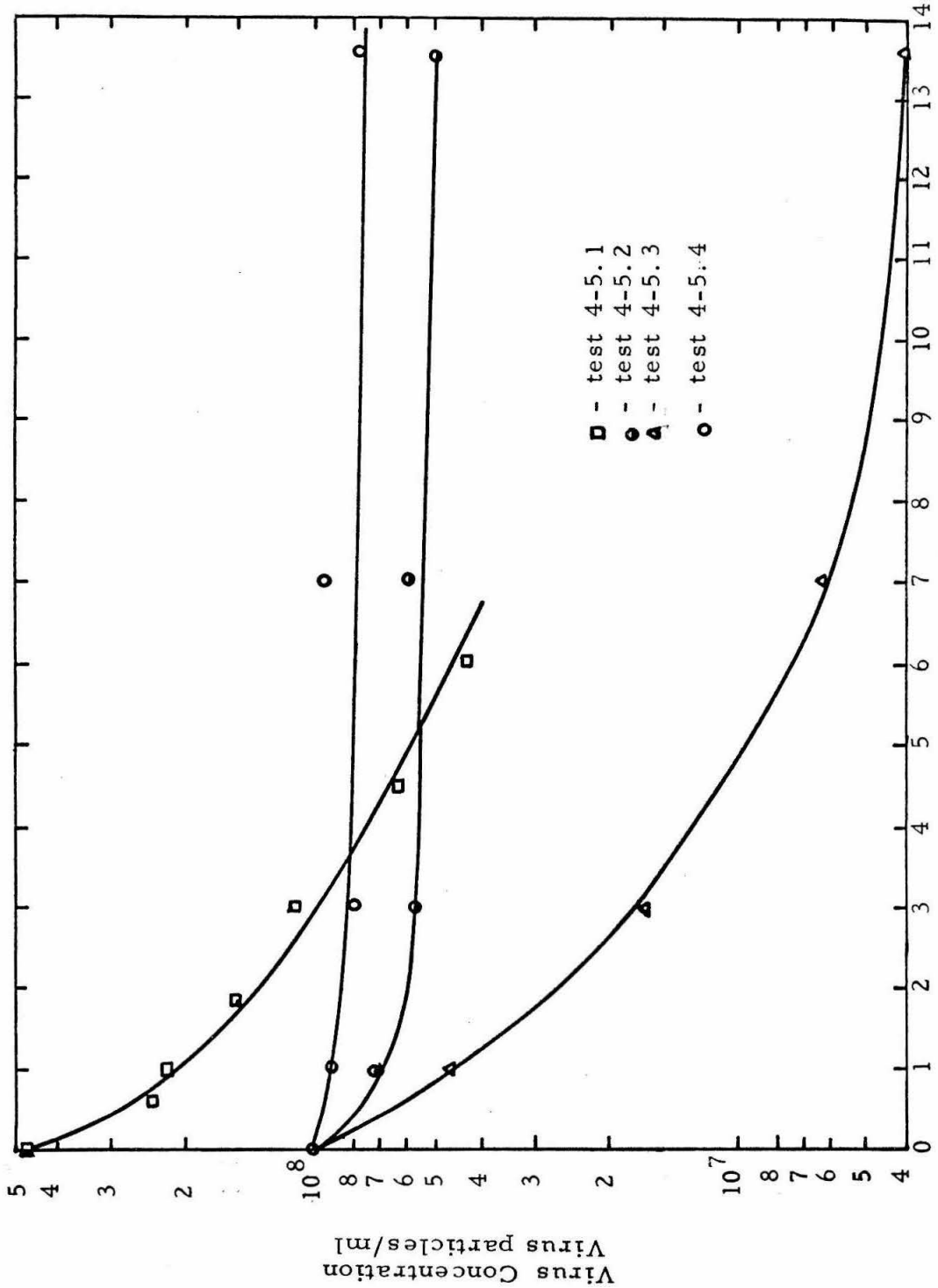


Figure 5-12. Virus concentration Remaining in Solution vs. Time for Different Concentrations of Reactants in Adsorption of T_4 Phage to Carbon

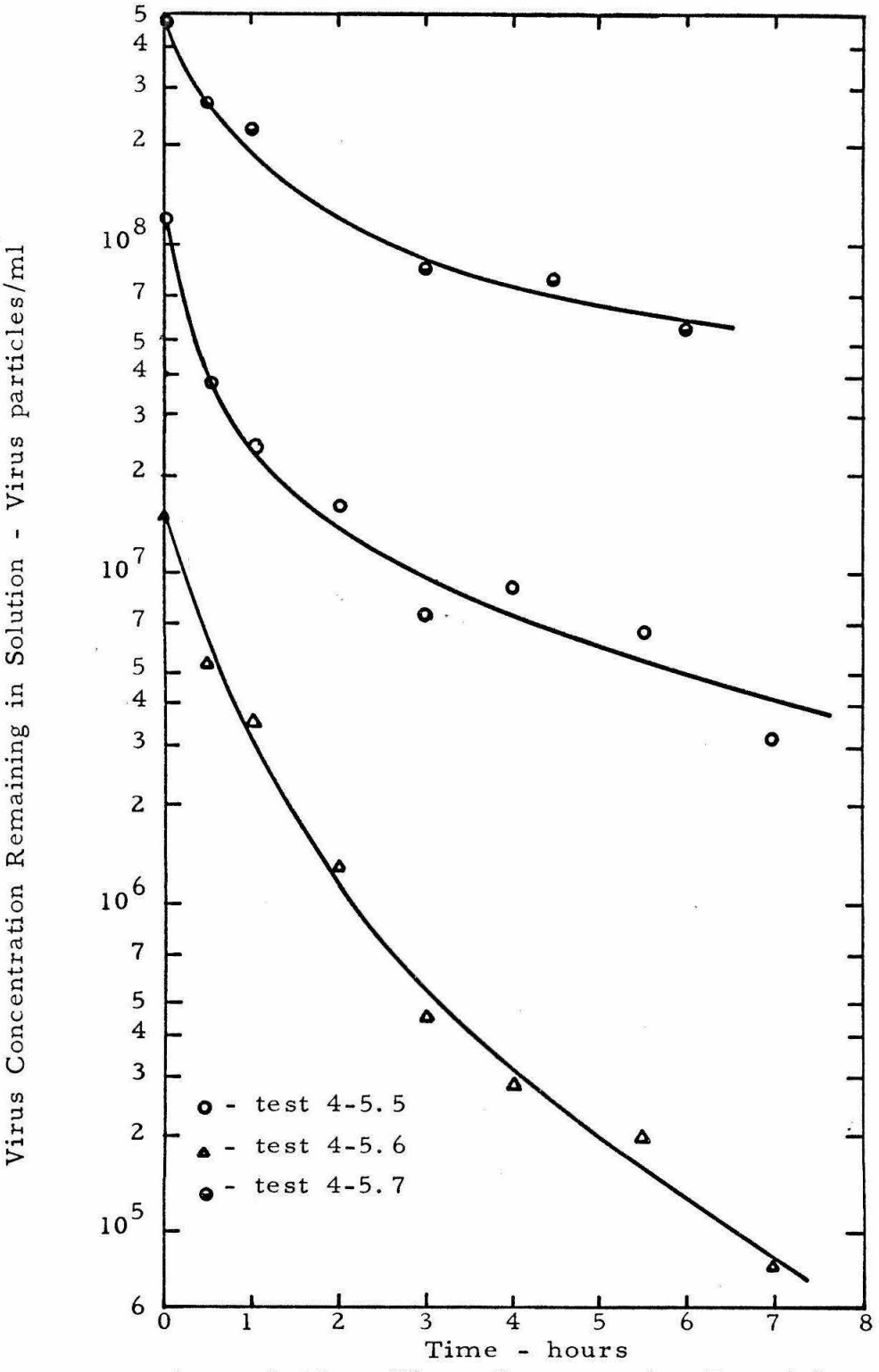


Figure 5-13. Virus Concentration Remaining in Solution vs. Time for Different Concentrations of Reactants in Adsorption of T_4 Phage to Carbon

represented by the following equation:

$$(\text{virus}) + (\text{site}) = (\text{virus-site complex})$$

TABLE 5-14

Ratios of Partial Reaction Times for T_4 Phage Adsorption to Carbon

Test No.	Initial carbon concentration mg/l	Initial Virus concentration virus particles/ml	$t_{3/4}$ min	$t_{1/2}$ min	$\frac{t_{3/4} - t_{1/2}}{t_{1/2}}$
4-5.1	250	4.8×10^8	129	44	1.90
4-5.3	100	1.0×10^8	126	53	1.40
4-5.5	250	1.2×10^8	43	9	3.8
4-5.6	250	1.5×10^7	15	6	1.50
4-5.7	250	4.7×10^8	120	39	2.08

This relationship is first order with respect to virus and site concentrations and second order with respect to the whole reaction. From the partial reaction times the reaction will appear as second order with respect to either reactant when they are present in equal concentrations. The ratios of partial reaction times for tests 4-5.1 and 4-5.7 gave values of approximately two (Table 5-14). This correlation with second order would indicate that the initial concentrations of virus and carbon sites were approximately equal. Test 4-5.1 had an initial virus concentration of 4.8×10^8 virus particles/ml, and test 4-5.7 contained 4.7×10^8 virus particles/ml. A carbon concentration of 250 mg/l was used in each test. If 4.8×10^8 sites/ml were also present, then each milligram of carbon contains 1.9×10^9 sites.

The relationship between partial reaction time and reaction order used in Table 5-14 is for an irreversible reaction. It will be shown that adsorption is weakly reversible. The weak reversibility enables data to be interpreted by irreversible kinetics during initial adsorption stages. The mathematical representation of an irreversible and reversible system as derived in Appendix 5 is given below.

Irreversible:

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

$$\ln a/b = (a_o - b_o)k_1 t + \ln a_o/b_o \qquad (5\text{-}4)$$

Reversible:

$$a + b \underset{k_2}{\overset{k_1}{\rightleftharpoons}} X$$

$$tk_1 m = \ln \left[\frac{0.5(a_o + b_o + K^{-1} + m) - X}{0.5(a_o + b_o + K^{-1} - m) - X}\right]\left[\frac{a_o + b_o + K^{-1} - m}{a_o + b_o + K^{-1} + m}\right] \qquad (5\text{-}3)$$

where:

a_o = initial concentration of virus (virus particles/ml)

b_o = initial concentration of carbon sites (sites/ml)

a = virus concentration at time t (virus particles/ml)

b = site concentration at time t (sites/ml)

t = time

k_1 = forward rate constant (ml/virus particle-sec)

k_2 = reverse rate constant (1/sec)

K = equilibrium constant ($K = k_1/k_2$) (ml/virus particle)

X = concentration of adsorbed virus at time t (virus particles/ml)

$m = \left[(a_o - b_o)^2 + K^{-1}\,(2a_o + 2b_o + K^{-1})\right]^{\frac{1}{2}}$

The irreversible system has two unknowns, the forward rate constant and the concentration of carbon sites. The reversible system has three unknowns, the forward and reverse rate constants and the concentration of carbon sites. The irreversible mathematical representation, therefore, is much simpler to use for approximating the concentration of carbon sites. It will be shown below that data conform closely to the assumption that the reaction is irreversible second order.

The irreversible equation was used to estimate the initial concentration of sites on the carbon. The two unknowns, forward rate constant and initial site concentration, can be found by using data from two adsorption tests in which the intial site concentrations are known to be different. If reaction conditions are identical the rate constants will be the same for each test. The known difference in site concentration between the two adsorption tests was realized by using the same carbon in a fresh condition and after it had adsorbed a known number of viruses. This technique was performed with the same carbon and reaction solution (4-3.1) in two steps of adsorption as explained in section 4-5.

Results of the first adsorption step were used to compare the effect of virus age on adsorption (Table 5-10). The initial concentration of virus was 4.2×10^8 virus particles/ml. The concentration was reduced to 3.3×10^7 virus particles/ml after 7.5 hours of adsorption. The virus concentration was then increased to 1.1×10^8 virus particles/ml. The test was continued for 5 more hours. A virus titer of 5.0×10^7 particles/ml was obtained after 5 hours of adsorption (Table 5-15 and Figure 5-14).

TABLE 5-15

Adsorption of T_4 Virus to 120/140 carbon in two steps: 1. Adsorption to fresh carbon, 2. Adsorption to partially exhausted carbon

Time Hr.		Virus Concentration Remaining in Solution virus particles/ml
First step	Second step	
0		4.2×10^8
0.25		2.7×10^8
1.0		1.6×10^8
2.0		1.2×10^8
3.7		6.2×10^7
5.0		4.1×10^7
7.5		3.3×10^7
Addition of more viruses:		
7.5	0	1.1×10^8
8.0	0.5	1.0×10^8
8.5	1.0	1.0×10^8
9.5	2.0	7.0×10^7
11.0	3.5	6.6×10^7
12.5	5.0	5.0×10^7

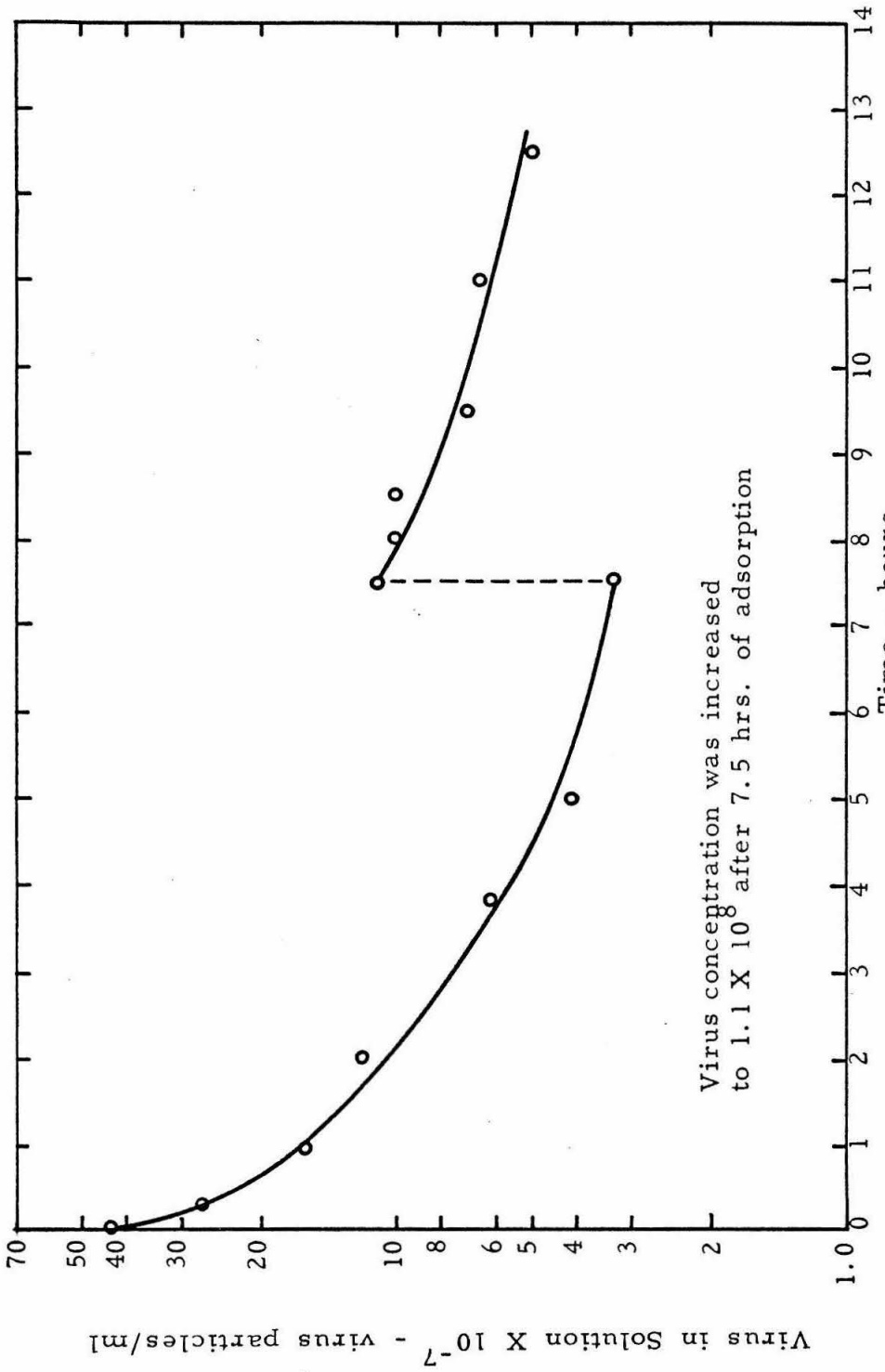


Figure 5-14. Viruses Remaining in Solution vs. Time of Contact With Activated Carbon.

A carbon concentration of 250 mg/l was used in both steps of the test. The site concentration, however, was reduced by 38.7×10^7 sites/ml at the initiation of the second step, equivalent to the amount of virus particles adsorbed in the first step. The calculated forward rate constant will be the same in both steps for a unique assumed value of the initial concentration of carbon sites. This condition assumes that all sites have equal ability to adsorb virus. Rate constants were calculated by plotting the experimental results in the form of the irreversible equation. A log plot of the ratio of virus-to-site concentration against time will give a straight line. The forward rate constant for the irreversible equation can be obtained from the slope.

Numerous values for initial site concentrations were used to calculate rate constants. One value, 4.8×10^8 sites/ml, gave the best interpretation of data by irreversible second-order kinetics. This site concentration corresponds to 1.9×10^9 sites/mg of carbon for a reaction solution containing 250 mg/l of carbon. Results for two other assumed site concentrations, 4.0×10^8 and 6.0×10^8 sites/ml, will be presented here to illustrate the effect of small changes in the assumed site concentration on the kinetic interpretation of data. Site concentrations of 4.0×10^8 and 6.0×10^8 sites/ml correspond to 1.6×10^9 and 2.4×10^9 sites/mg of carbon respectively.

Kinetic interpretation of data in Table 5-15 gave a slight curve when using the assumed value of 6.0×10^8 sites/ml (Figures 5-15 and 5-16). The value of 4.0×10^8 sites/ml gave a much greater curve for the first step. It could not be applied to the second step since more total virus particles were adsorbed than the assumed number of initial

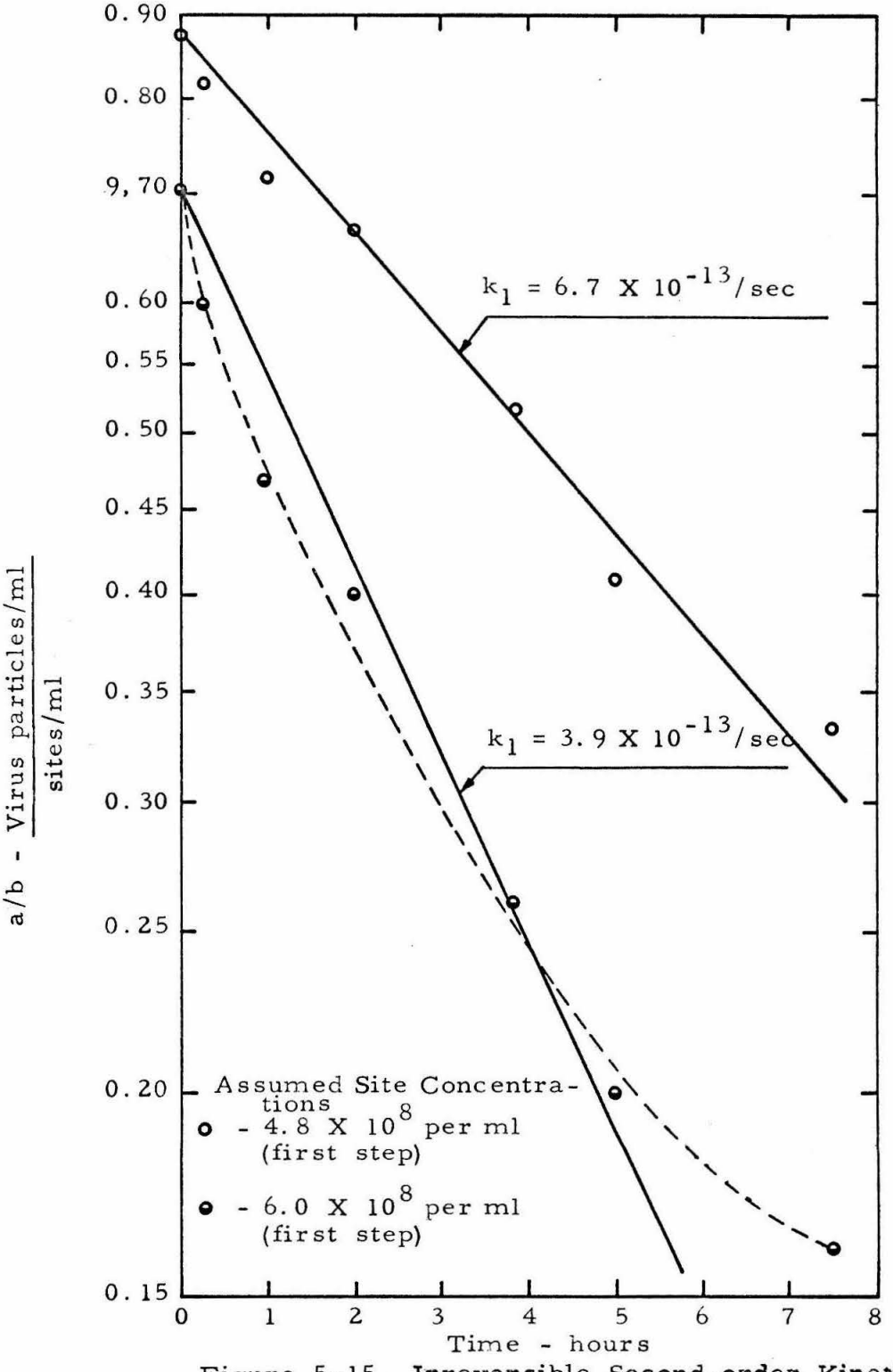


Figure 5-15. Irreversible Second-order Kinetic Plot of Two-step Adsorption Test Using Assumed Initial Site Concentrations

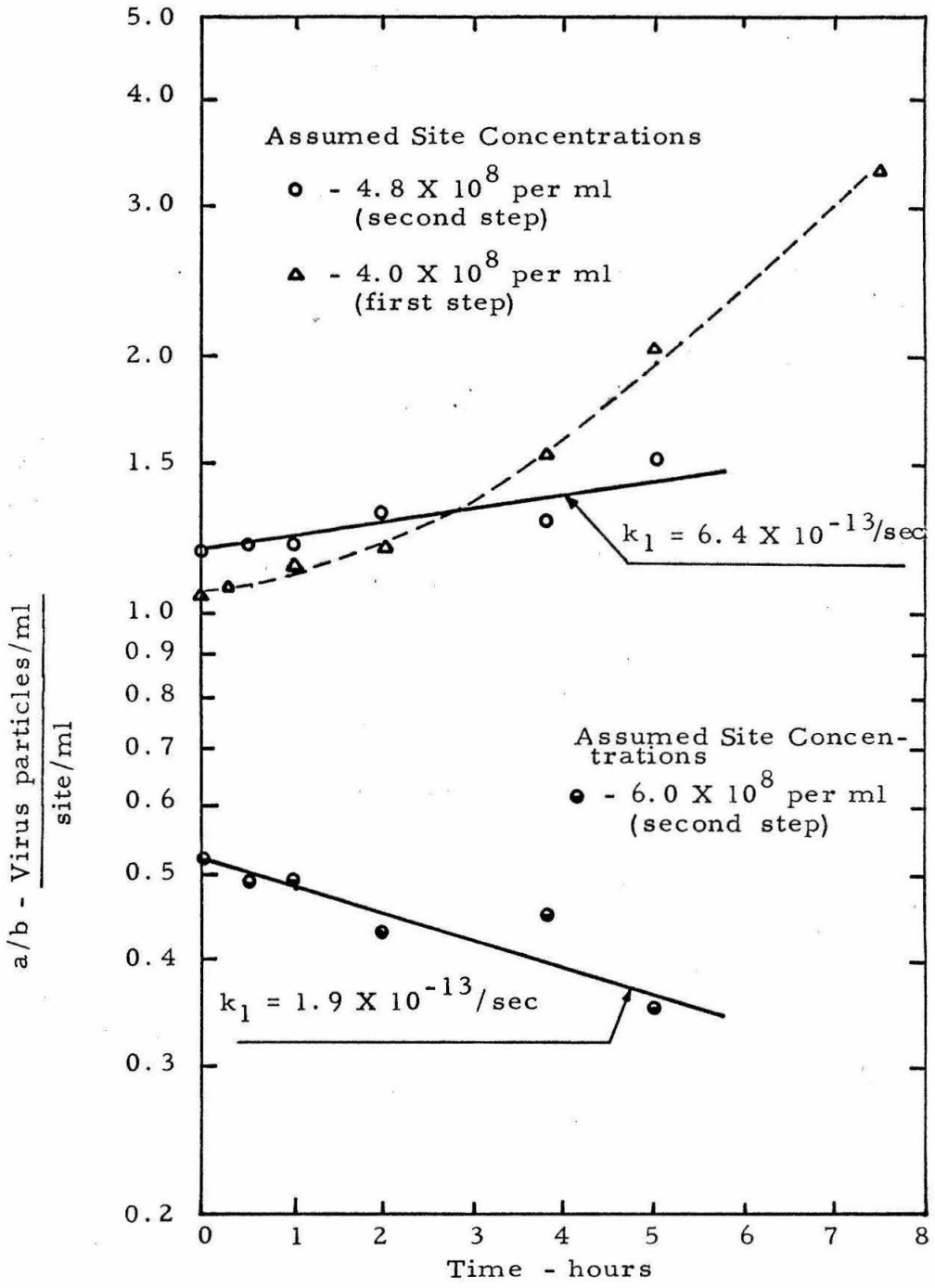


Figure 5-16. Irreversible Second-order Kinetic Plot of Two-step Adsorption Test Using Assumed Initial Site Concentrations

sites. With the assumed value of 4.8×10^8 sites/ml, rate constants of 6.7×10^{-13} and 6.4×10^{-13} ml/virus particle-sec were obtained for the first and second adsorption steps. These values are not considered significantly different and indicate that the assumed initial site concentration was reasonable. The value of 6.0×10^8 sites/ml gave rate constants of 3.9×10^{-13} and 1.9×10^{-13} ml/virus particle-sec for the first and second steps respectively. This variation in rate constants indicates that the assumption of 6.0×10^8 sites/ml for initial site concentration was incorrect.

In summary, the reaction was found to be first order with respect to both virus concentration and carbon sites. The overall reaction was second order. By using the relation between partial reaction time and reaction order an approximate value of 1.9×10^9 sites per milligram was obtained for the 120/140 carbon. This value was also obtained by the kinetic representation of experimental data with the irreversible second-order equation. A forward rate constant of 6.4 to 6.7×10^{-13} ml/virus particle-sec was obtained for this representation.

Values of 1.6×10^9 and 2.4×10^9 sites/mg were either too low or too high to apply to the experimental data and mathematical representation. The experimental data were represented very well by the irreversible second-order equation. The close agreement can be seen by the straight-line plots when using 4.8×10^8 sites/ml in Figures 5-15 and 5-16. The good agreement between the data and the irreversible equation indicates that the reverse rate constant in the equilibrium is small with respect to the forward rate constant.

Each section into which this study has been divided is dependent on the results obtained from the rest, and they cannot be discussed separately. After consideration of the reversible nature of the adsorption process these tests will be discussed with respect to reversible second-order kinetics.

5-6 Equilibrium Without Competitive Adsorbate

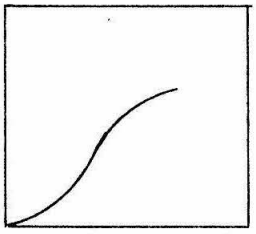
Tests run with various initial concentrations of reactants have been described in Chapter 4 (Table 4-6). Virus adsorbed per unit of carbon and the virus concentration in solution at equilibrium were determined for each of these tests (Table 5-16). Tests 4-6.1 and 4-6.2 were periodically assayed for 28.5 hours to determine the time required to achieve equilibrium. Tests 4-6.1 and 4-6.2 assayed 4.5×10^6 and 4.2×10^5 virus particles/ml respectively after 12 hours and 4.0×10^6 and 3.5×10^5 virus particles/ml respectively after 28.5 hours. A natural inactivation rate of 0.004/hr (Figure 5-11) for those testing conditions gave a virus reduction of 0.3×10^6 and 0.3×10^5 virus particles/ml between the period of 12 and 28.5 hours for tests 4-6.1 and 4-6.2 respectively. Adsorption over this period, therefore, only amounted to 0.2×10^6 and 0.4×10^5 virus particles/ml. The change in virus concentration beyond 12 hours of adsorption is small compared to the initial virus concentrations of 5.8×10^7 and 1.1×10^7 virus particles/ml. Tests 4-5.3 and 4-5.4 also indicated very little change in virus concentration after 13.5 hours of adsorption (Table 5-13). Test runs as long as 24 hours usually developed contamination. Equilibrium values, therefore, were presumed to exist after 12 hours of adsorption (Table 5-16).

TABLE 5-16

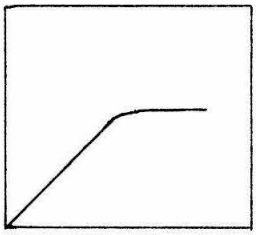
Results of Equilibrium Tests and Determination of Parameters for the Langmuir Equation

Test No.	Equilibrium Condition: Adsorbed virus virus particles/ml (q) X 10^{-9}	Equilibrium Condition: Virus in Solution virus particles/ml c_e X 10^{-6}	Ratio c_e/q X 10^3	$\frac{(Z-q)}{q} c_e$ X 10^{-6}
4-6.1	1.07	4.50	4.20	2.2
4-6.2	0.21	0.42	1.98	2.6
4-6.3	1.44	75.0	52.0	3.3
4-6.4	1.60	56.0	35.0	0.0
4-6.5	1.47	28.0	19.0	4.8
4-6.6	1.27	19.0	15.0	2.1
4-6.7	1.33	12.0	9.0	2.6
4-6.8	1.40	52.0	37.1	9.2
4-6.9	1.50	83.0	55.0	5.3
Previous tests (Table 5-13)				
4-5.3	1.06	3.9	3.7	2.0
4-5.4	1.40	70.0	50.0	0.0

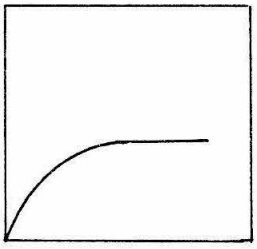
The equilibrium conditions of adsorption can be represented by isotherms. Giles and MacEwan (142) have classified adsorption isotherms from dilute solutions as four main types. These are illustrated below. The ordinate represents the concentration of adsorbed solute



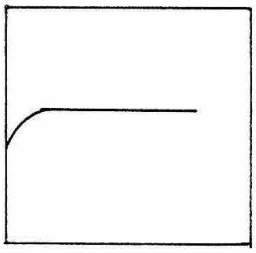
S



Ln



L



HA

per unit of adsorbent and the abscissa the concentration of solute in solution. The S type is typical of solutes that have a low affinity for the adsorbent. The Ln type has only been observed in two cases. These have been the adsorption of non-ionic polar dyes on cellulose acetate and simple amino-acids on powdered silica. The theoretical explanation of the Ln type is that the number of available sites remain constant even though the amount of solute adsorbed increases. This phenomenon occurs when the presence of the solute increases the available surface area. The L type is the most common and theoretically is represented by the Langmuir isotherm. The HA type is a special form of the L curve. The HA curve represents a solute with very high affinity

for the adsorbent in a solvent of low affinity. It is rare and has only been observed in adsorption from water of anionic dyes by graphite and phenol by charcoal.

5-6-1 Adsorption Isotherm Obtained for T_4, Carbon System

The adsorption isotherm for bacteriophage T_4 to activated carbon resembles type L (Table 5-16 and Figure 5-17). The virus has a high affinity for the carbon. The site concentration decreases with adsorption of viruses. The adsorption isotherm becomes horizontal at 1.5×10^9 virus particles per mg of carbon. The curve becomes horizontal when the equilibrium capacity of the carbon has been reached. It should be possible to represent this type of curve by the Langmuir isotherm.

The Langmuir adsorption isotherm is based on the formation of a unimolecular film. Molecules are considered to bind at discrete sites on the surface of a solid. Each site holds only one molecule by adsorption forces. All surface sites are assumed to be equivalent and an equilibrium condition exists between the adsorbed and free solute. The Langmuir equation (derived in Appendix 3) can be expressed by

$$c_e/q = \frac{1}{KZ} + \frac{1}{Z} c_e \qquad (5\text{-}5)$$

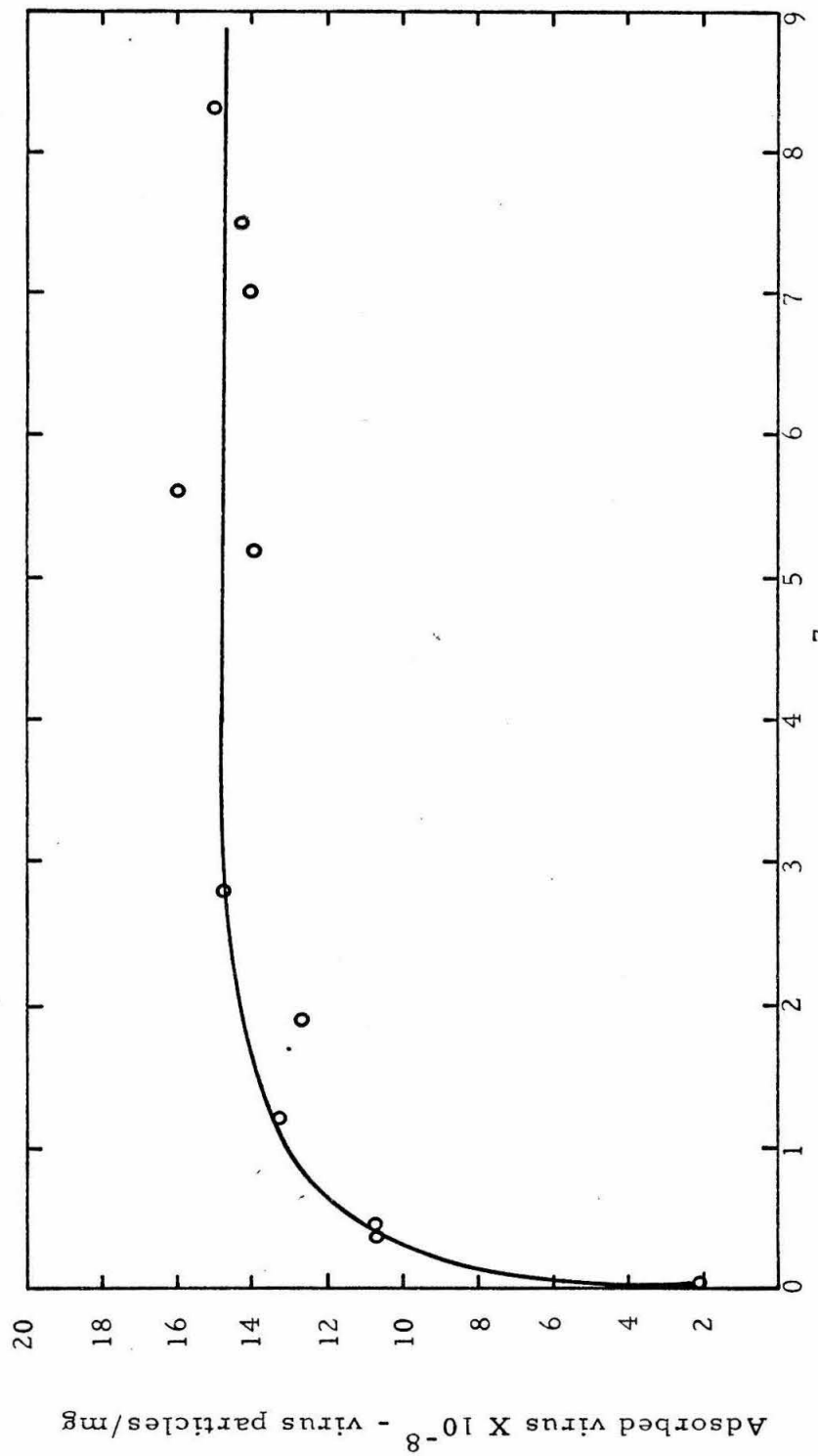


Figure 5-17. Adsorbed Virus per Unit Weight of Carbon vs. Virus Concentration in Solution at Equilibrium

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)

K = ratio of forward rate constant to reverse rate constant ($K=k_1/k_2$) (ml/virus particle)

Experimental data obeying the Langmuir isotherm will plot as a straight line in a graph of c_e/q (ratio of virus in solution to virus adsorbed) versus virus concentration in solution (Figure 5-18). From the slope the initial number of sites per milligram of carbon was found to be 1.6×10^9 sites/mg. In section 5-5, using the irreversible second-order equation, a value of 1.9×10^9 sites/mg was obtained. These values were determined by two different methods and compare very well.

The ratio of the forward to reverse rate constant can be obtained from the intercept of the Langmuir plot. In Figure 5-18 the intercept of 1/KZ is difficult to evaluate with any accuracy. The data, however, can be plotted in a different form for determining the ratio of the two rate constants. Rearranging the Langmuir equation gives:

$$\frac{k_2}{k_1} = c_e \frac{Z-q}{q}$$

The term $(Z-q)/q$ can be accurately determined at low concentrations of virus particles (c_e). As c_e increases, the amount of virus adsorbed (q) increases and approaches the total number of sites (Z). As q approaches Z the accuracy of the calculated value for k_2/k_1 decreases. At satura-

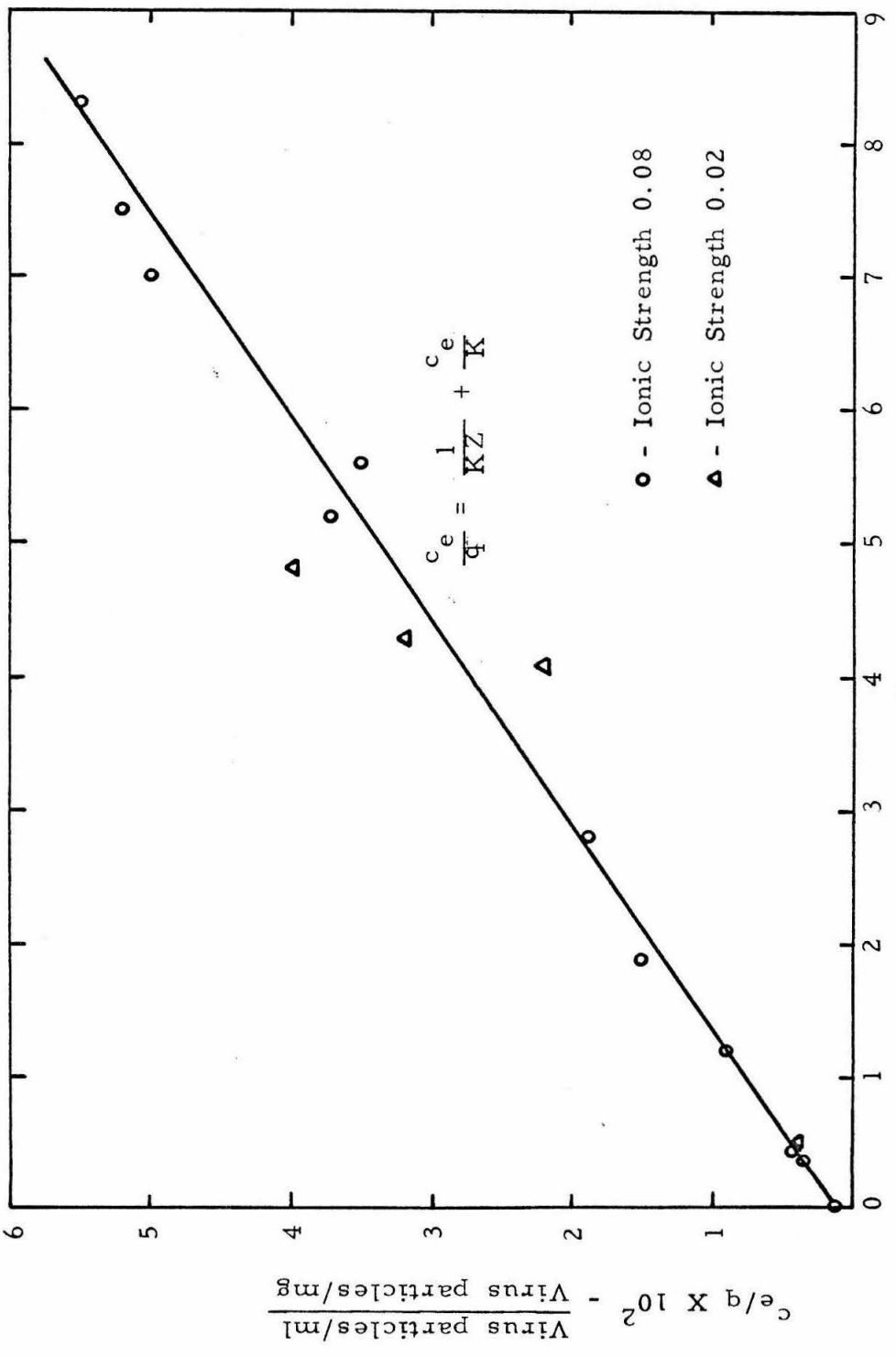


Figure 5-18. Langmuir Plot for Adsorption of T_4 Phage to 120/140 Activated Carbon by Least Squares Analysis

tion of the adsorbent the quantity (Z-q) is zero. A plot of $c_e(Z-q)/q$ versus c_e should give a scattering of points through which a horizontal line can be drawn for the most accurate value of k_2/k_1. The degree of scattering should increase with virus concentration. This procedure gave a value of 2.5×10^6 virus particles/ml for k_2/k_1 (Table 5-16 and Figure 5-19). The equilibrium constant is the reciprocal, 4×10^{-7} ml/virus particle. These units occur since the forward rate constant is second order and the reverse rate constant is first order.

The Langmuir isotherm represents the data obtained for adsorption of bacteriophage T_4 on activated carbon. Typical unimolecular adsorption of gases to carbon is represented by the Langmuir isotherm. In many cases of adsorption from solution, data are better represented by the Freundlich isotherm. The Freundlich isotherm is an empirical development represented by $q = k\ (c_e)^{1/n}$. Where q and c_e are the same as before, and k and 1/n are constants. This isotherm is only valid over small changes in concentration. Unlike the Langmuir equation it does not reach any limiting or saturation value. Conformance of data to the Freundlich isotherm may imply that the surface of the adsorbent is heterogeneous.

The results in Table 5-16 do not conform to the Freundlich isotherm since one should obtain a straight line (Figure 5-20). In these experiments better agreement is obtained with the Langmuir isotherm. In this respect the adsorption of bacteriophage T_4 differs from adsorption of amino acids to activated carbon. The Freundlich isotherm was

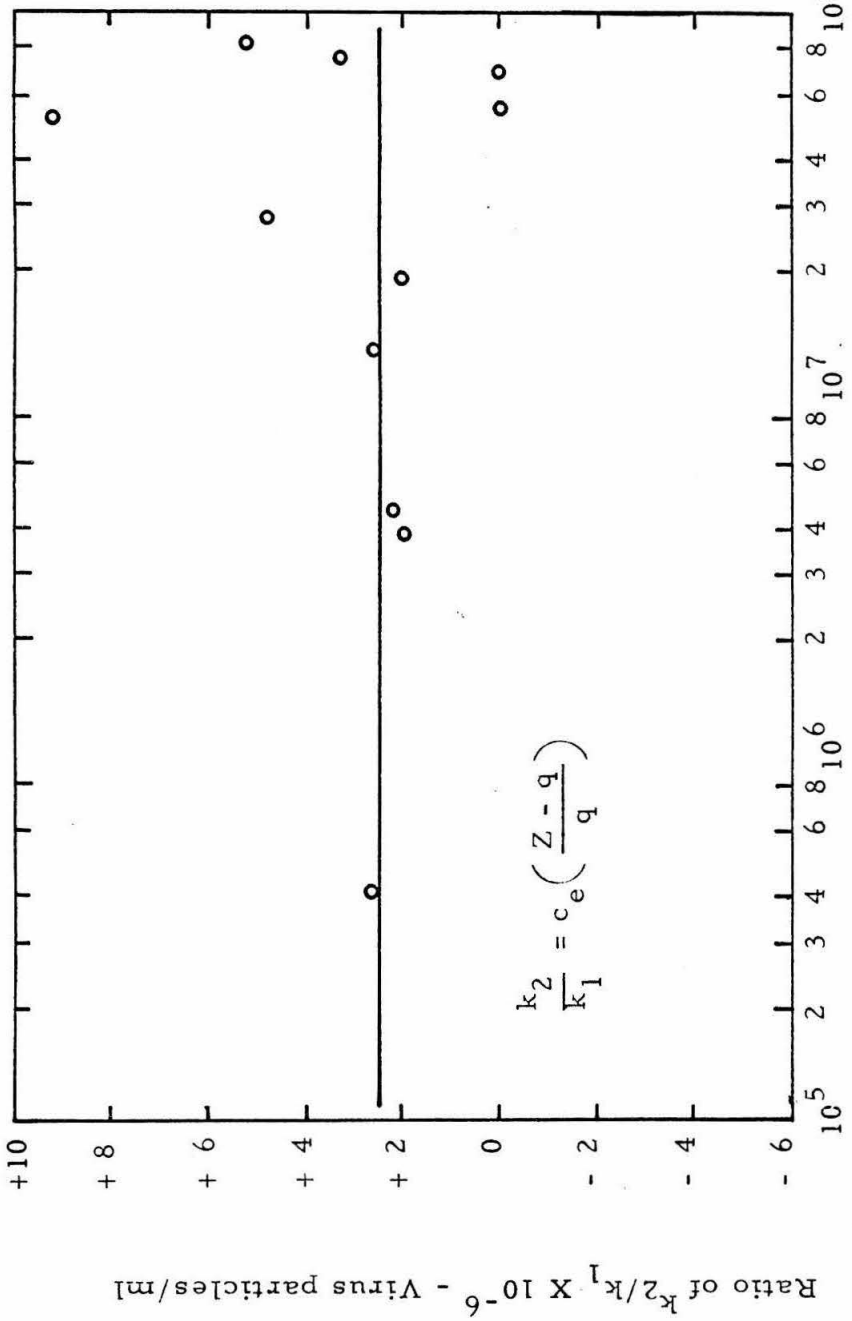


Figure 5-19. Ratio of Reverse to Forward Rate Constant vs. Virus Concentration in Solution at Equlibrium

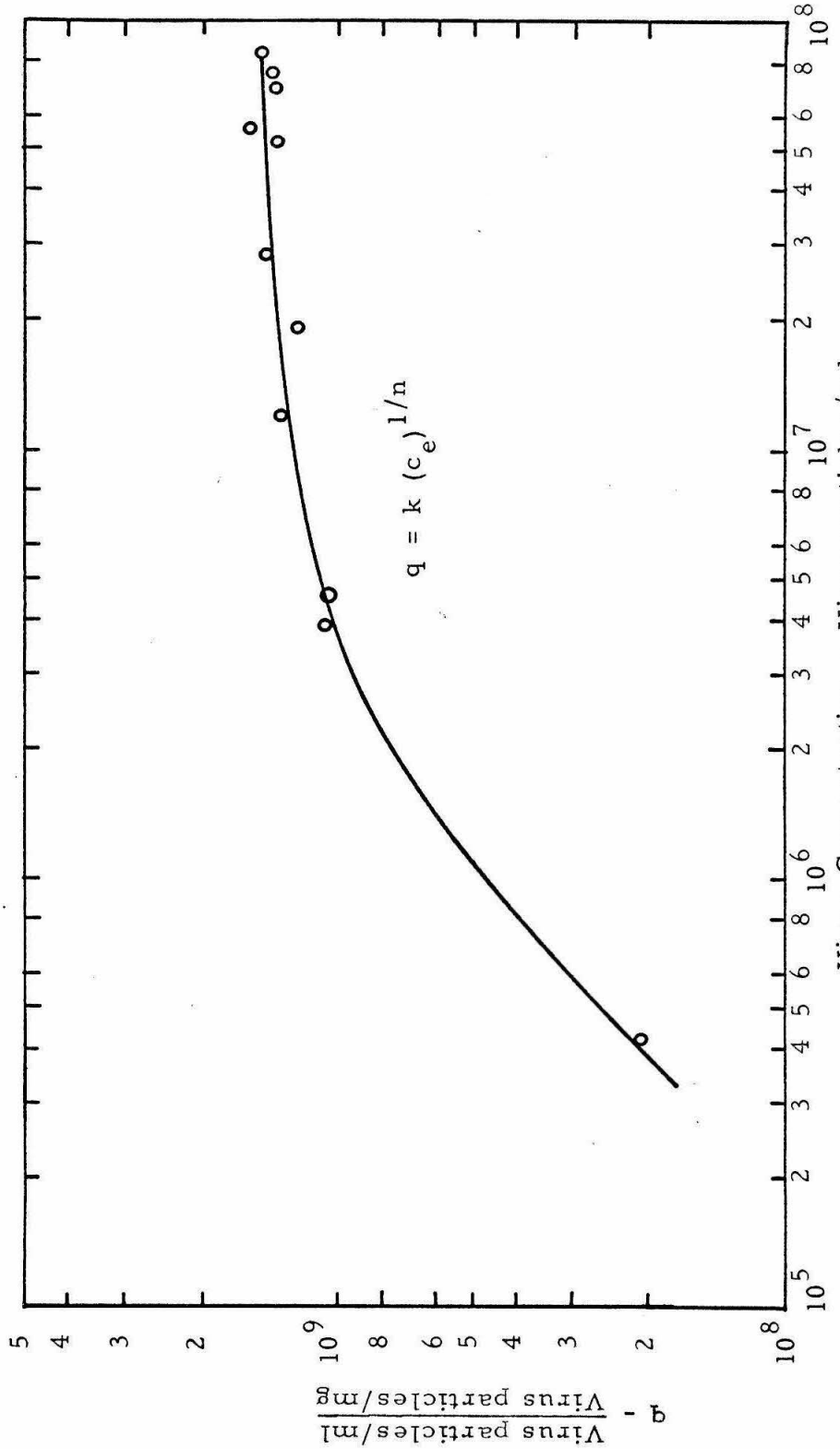


Figure 5-20. Freundlich Plot for Adsorption of T_4 Phage to 120/140 Activated Carbon

found applicable in almost all cases for amino acids (143). Amino acids show great individual variation in their adsorption properties (144). The position of the amino group, chain length, and branched chains all influence adsorption. The aromatic and heterocyclic groups adsorb very readily. Proteins, such as undigested casein, were found to adsorb very little on charcoal. Tiselius (144) suggested that most of the active surface of the adsorbent was inaccessible to large protein molecules. Although the surfaces of viruses are composed of amino acids, they apparently do not adsorb in a fashion similar to a single pure amino acid.

A special type of adsorption behavior has been proposed for adsorption of high-molecular-weight polymers (145)(146). Viruses have very high molecular weights, but they are not flexible like long-chain polymers. Bacteriophage T_4, however, does possess semi-flexible tail fibers (Figure 3-3). Each fiber is about 1500 Å long.

During adsorption to host cells, the tail fibers have been found to be the attaching unit (100)(147)(148). Purified isolated units of viruses have been tested for adsorption to host cells (148)(149). The head and tail cores did not adsorb to the bacterial cell under any of the tested conditions. Only the separate segments of the tail and tail fibers were found to adsorb. In particular, the fibers were found to have a high affinity for host cells (148). Tail fibers can also cause clumping of bacterial cells (149). They undoubtedly have more than one site for attaching.

Tail fibers 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. Separate fibers, therefore, might occupy different quantities of sites. Such an adsorption system might be expected to deviate from the condition represented by the Langmuir isotherm. If the sites were separated from each other by distances of the order of an extended fiber, the isotherm might be expected to follow the Langmuir equation. In Chapter 6 it will be shown that the virus particles adsorbed on carbon are separated by large distances.

Since the adsorption data follow the Langmuir isotherm, three possibilities exist: 1) The tail fibers are not the adsorbing unit. 2) The sites are separated by distances greater than a fiber length. 3) An average of N sites are occupied by the fibers of a virus, and the variance from N is too small to be significant. In the latter case the previous definition of a site as that which adsorbs one virus particle would be the same as N sites in the present definition.

The results of pH and ionic-strength experiments (discussed below) support the hypothesis that tail fibers adsorb to activated carbon as to host cells. The first hypothesis, thus, is not likely. In section 5-10 it will be shown that the adsorption site on carbon has the characteristics of a carboxyl group. To hold the virus particle on the carbon surface a number of carboxyl groups are probably necessary, and they must therefore be spaced at distances less than 1500 Å. The third hypothesis appears the most probable. 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 definite configuration when extended. Electron micrographs of extended fibers consistantly reveal them as two straight segments with a bend about 900 Å from the end (see

Figure 3-3). The existence of this configuration indicates that they are not as flexible as long-chain polymers. During adsorption, fibers probably occupy about the same number of attaching groups on the carbon as a result of this extended configuration. No significant deviation, therefore, would be expected from the Langmuir isotherm.

Hansen, Fu, and Bartell (150) found that in many cases, adsorption from solution by graphites and carbon blacks was multimolecular. In identical studies with high porosity carbons they found only unilayer adsorption. Their high porosity carbons had a specific area of 900 m^2/gm. Multilayer adsorption would not be expected with the virus and carbon system studied. The carbon used in these experiments for adsorbing T_4 phage had a specific area of 700-900 m^2/gm.

The equilibrium studies do not suggest that multimolecular adsorption occurred. This follows from agreement of the data with the Langmuir isotherm and the shape of the isotherm curve in Figure 5-17. Multimolecular adsorption isotherms resemble the Langmuir equation at low equilibrium concentrations, but as the concentration is increased the curve does not become horizontal as in Figure 5-17. It passes through a point of inflection as the adsorption layer becomes more than one molecule thick. Additional support for unilayer adsorption of T_4 phage to activated carbon will be given below when discussing the surface area that a virus particle occupies on the carbon (Chapter 6).

5-6-2 Desorption Isotherm Obtained for T_4, Carbon System

If true thermodynamic equilibrium was achieved in the above adsorption tests, the same equilibrium would be obtained by desorption.

T_4 phage was desorbed from carbon used in the previous tests and equilibrium values determined (Table 5-17). The data corresponded to the Langmuir isotherm (Figure 5-21). A greater scattering of points was obtained for the plot of the isotherm obtained by desorption than for the adsorption isotherm. This scattering 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 centrifugation period for removing carbon prior to resuspension probably had a small influence on the virus titer. Centrifugation for 20 minutes has a definitely noticeable effect, as discussed in section 5-1.

The method of least squares was used to fit a curve to the desorption equilibrium data (Figure 5-21). From the slope a value of 1.3×10^9 sites per milligram of carbon was obtained. For the same carbon, a value of 1.6×10^9 sites/mg was obtained when equilibrium was approached by adsorption. The close similarity of these values indicate that essentially the same equilibrium data are obtained by either adsorption or desorption.

5-7 Equilibrium in the Presence of a Competitive Adsorbate

Equilibrium was obtained between adsorption and desorption of viruses in tryptone broth. Tryptone was previously found to elute more viruses from carbon than a buffered salt solution. If this eluting effect resulted from competition between tryptone and virus particles for sites, then a Langmuir plot would give less available sites with respect to virus adsorption. As explained in section 4-7.2, carbon samples with

TABLE 5-17

Equilibrium Data Obtained by Desorption of T_4 Phage from Activated Carbon

Test No.	Virus Remaining Adsorbed virus particles/mg X 10^{-9}	Virus in Solution X 10^{-5} virus particles/ml	Ratio of Virus in solution to virus adsorbed X 10^{-4}
4-6.3	1.25	8.1	6.5
4-6.4	0.95	8.2	8.7
4-6.5	1.14	4.9	4.3
4-6.6	1.09	4.2	3.9
4-6.7	0.78	3.7	4.8
4-6.8	1.39	5.0	3.6
4-6.9	1.12	7.3	6.5

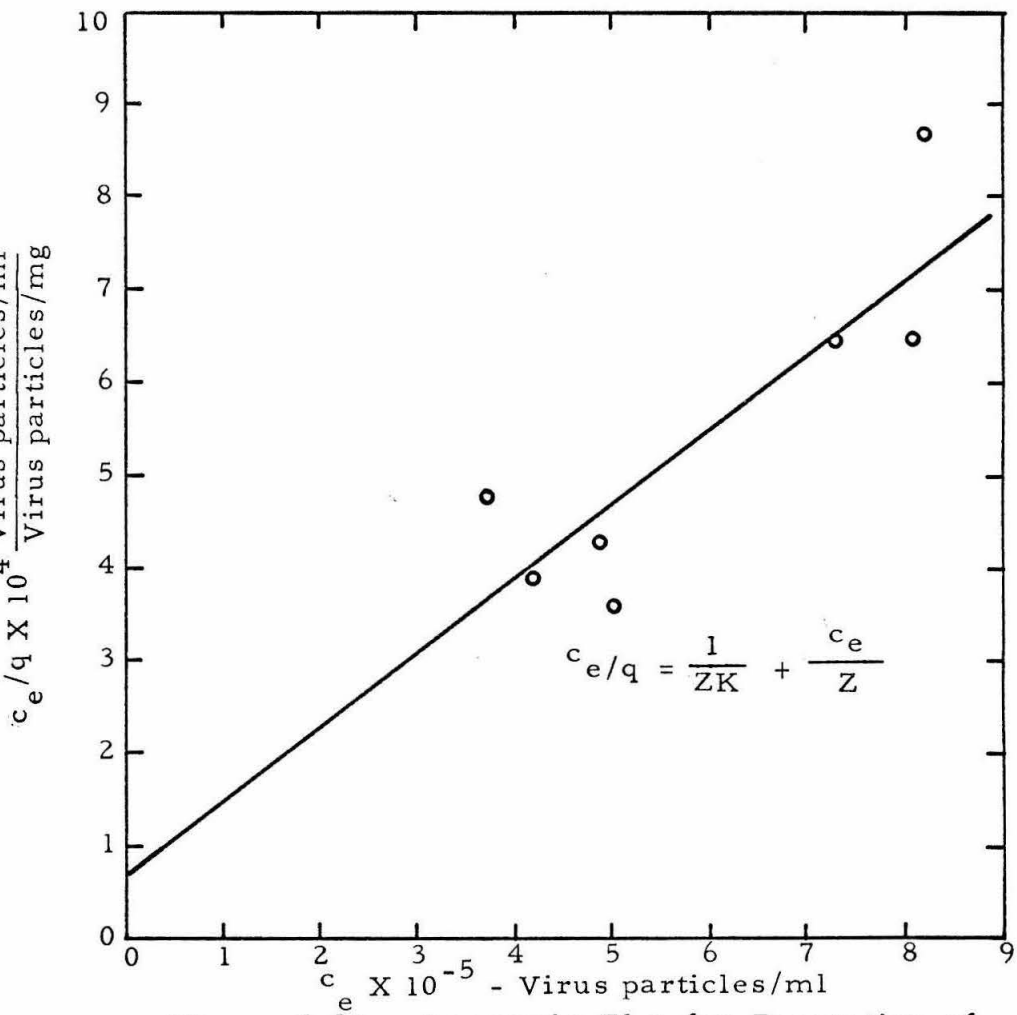


Figure 5-21. Langmuir Plot for Desorption of T_4 Phage from 120/140 Activated Carbon by Least Squares Analysis

the reaction solution were taken at different adsorption times and diluted in tryptone broth by 1/10, 1/100, 1/500, and 1/1000. The series of dilutions were made to obtain a series of equilibrium observations.

Dilutions of the reaction solution were made after adsorption had taken place for 30, 60, 180, and 420 minutes. From the dilution factor, the total virus concentration (adsorbed and in solution) for each desorption solution was known. From this information and the concentration of viruses in solution at equilibrium, the amount of virus adsorbed per milligram of carbon was determined for each equilibrium (Tables 5-18, 5-19, 5-20, and 5-21). The dilutions were assayed after 2, 7, and 24 hours to verify that equilibrium was established.

A Langmuir plot was made with assay values for the longest desorption times (Figure 5-22). By the method of least squares a value of 4.2×10^8 was obtained for the number of sites per milligram of carbon. The presence of tryptone reduced the number of sites available for virus particles from 16×10^8 to 4×10^8 per milligram of carbon. Tryptone, therefore reduces the equilibrium capacity for adsorption of bacteriophage T_4. The adsorption rate for viruses is probably also reduced. These phenomena are generally typical of binary systems.

Equilibrium values for low virus concentrations have been plotted to a larger scale to illustrate their conformity to the Langmuir isotherm (Figure 5-23). These low concentration values gave an equilibrium capacity of 3×10^8 virus particles/mg of carbon. This compares reasonably well with the value 4×10^8 virus particles/mg obtained from the complete curve (Figure 5-22).

TABLE 5-18

Desorption in Tryptone Dilutions
After Adsorption for 30 Minutes

Dilution factor	Time allowed for desorption Hr.	Virus concentration (c_e) virus particles/ml	Virus adsorbed per mg of carbon (q) X 10^{-8}	$\frac{(c_e/q)\ \text{virus particles/ml}}{\text{virus particles/mg}}$ X 10^4
1/10	2	5.3×10^6	3.5	
	7	3.5×10^6	4.2	83.3
1/100	2	5.6×10^5	3.4	
	7	5.6×10^5	3.4	16.6
	24	6.2×10^5	3.2	19.4
1/500	2	1.2×10^5	3.2	
	7	1.4×10^5	2.8	5.0
1/1000	2	6.1×10^4	3.2	
	7	5.8×10^4	3.3	1.8
	24	6.2×10^4	3.2	1.9

TABLE 5-19

Desorption in Tryptone Dilutions
After Adsorption for 1 Hour

Dilution factor	Time allowed for desorption Hr.	Virus concentration (c_e) virus particles/ml	Virus adsorbed per mg of carbon (q) $\times 10^{-8}$	$\frac{c_e/q)\ \text{virus particles/ml}}{\text{virus particles/mg} \times 10^4}$
1/10	2	3.4×10^6	4.2	
	7	3.7×10^6	4.1	90.0
1/100	2	3.8×10^5	4.1	
	7	3.7×10^5	4.1	9.0
	24	4.6×10^5	3.7	12.5
1/500	2	7.8×10^4	4.0	
1/1000	2	3.6×10^4	4.2	
	7	3.4×10^4	4.2	0.8
	24	5.9×10^4	3.2	1.8

TABLE 5-20

Desorption in Tryptone Dilutions
After Adsorption for 3 Hours

Dilution factor	Time allowed for desorption Hr.	Virus concentration (c_e) virus particles/ml	Virus adsorbed per mg of carbon (q) X 10^{-8}	$\frac{c_e/q)\ \text{virus particles/ml}}{\text{virus particles/mg}}$ X 10^4
1/10	2	1.9×10^6	4.8	
	7	1.8×10^6	4.9	36.6
1/100	2	2.2×10^5	4.8	
	7	2.0×10^5	4.8	4.2
	24	2.4×10^5	4.7	5.1
1/1000	2	2.3×10^4	4.7	
	7	3.0×10^4	4.4	0.7
	24	3.0×10^4	4.4	0.7

TABLE 5-21

Desorption in Tryptone Dilutions
After Adsorption for 7 Hours

Dilution factor	Time allowed for desorption Hr.	Virus concentration (c_e) virus particles/ml	Virus adsorbed per mg of carbon (q) X 10^{-8}	$\frac{c_e/q)\ \text{virus particles/ml}}{\text{virus particles/mg X } 10^4}$
1/10	2	6.9 X 10^5	5.3	
	7	1.6 X 10^6	5.0	32.6
1/100	2	8.1 X 10^4	5.3	
	7	1.6 X 10^5	5.0	3.3
	24	2.4 X 10^5	4.6	5.2
1/500	2	2.1 X 10^4	5.3	
	7	3.1 X 10^4	5.0	0.6
1/1000	2	1.1 X 10^4	5.2	
	7	2.7 X 10^4	4.5	0.6
	24	2.8 X 10^4	4.4	0.6

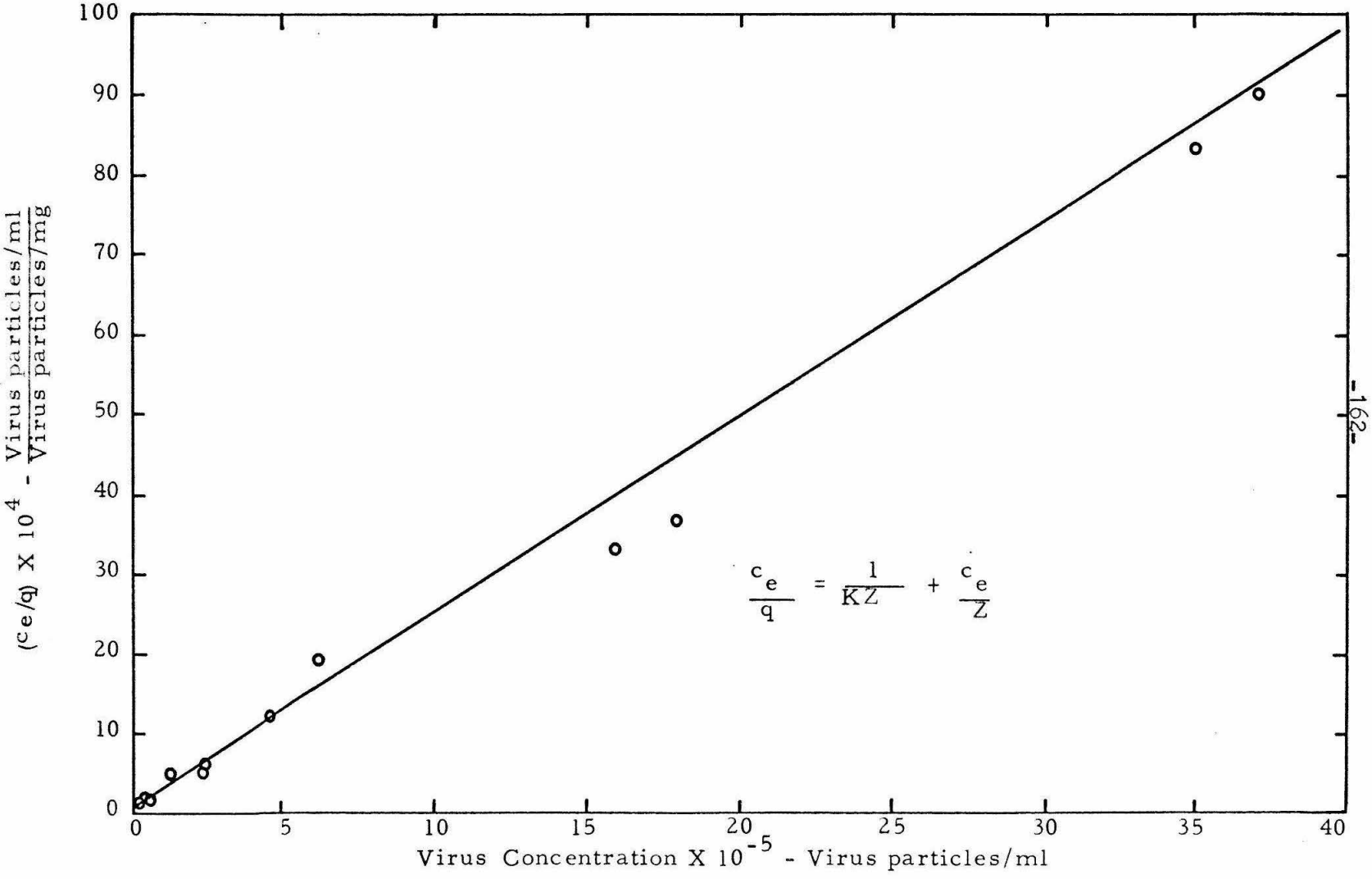


Figure 5-22. Langmuir Plot for Desorption Equilibrium in Tryptone Broth by Least Squares Analysis

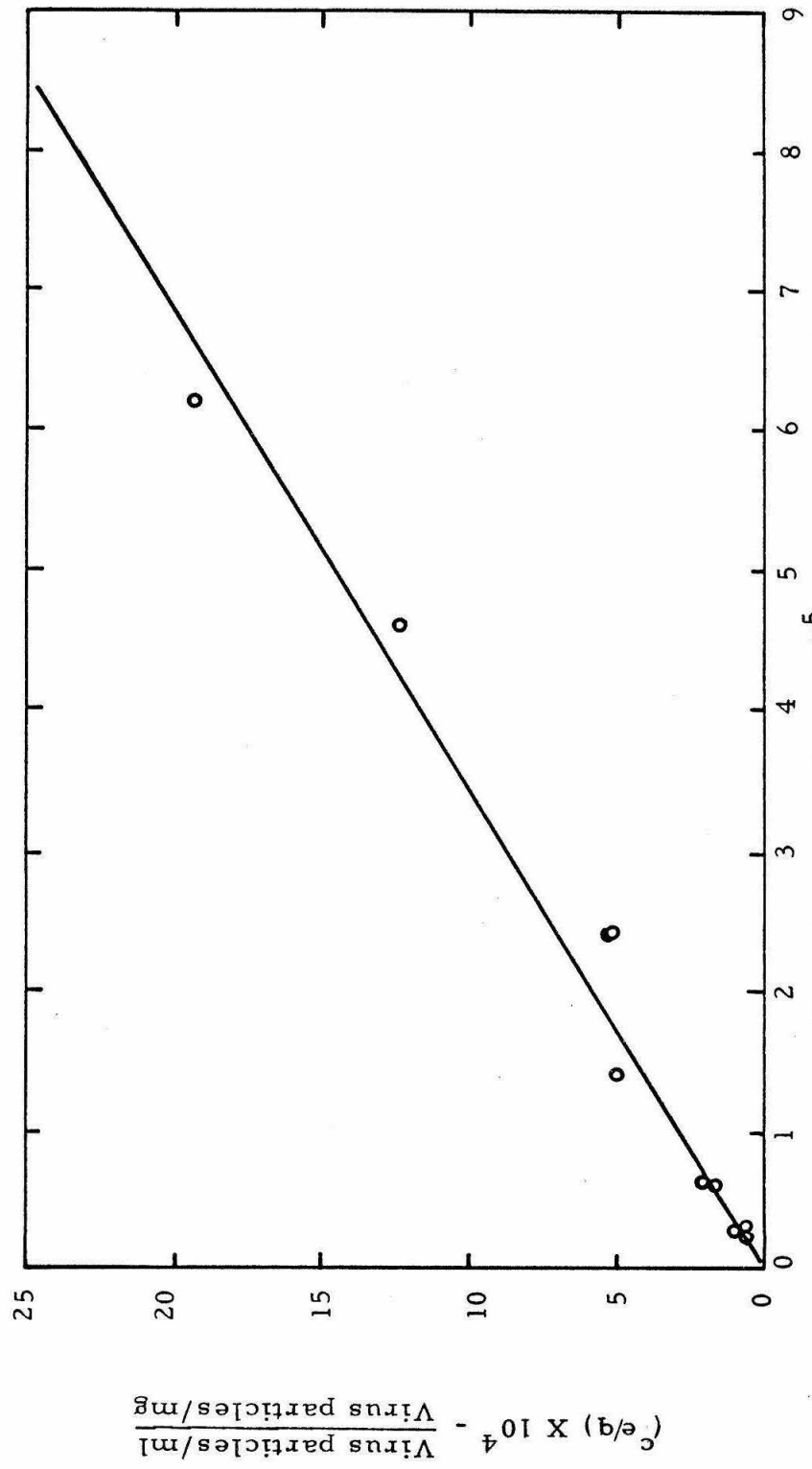


Figure 5-23. Langmuir Plot for Desorption Equilibrium in Tryptone Broth by Least Squares Analysis (Enlarged scale)

Values of q higher than 4×10^8 virus particles/mg were obtained from the 3 and 7 hr adsorption samples (Tables 5-20 and 5-21). Values presented in these tables were probably not at equilibrium. In the samples taken after 7 hours of adsorption the virus concentration of each dilution increased over the assay time of 24 hours. These results show that viruses were desorbed between 7 and 24 hours, and no later assay was made to establish that equilibrium had occurred at 24 hours.

5-8 Interpretation of Adsorption Data According to Reversible Second-order Kinetics

In section 5-5 the irreversible second-order equation was used to obtain an estimate of the total sites per milligram of carbon. Adsorption of bacteriophage T_4 to carbon, however, is thermodynamically reversible and would be represented better by the reversible second-order equation.

$$k_1 tm = \ln \left[\frac{0.5\ (a_o+b_o+K^{-1}+m)-X}{0.5\ (a_o+b_o+K^{-1}-m)-X} \right] \left[\frac{a_o+b_o+K^{-1}-m}{a_o+b_o+K^{-1}+m} \right] \qquad (5\text{-}3)$$

A value of 4.0×10^{-7} ml/virus particle was obtained for the equilibrium constant, K, and the number of sites per milligram of carbon was 1.6×10^9 (Figure 5-18). The initial concentration of carbon sites, b_o, can be calculated from the number of sites per milligram and the carbon concentration of the reaction solution.

A plot of the quantities in brackets of equation (5-3) versus time on semi- logarithmic paper should give a straight line for the proper values for b_o and K. This procedure allows calculation of the forward rate constant, k_1, from the slope of the line. The forward rate constant

should be identical for all tests made under the same adsorption conditions of pH, ionic strength and temperature.

Tests 4-5.1 through 4-5.8 were made at pH 6.9 and ionic strengths of 0.08 and 0.10. The data (Table 5-13) for these seven tests were substituted into the reversible second-order equation for determination of the rate constants (Table 5-22). A value of 4.0×10^{-7} ml/virus particle was used for K and 1.9×10^{9} for the total sites per milligram of carbon. A carbon capacity of 1.9×10^{9} sites/mg was used instead of 1.6×10^{9} since in two tests slightly more viruses were adsorbed than the 1.6×10^{9} value allowed for available sites. Rate constants were determined from the slopes of the plotted data (Figures 5-24 through 5-27). The forward rate constants for the tests at an ionic strength of 0.08 and 0.10 ranged from 8.3×10^{-13} to 8.8×10^{-13} ml/virus particles-sec and 7.2×10^{-13} to 7.9×10^{-13} ml/virus particles-sec respectively (Table 5-23). The variance of these values for a particular ionic strength is reasonable and indicates that reversible second-order kinetics can be used to represent the adsorption process. This kinetic representation, however, may not be proper, as will be discussed in Chapter 6.

Test 4-3.1, which was used to determine the effect of virus age on adsorption, previously was assumed to comply with reversible second-order kinetics (section 5-3). A second phase of this test was used to estimate the initial number of carbon sites by the irreversible second-order equation (section 5-5). This second step of the test is also represented well by reversible second-order kinetics (Table 5-24, Figure 5-27.

TABLE 5-22

Interpretation of Adsorption Data by Reversible Second-order Kinetics (tests 4-5.1 through 4-5.7)

Time t min.	Virus adsorbed X virus particles/ml	$\frac{0.5\,(a_o+b_o+K^{-1}+m)-X}{0.5\,(a_o+b_o+K^{-1}-m)-X}$	$\frac{a_o+b_o+K^{-1}-m}{a_o+b_o+K^{-1}+m}$
Test 4-5.1 ($b_o = 4.8 \times 10^8$, $a_o = 4.8 \times 10^8$, $m = 6.2 \times 10^7$)			
35	24×10^7		1.10
60	26×10^7		1.17
110	33×10^7		1.33
180	37×10^7		1.56
270	41.7×10^7		2.54
360	42.7×10^7		3.25
Test 4-5.2 ($b_o = 9.4 \times 10^7$, $a_o = 10 \times 10^7$, $m = 2.5 \times 10^7$)			
60	3×10^7		1.12
180	4.3×10^7		1.24
Test 4-5.3 ($b_o = 18.8 \times 10^7$, $a_o = 10.0 \times 10^7$, $m = 9.6 \times 10^7$			
60	5.3×10^7		1.52
180	8.3×10^7		3.12
420	9.4×10^7		6.72

TABLE 5-22 (Cont'd)

Time t min.	Virus adsorbed X virus particles/ml	$\frac{0.5\ (a_o+b_o+K^{-1}+m)-X}{0.5\ (a_o+b_o+K^{-1}-m)-X}$	$\frac{a_o+b_o+K^{-1}-m}{a_o+b_o+K^{-1}+m}$
Test 4-5.4 (b_o = 4.7 X 10^7, a_o = 10.5 X 10^7, m = 6.4 X 10^7)			
60	1.4 X 10^7	1.26	
180	2.5 X 10^7	1.73	
Test 4-5.5 (b_o = 4.7 X 10^8, a_o = 1.2 X 10^8, m = 3.5 X 10^8			
30	8.2 X 10^7	2.66	
60	9.6 X 10^7	4.13	
120	10.4 X 10^7	6.20	
180	11.24 X 10^7	13.80	
Test 4-5.6 (b_o = 4.7 X 10^8, a_o = 1.5 X 10^7, m = 4.6 X 10^7			
30	9.6 X 10^6	2.70	
60	1.14 X 10^7	4.06	
120	1.37 X 10^7	11.2	
180	1.45 X 10^7	29.0	
Test 4-5.7 (b_o = 4.7 X 10^8, a_o = 4.7 X 10^8, m = 6.1 X 10^7)			
30	2.0 X 10^8	1.10	
60	2.5 X 10^8	1.16	
180	3.84 X 10^8	1.82	
240	3.9 X 10^8	1.94	
360	4.15 X 10^8	2.99	

Application of the reversible second-order equation to the first step of adsorption yielded a forward rate constant of 7.2×10^{-13} ml/virus particles-sec (Figure 5-8). The same carbon was used in the second step. The number of available sites, thus, was reduced by the number of virus particles adsorbed in the first step. Using this reduced value for b_o (8.3×10^7 sites/ml) a forward rate constant of 7.0×10^{-13} ml/virus particles-sec was obtained (Figure 5-27). The two rate constants for both steps show good agreement. These constants are lower than those obtained for tests 4-5.1 through 4-5.7. This difference presumably resulted from different testing conditions. Test 4-3.1 with the two steps was made with an ionic strength of 0.06, where tests 4-5.1 through 4-5.7 used ionic strengths of 0.08 and 0.10. The effect of ionic strength will be examined in section 5-11.

In summary, reversible second-order kinetics were found to represent adsorption of bacteriophage T_4 to activated carbon satisfactorily. The values for carbon capacity and equilibrium constant obtained from the Langmuir equation were employed successfully in interpreting adsorption data by reversible second-order kinetics. The linearization of data by the reversible second-order equation was very sensitive to the value used for the carbon capacity. The best fit of data to a linear form was obtained by assuming a carbon capacity of 1.9×10^9 virus particles/mg.

The data of test 4-3.1 were also well represented by the irreversible second-order equation, yielding forward rate constants of 6.7×10^{-13} and 6.4×10^{-13} ml/virus particles-sec. Values of 7.2×10^{-13} and 7.0×10^{-13} ml/virus particles-sec were obtained

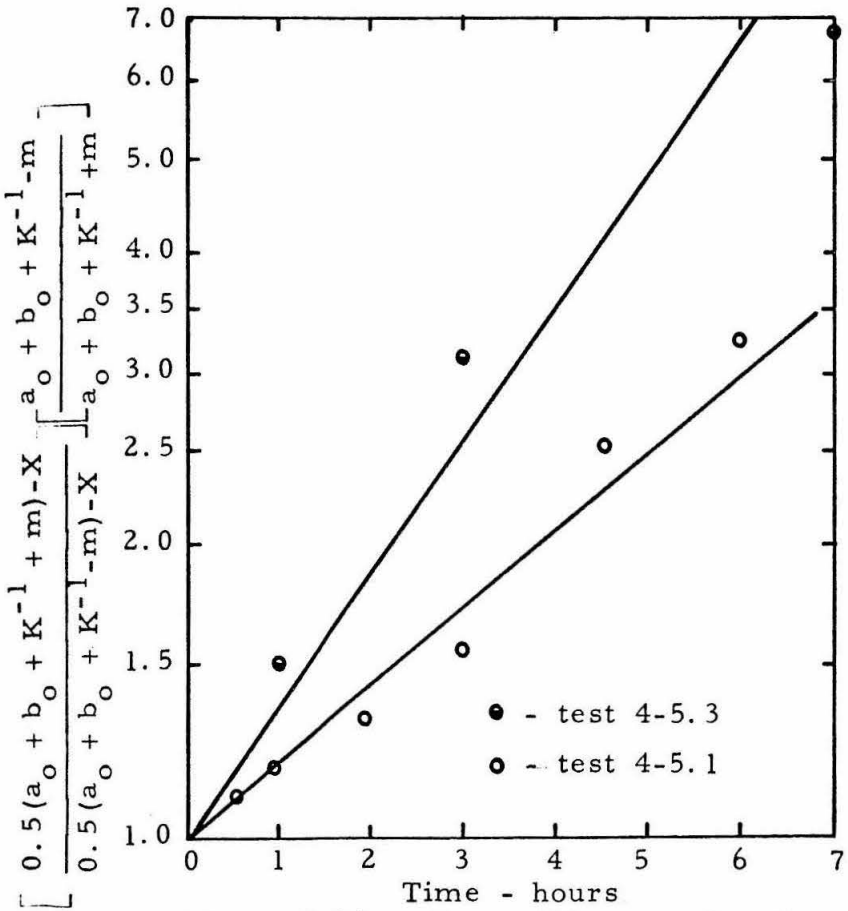


Figure 5-24. Reversible second-order kinetic plot of tests 4-5.1 and 4-5.3 for adsorption of T_4 phage to carbon

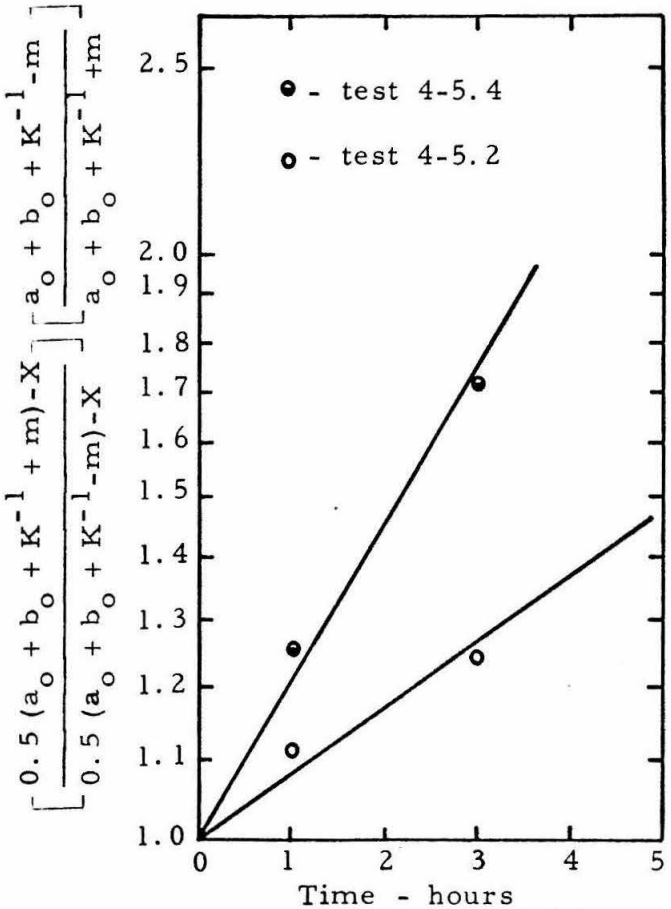


Figure 5-25. Reversible second-order kinetic plot of tests 4-5.2 and 4-5.4 for adsorption of T_4 phage to carbon

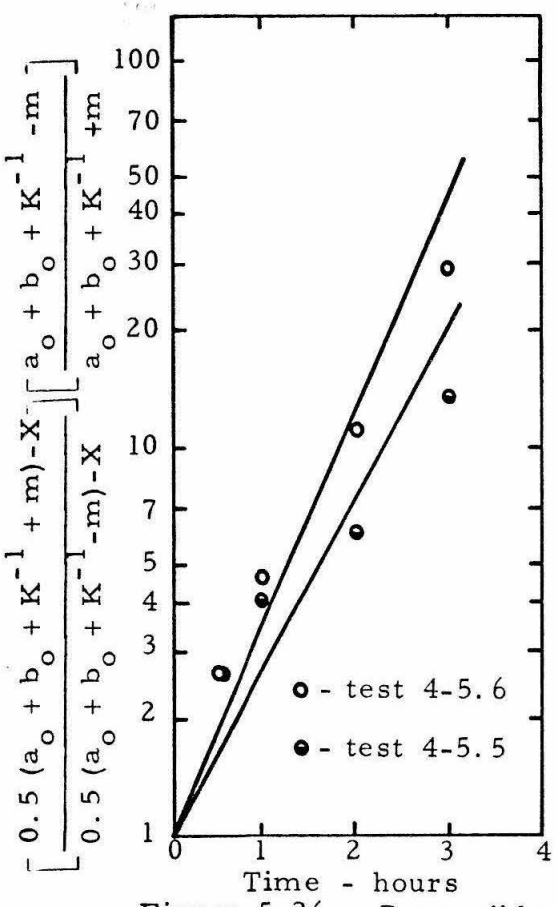


Figure 5-26. Reversible second-order kinetic plot of tests 4-5.5 and 4-5.6 for adsorption of T_4 phage to carbon

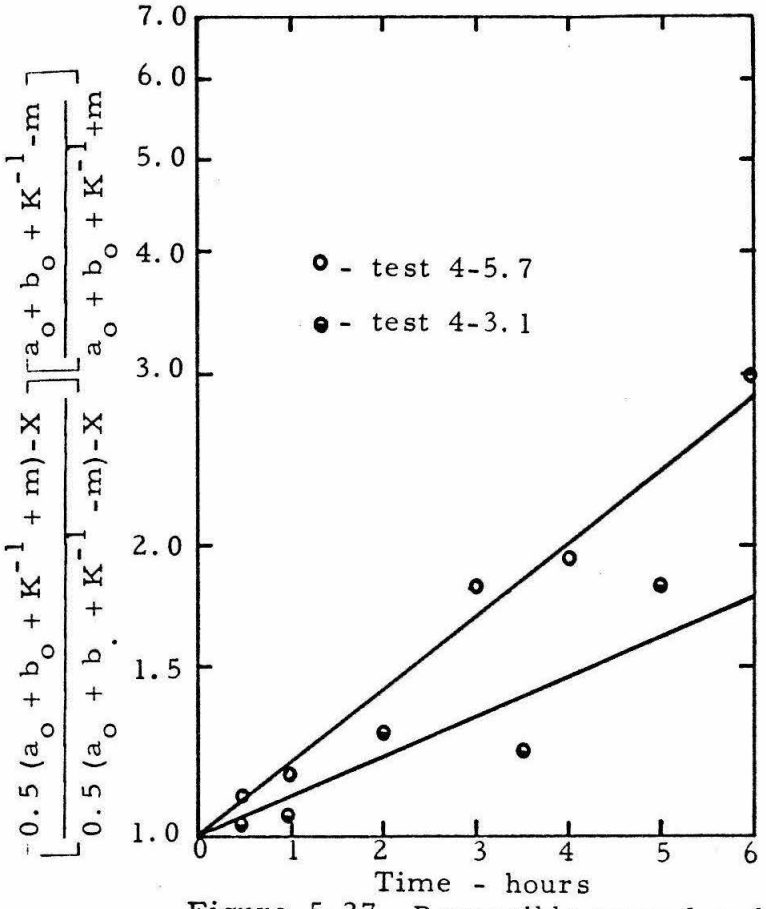


Figure 5-27. Reversible second-order kinetic plot of test 4-3.1 (second step) and test 4-5.7 for adsorption of T_4 phage to carbon

TABLE 5-23

Rate Constants for Adsorption of Bacteriophage T_4 to Activated Carbon at pH 6.9 and Ionic Strengths of 0.08 and 0.10

Test No.	Ionic Strength	Figure	Slope mk_1 1/hr	Rate Constants: forward $k_1 \times 10^{13}$ ml / virus particle-sec	Rate Constants: reverse $k_2 \times 10^{7}$ 1 / sec
4-5.1	0.10	5-24	0.161	7.2	18.0
4-5.2	0.08	5-25	0.091	8.3	20.5
4-5.3	0.08	5-24	0.304	8.8	22.0
4-5.4	0.08	5-25	0.192	8.3	20.8
4-5.5	0.08	5-26	1.022	8.1	20.3
4-5.6	0.10	5-26	0.131	7.9	19.8
4-5.7	0.08	5-27	0.188	8.6	21.5

TABLE 5-24

Interpretation of Data by Reversible Second-order Kinetics for the Second Step in Two-Step Adsorption Experiments (test 4-3.1)

Time t min	Virus Adsorbed X virus particles/ml	$\frac{0.5(a_o+b_o+K^{-1}+m)-X}{0.5(a_o+b_o+K^{-1}-m)-X}$	$\frac{a_o+b_o+K^{-1}-m}{a_o+b_o+K^{-1}+m}$
30	5×10^6	1.02	
60	1.0×10^7	1.05	
120	3.5×10^7	1.28	
210	3.0×10^7	1.22	
300	5.5×10^7	1.87	

$b_o = 8.3 \times 10^7$

$a_o = 10.5 \times 10^7$

$m = 3.8 \times 10^7$

$K = 2.5 \times 10^6$

from the reversible representation. The representation of data by these different kinetic models results from the weak reversibility of the adsorption process.

5-9 Effects of pH on Adsorption

The pH stability range of bacteriophage T_4 was determined by measuring the amount of virus inactivation in suspensions at different pH values. The results show little inactivation of T_4 phage at pH 7.0. Virus stability rapidly decreased below pH 5 and above pH 11 (Table 5-25).

Preliminary adsorption tests were made with 26/35 carbon and T_4 phage. Four adsorption tests were made in buffer solutions at pH levels of 5.0, 7.0, 8.6, and 10.4. No significant loss of virus stability was found in the control at pH 5.0 and 7.0 during the testing period. Appreciable inactivation, however, occurred at pH 10.4 (Table 5-26).

The adsorption results can be plotted as a pseudo-first-order reaction (Figure 5-28) because of high carbon concentrations (10,000 mg/l) used in the tests. First-order reaction rates of 1.93, 1.48, 1.46, and 1.95 per hour were obtained for pHs of 5.0, 7.0, 8.6, and 10.4 respectively. Previous tests at pH 7.0 with 26/35 carbon gave a first-order rate of 0.26/hr (Table 5-6, and Figure 5-5). The reaction solution yielding a rate of 0.26/hr consisted of deionized water. The higher rate of 1.48/hr occurred in the presence of phosphate buffer. This effect of the buffer was then investigated before additional studies on pH effect were made. Optimum adsorption occurred at an ionic strength of 0.08 to 0.10 (section 5-11).

TABLE 5-25

Effect of pH on Stability of T_4 Bacteriophage

pH	Virus Concentration virus particles/ml		Percent Drop
	Initial	After 8.5 hours	
3.2	4.8×10^{10}	Zero	100
4.02	4.8×10^{10}	1.3×10^{10}	73
5.12	4.7×10^{10}	3.9×10^{10}	17
5.96	4.7×10^{10}	4.2×10^{10}	10
7.00	4.8×10^{10}	4.7×10^{10}	2
8.33	4.8×10^{10}	--	
11.10	4.8×10^{10}	2.5×10^{10}	48
11.38	4.7×10^{10}	Zero	100

TABLE 5-26

Preliminary Testing of pH
Effect on Adsorption

Time Hr.	Virus Remaining in Solution Virus particles per ml Test No. 4-8.1 pH 10.4	4-8.2 pH 8.6	4-8.3 pH 7.0	4-8.4 pH 5.0
0	2.9×10^9	2.9×10^9	3.0×10^8	2.9×10^8
0.25	2.0×10^9	2.2×10^9	2.1×10^8	2.3×10^8
0.50	2.3×10^9	1.4×10^9	1.5×10^8	1.6×10^8
1.50	2.0×10^8	8.5×10^8	2.9×10^7	1.9×10^7
3.50	4.1×10^6	1.5×10^7	1.9×10^6	4.7×10^5
7.50	--	1.5×10^6	8.0×10^5	5.0×10^4
17	4.4×10^5	2.9×10^4	2.0×10^4	1.0×10^4
24	6.6×10^4	1.5×10^4	4.0×10^4	9.0×10^3
		Controls		
0	2.9×10^9	2.7×10^9	2.9×10^9	2.5×10^9
0.25	3.3×10^9	2.6×10^9	2.3×10^9	2.5×10^9
0.50	1.9×10^9	2.6×10^9	2.4×10^9	2.3×10^9
1.50	--	--	2.9×10^9	2.4×10^9
3.50	2.2×10^9	2.3×10^9	2.9×10^9	2.6×10^9
7.50	1.8×10^9	2.5×10^9	2.6×10^9	2.5×10^9
17	1.9×10^9	2.3×10^9	2.9×10^9	2.5×10^9
24	1.8×10^9	2.4×10^9	2.8×10^9	--

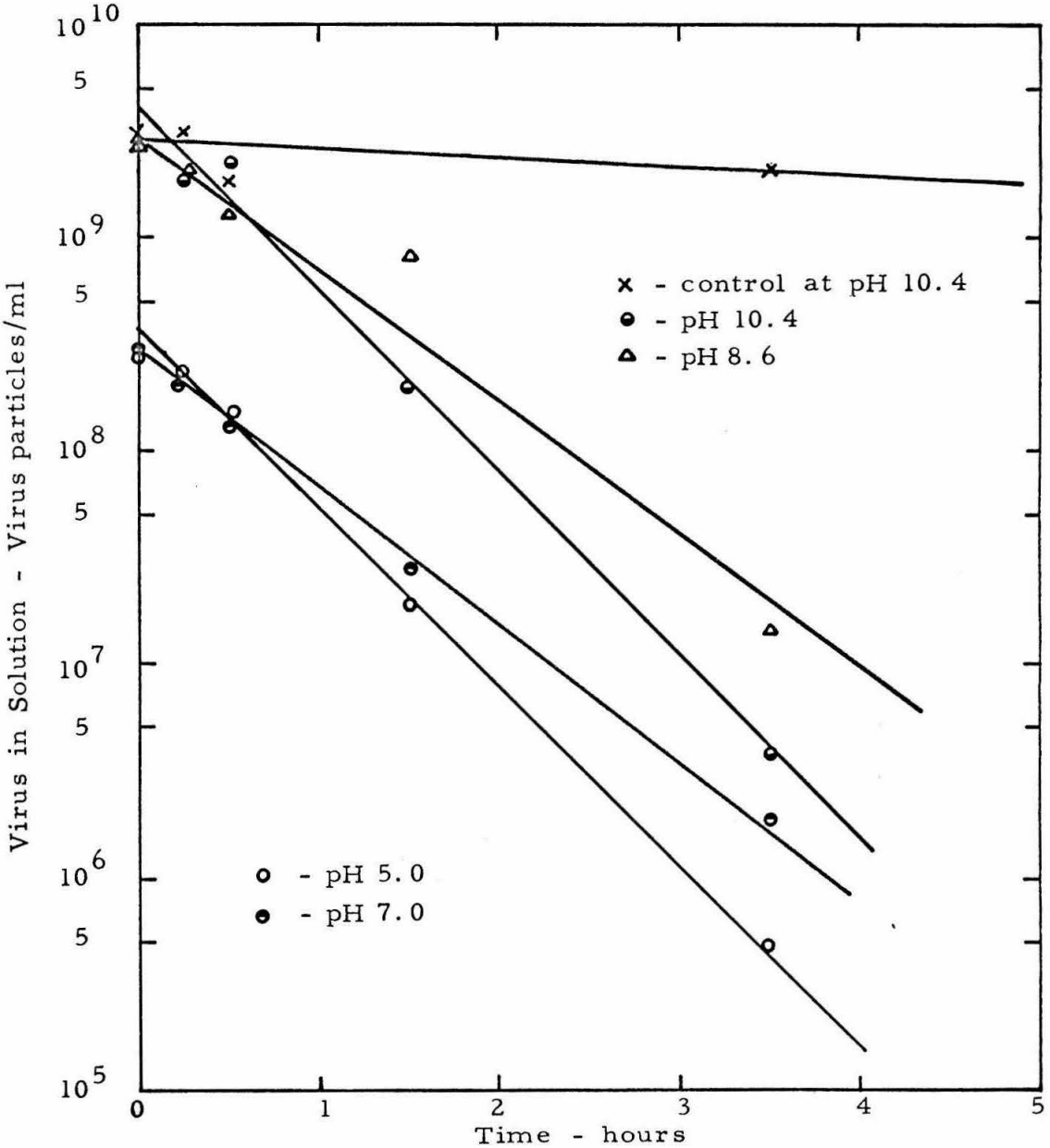


Figure 5-28. Preliminary Testing of pH Effect on Adsorption by Least Squares Analysis

Six adsorption tests at different pH values and a constant ionic strength of 0.10 were made with 120/140 carbon (Table 4-7 and 4-8). The results are given in Table 5-27. The solutions employed in tests, 4-8.9 and 4-8.10, which were buffered at pH 8.8 and 9.7 respectively, did not remain at constant pH during adsorption. Upon completion (7 hours of adsorption) the reaction solutions had pHs of 8.4 and 8.2 for test 4-8.9 and 4-8.10 respectively. This drop resulted from inadequate buffering. Sodium hydroxide with dipotassium phosphate had been used to obtain the pH of 9.7.

To determine the rate of pH change in test 4-9.10 an identical reaction solution was placed on the Gyrotory shaker, and pH was periodically measured (Table 5-28). The pH of the reaction solution dropped from 9.7 to 8.25 at a constant rate over a 4-hour period (Figure 5-29). Adsorption for the first 3.5 hours occurred at a pH above 8.25. After 3.5 hours adsorption proceeded at pH 8.25. This change in pH caused only a slight shift of 0.0006 in the ionic strength of the test solution.

For the 7 hours of adsorption the corrections for virus titer resulting from inactivation were insignificant. The only significant change in virus concentration for the controls was test 4-8.15. The assay values in this test are probably in error since the drop in virus concentration occurred in the first hour, and the higher and lower pH solutions showed no significant decrease (Table 5-27).

TABLE 5-27

Results of Tests Designed to Determine Effect of pH on Adsorption of T_4 Phage to Carbon

Time Hr.	Test No. pH	Virus Remaining in Solution, Virus particles per ml 4-8.5 4.6	4-8.6 5.7	4-8.7 6.5
0		4.5×10^8	4.3×10^8	4.5×10^8
0.5		4.0×10^8	3.4×10^8	3.5×10^8
1.0		4.0×10^8	2.0×10^8	2.3×10^8
2.0		4.0×10^8	1.9×10^8	1.7×10^8
3.0		--	1.2×10^8	--
3.5		4.2×10^8	--	7.0×10^7
4.0		--	1.1×10^8	---
5.0		--	8.7×10^7	6.6×10^7
7.0		3.7×10^8	5.7×10^7	3.5×10^7
Controls		4-8.11	4-8.12	4-8.13
0		4.4×10^8	4.6×10^8	4.6×10^8
1.0		2.8×10^8	4.3×10^8	--
3.0		--	4.3×10^8	4.6×10^8
3.5		4.7×10^8	--	--
5.0		4.3×10^8	4.7×10^8	--
7.0		4.4×10^8	4.0×10^8	--

TABLE 5-27 (Cont'd)

Time Hr.	Virus Remaining in Solution, Virus particles per ml Test No. 4-8.8 pH 7.85	4-8.9 8.8	4-8.10 9.7
0	4.6×10^8	4.35×10^8	4.6×10^8
0.5	3.0×10^8	2.4×10^8	4.1×10^8
1.0	2.2×10^8	1.5×10^8	3.3×10^8
2.0	1.3×10^8	1.2×10^8	2.3×10^8
3.0	1.1×10^8	--	--
3.5	--	7.4×10^7	1.3×10^8
4.0	8.6×10^7	--	--
5.0	5.6×10^7	5.0×10^7	7.4×10^7
7.0	3.8×10^7	2.9×10^7	5.5×10^7
Controls	4-8.14	4-9.15	4-8.16
0	4.6×10^8	4.3×10^8	4.5×10^8
1.0	4.9×10^8	3.7×10^8	4.6×10^8
3.0	4.6×10^8	--	--
3.5	--	--	4.6×10^8
5.0	--	3.7×10^8	4.7×10^8
7.0	4.9×10^8	--	4.3×10^8

TABLE 5-28

Rate of pH Change in Reaction Solution of Test 4-8.10

Time Hr.	pH
0	9.70
0.75	9.50
2	8.80
3.75	8.25
5.75	8.25

The forward rate constant for each test was determined as in previous tests. A kinetic plot of the data was made in the form of the reversible second-order equation (Table 5-29, Figures 5-30 through 5-34). As in previous calculations, values of 1.9×10^9 sites/mg and 4.0×10^{-7} ml/virus particle were used as the carbon capacity and equilibrium constant, respectively.

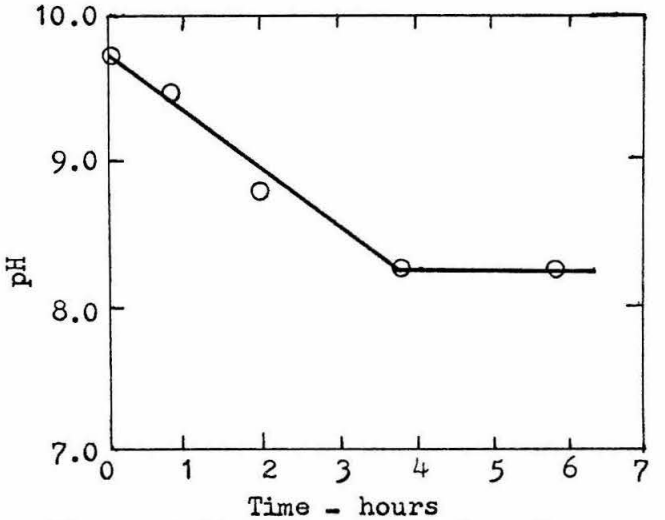


Figure 5-29. Change of pH in Reaction Solution 4-8.10

TABLE 5-29

Interpretation of Adsorption Results at Various pH Values by Reversible Second-order Kinetics

Time t Hr.	Virus adsorbed x virus particles/ml	$\frac{0.5(a_o+b_o+K^{-1}+m)-X}{0.5(a_o+b_o+K^{-1}-m)-X}$	$\frac{a_o+b_o+K^{-1}-m}{a_o+b_o+K^{-1}+m}$
Test 4-8.5, pH 4.6, ($a_o = 4.5 \times 10^8$, $b_o = 4.7 \times 10^8$, $m = 7.1 \times 10^7$)			
0.5	5.0×10^7	1.02	
1.0	0	0	
2.0	5.0×10^7	1.02	
3.5	3.0×10^7	1.01	
7.0	8.0×10^7	1.03	
Test 4-8.6, pH 5.7 ($a_o = 4.3 \times 10^8$, $b_o = 4.7 \times 10^8$, $m = 7.8 \times 10^7$)			
0.5	9.0×10^7	1.05	
1.0	2.3×10^8	1.20	
2.0	2.45×10^8	1.24	
3.0	3.1×10^8	1.49	
4.0	3.1×10^8	1.56	
5.0	3.43×10^8	1.80	
7.0	3.73×10^8	2.56	

TABLE 5-29 (Cont'd)

Time t Hr.	Virus adsorbed x virus particles/ml	$\frac{0.5(a_o+b_o+K^{-1}+m)-X}{0.5(a_o+b_o+K^{-1}-m)-X}$	$\frac{a_o+b_o+K^{-1}-m}{a_o+b_o+K^{-1}+m}$
Test 4-8.7, pH 6.5 (a_o = 4.5 X 10^8, b_o = 4.7 X 10^7, m = 6.8 X 10^7)			
0.5	1.0 X 10^8	1.04	
1.0	2.2 X 10^8	1.14	
2.0	2.8 X 10^8	1.25	
3.5	3.8 X 10^8	1.99	
5.0	3.94 X 10^8	2.41	
Test 4-8.8, pH 7.85 (a_o = 4.6 X 10^8, b_o = 4.7 X 10^8, m = 6.9 X 10^7)			
0.5	1.6 X 10^8	1.08	
1.0	2.4 X 10^8	1.17	
2.0	3.3 X 10^8	1.45	
3.0	3.5 X 10^8	1.60	
4.0	3.74 X 10^8	1.91	
5.0	4.04 X 10^8	3.07	
7.0	4.22 X 10^8	7.45	
Test 4-8.9, pH 8.8 (a_o = 4.35 X 10^8, b_o = 4.7 X 10^8, m = 8.1 X 10^7)			
0.5	19.5 X 10^7	1.13	
1.0	28.5 X 10^7	1.32	
2.0	31.5 X 10^7	1.45	
3.5	36.1 X 10^7	1.91	
5.0	38.5 X 10^7	2.62	
7.0	40.6 X 10^7	5.10	

TABLE 5-29 (Cont'd)

Time t Hr.	Virus adsorbed x virus particles/ml	$\frac{0.5(a_o+b_o+K^{-1}+m)-X}{0.5(a_o+b_o+K^{-1}-m)-X}$	$\frac{a_o+b_o+K^{-1}-m}{a_o+b_o+K^{-1}+m}$
Test 4-8.10, pH 9.7, ($a_o = 4.6 \times 10^8$, $b_o = 4.7 \times 10^8$, $m = 6.9 \times 10^7$)			
0.5	5.0×10^7	1.02	
1.0	$13,0 \times 10^7$	1.06	
2.0	23.5×10^7	1.17	
3.5	33.0×10^7	1.45	
5.0	38.6×10^7	2.18	
7.0	40.5×10^7	3.15	

TABLE 5-30

Determination of Rate Constant
for Test 4-8.10 for the Adsorption Period
After 3.5 Hours at Constant pH 8.25

Time zero $a_o = 4.6 \times 10^8$ $b_o = 4.7 \times 10^8$

Time 3.5 $a'_o = 1.3 \times 10^8$ $b'_o = 1.4 \times 10^8$

where $X' = X - X$ at 3.5 hrs. $m = 3.8 \times 10^7$

Time t min.	Virus Adsorbed x′ virus particles/ml	$\frac{0.5(a'_o+b'_o+K^{-1}+m)-X}{0.5(a'_o+b'_o+K^{-1}-m)-X}$	$\frac{a'_o+b'_o+K^{-1}-m}{a'_o+b'_o+K^{-1}+m}$
3.5	0	0	
5.0	5.6×10^7	1.23	
7.0	7.5×10^7	1.44	

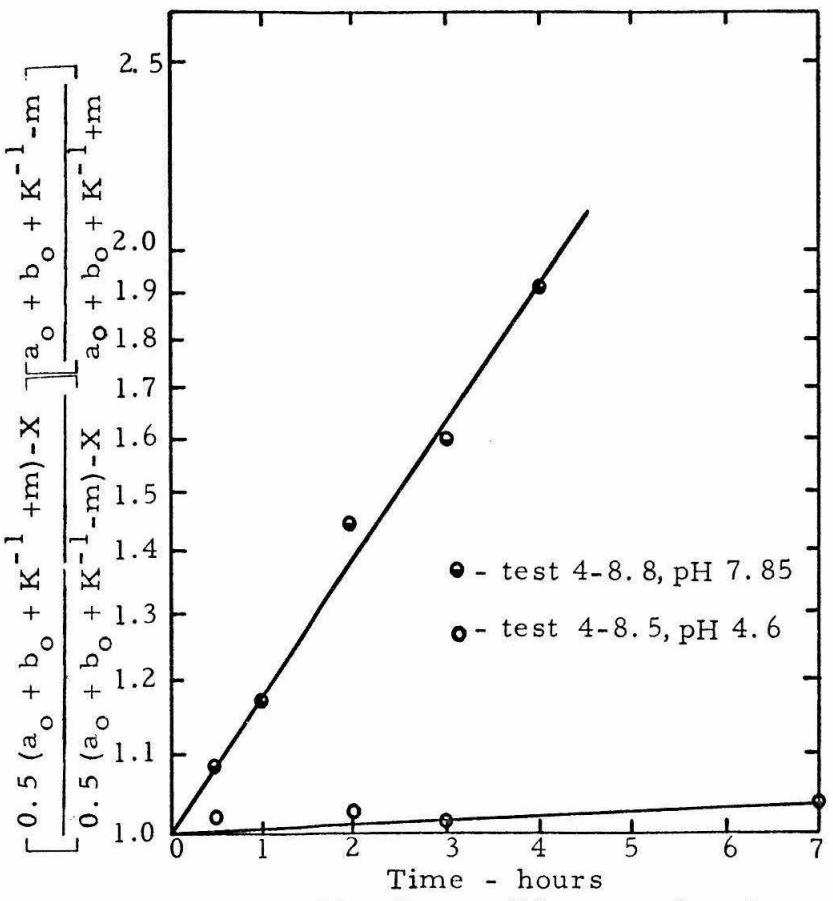


Figure 5-30. Reversible second-order kinetic plot of tests 4-8.5 and 4-8.8; adsorption of T_4 phage to carbon at pH 4.6 and 7.85

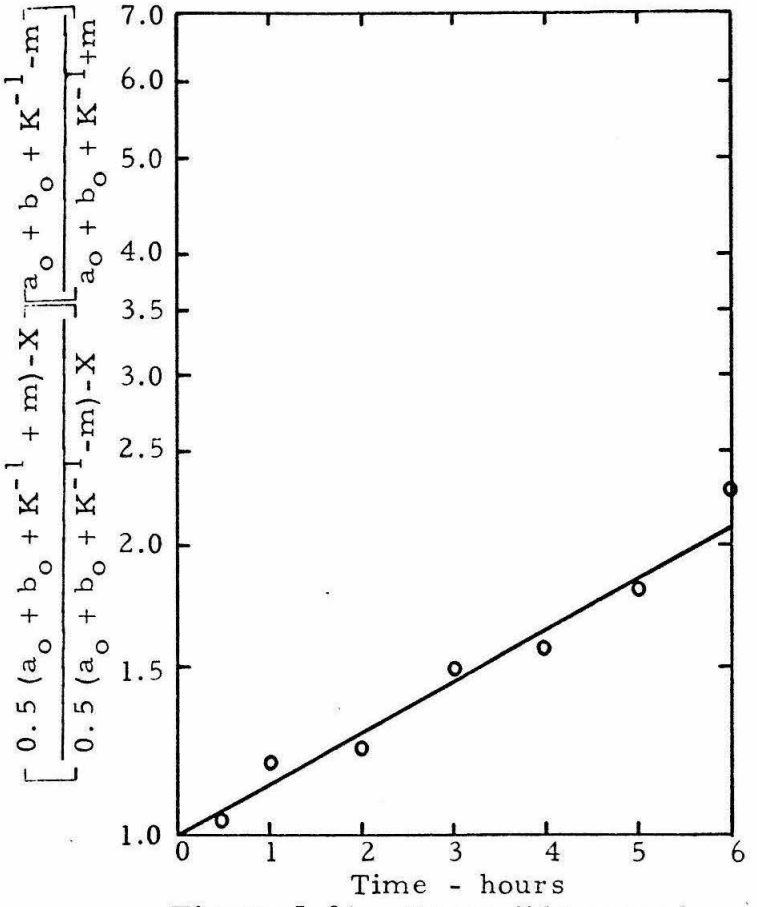


Figure 5-31. Reversible second-order kinetic plot of test 4-8.6; adsorption of T_4 phage to carbon at pH 5.7

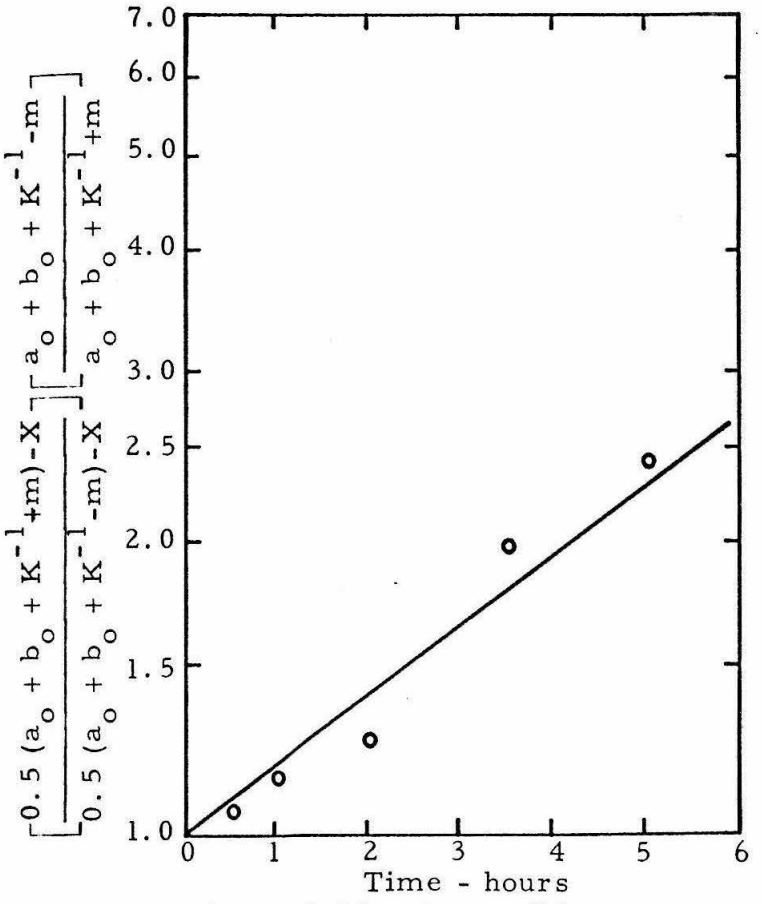


Figure 5-32. Reversible second-order kinetic plot of test 4-8.7; adsorption of T_4 phage to carbon at pH 6.5

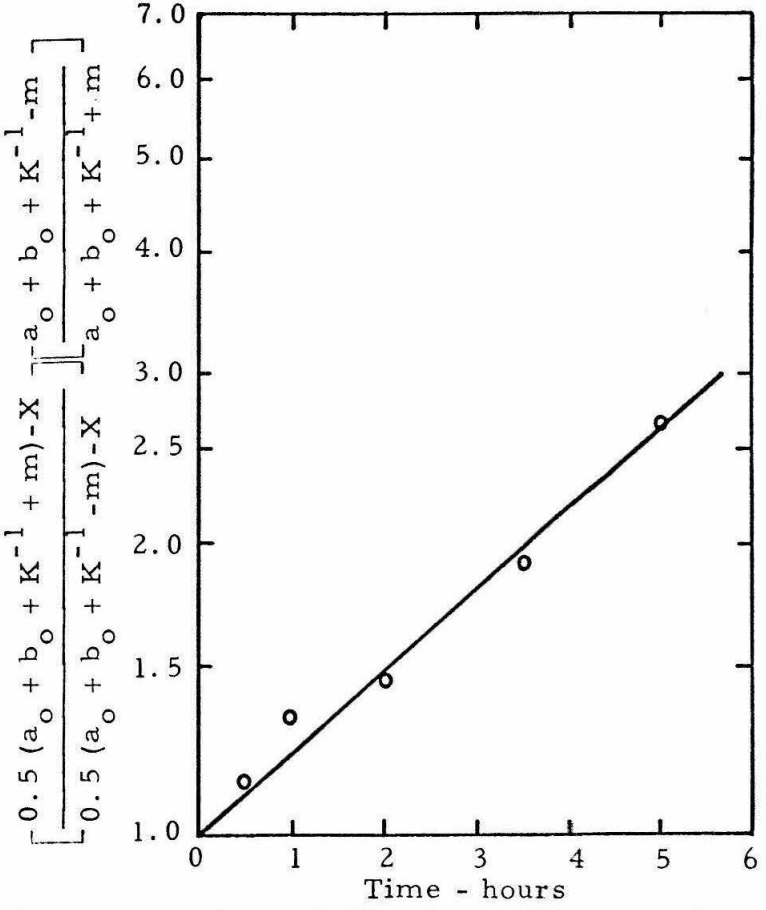
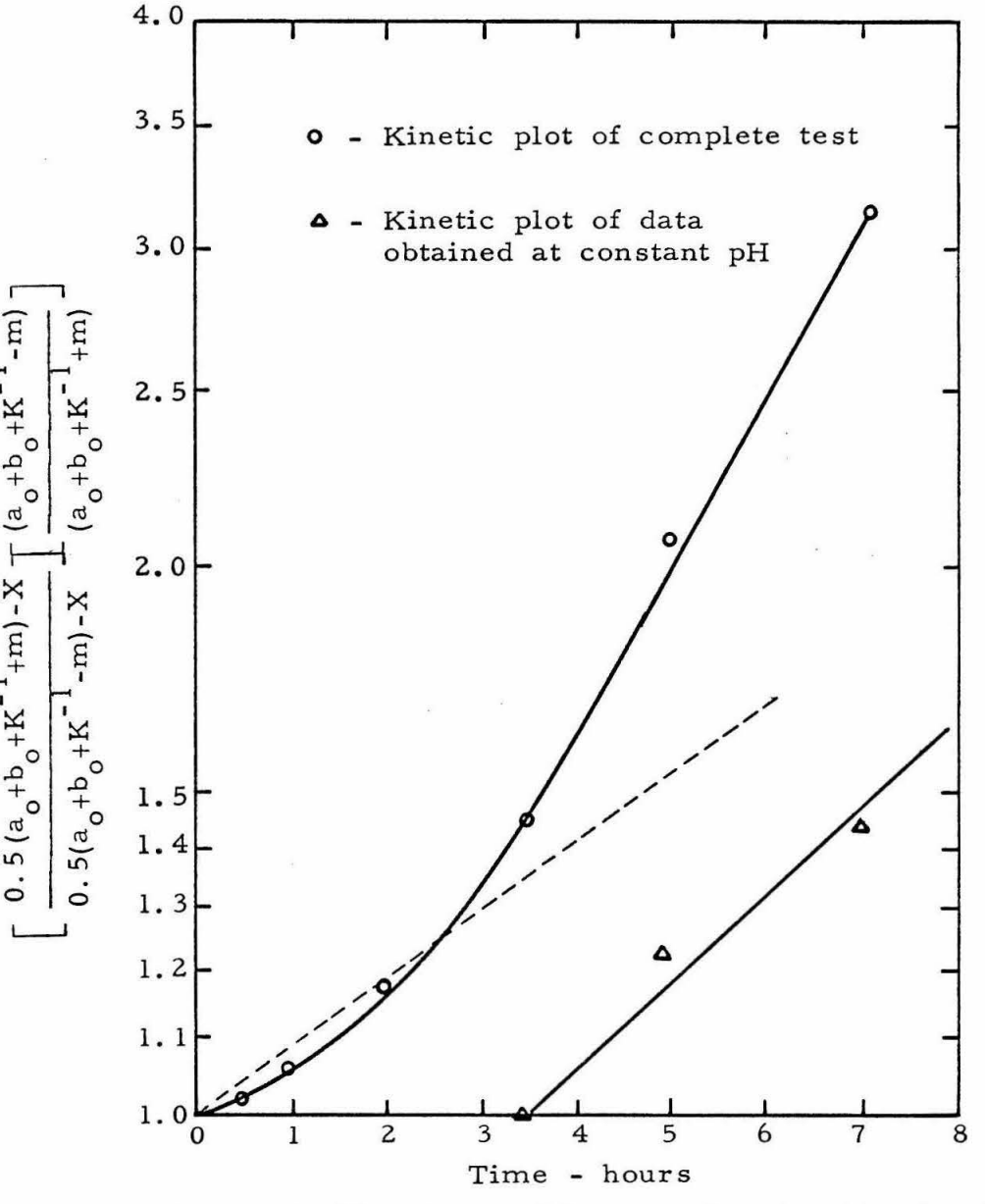


Figure 5-33. Reversible second-order kinetic plot of test 4-8.9; adsorption of T_4 phage to carbon at pH 8.8



Figures 5-34. Reversible second-order kinetic plot of test 4-8.10; adsorption of T_4 phage to carbon at pH 9.7

All of the results plotted as a straight line except for test 4-8.10 at pH 9.7. The curve in this test occurred only in the first 3.5 hours (Figure 5-34). This time range corresponds with the pH drop in the reaction solution from 9.7 to 8.25 (Figure 5-29). The drop in pH from 8.8 to 8.4 in test 4-8.9 showed no noticeable influence in the kinetic plot (Figure 5-33).

In test 4-8.10 the pH remained constant at 8.25 after 3.5 hours (Figure 5-29). After 3.5 hours the data plotted as a straight line (Figure 5-34). The initiation of adsorption can be considered to begin after 3.5 hours at a constant pH of 8.25. This procedure requires new initial concentrations for adsorption commencing at 3.5 hours. These concentrations for carbon sites and viruses were obtained by reducing the initial concentrations by the amount of viruses adsorbed at 3.5 hours. By using this procedure the rate constant was determined for the portion of the test after 3.5 hours and at a pH of 8.25 (Table 5-30, Figure 5-34). In the first 3.5 hours pH changed from 9.7 to 8.3 (Figure 5-29), the average being about pH 9.0. An approximate value for the rate constant was obtained over this pH range by fitting a straight line through the first 3.5 hours of data (Figure 5-34).

The application of the data in the above tests to straight-line plots indicates that the same values for carbon capacity and equilibrium constant apply over the tested pH range. The pH, however, has a significant effect on adsorption, as seen in the rate constants as determined from the slopes of their corresponding kinetic plots (Table 5-31). The reverse rate constants were calculated from the equilibrium constant and the forward rate constants. It should be noted that this equili-

brium constant was determined at a pH of 7.0. The equilibrium constant may vary with pH.

The forward rate constant is maximum at pH 7.2 and drops off to zero below pH 4.5 and above pH 10 (Figure 5-35). Puck and Tolmach (100) found that the attachment of Escherichia coli bacteriophage T_2 to its host cell is maximum at pH 7 and drops to zero at pHs below 4.8 or above 10.5. The similarity of the two adsorption systems with respect to pH indicates three possibilities. The attachment of bacteriophage to host cells and activated carbon may involve similar groups on each adsorbent, or the effect of pH may be solely on the virus, or both of these factors may influence the adsorption rate.

The main groups on viruses that can be affected by pH are the free side chains of amino acids. The amino-acid composition of bacteriophages T_2 and T_3 has been accurately determined (147). Only the amino-acid composition for T_2 phage has been given in Table 5-32 since it is almost identical for T_2 and T_3. Bacteriophage T_4 should have an amino-acid content almost identical to that given in Table 5-32. Of the listed amino acids only nine have side chains of importance. These amino acids and their corresponding side chains are listed below.

amino group:

Asparagine
Glutamine
Lysine

$$-CHNH_3^+ \rightleftharpoons -CHNH_2 + H^+$$

carboxyl group:

Aspartic acid
Glutamic acid
Serine

$$-COOH \rightleftharpoons -COO^- + H^+$$

TABLE 5-31

Rate Constants for Adsorption of Bacteriophage T_4 to Activated Carbon at Different pH Values

Test No.	pH	Figure	Slope mk_1 hr.	Rate Constants forward $k_1 \times 10^{13}$ $\frac{ml}{\text{virus particles} - sec}$	Rate Constants reverse $k_2 \times 10^7$ $\frac{1}{sec}$
4-8.5	4.6	30	0.0054	0.22	0.55
4-8.6	5.7	31	0.125	4.45	11.1
4-8.7	6.5	32	0.176	6.10	15.3
4-8.8	7.85	30	0.167	6.66	16.7
4-8.9	8.8-8.4	33	0.178	6.12	15.3
4-8.10	9.8-8.3	34	0.090	3.61	9.0
4-8.10	8.25	34	0.112	8.1	20.2

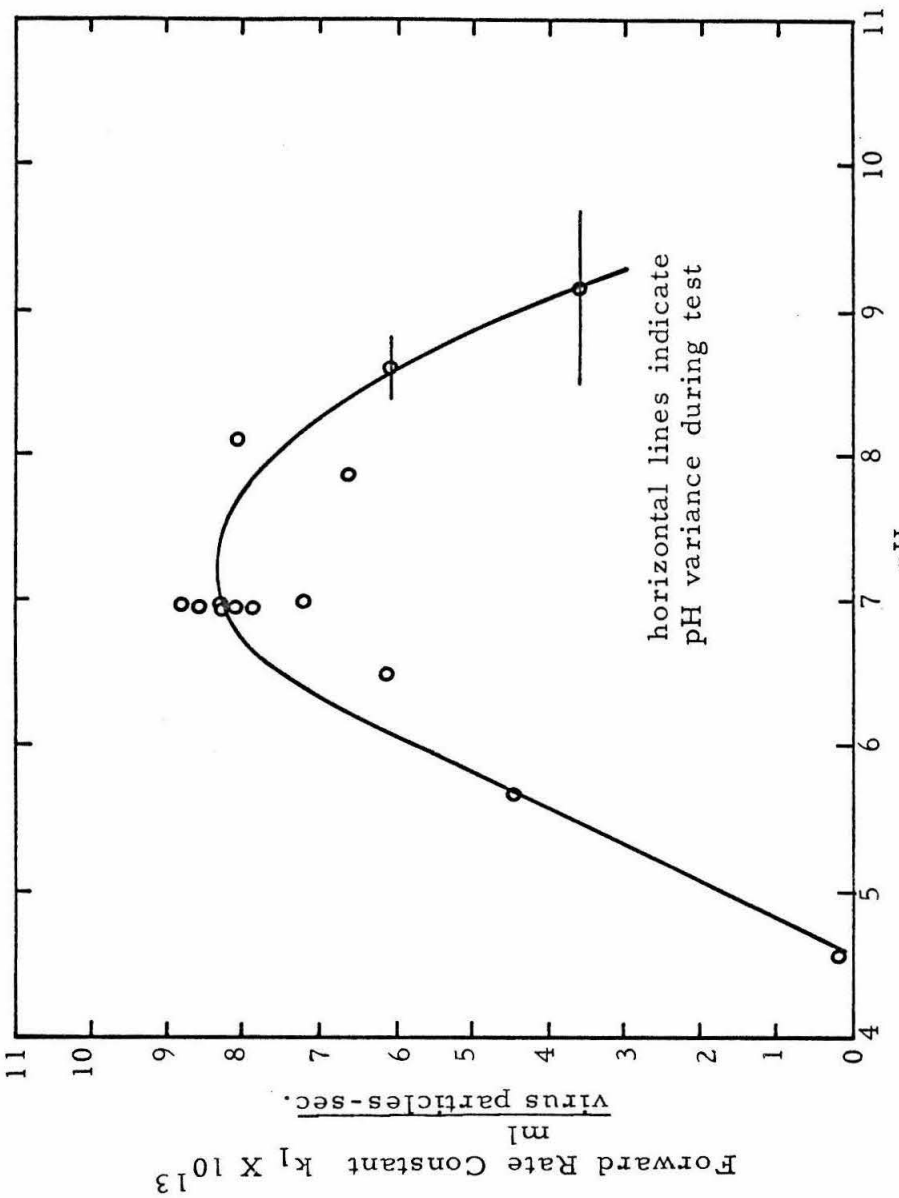
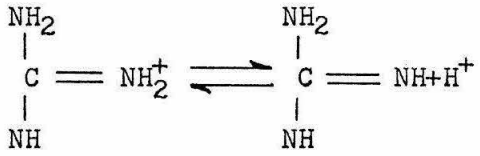


Figure 5-35. Forward Rate Constant (k_1) vs. pH for adsorption of T_4 phage to carbon

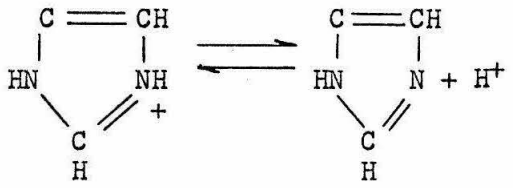
guanidino group:

Arginine



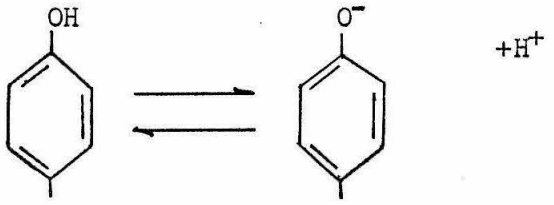
imidazolyl group:

Histidine



phenolic group:

Tyrosine



Another important side chain is the sulfhydryl group of cysteine. No value for the cysteine content of T_2 phage is available, but the cysteine content is not expected to be significant.

The dry weight of T_2 phage is approximately 45 percent amino acid and 45 percent DNA (147). Of the nine amino acids listed above, amino and carboxyl groups are present in 74 percent of the ionizable amino acids of T_2 phage. About 10 and 14 percent of them contain guanidino and phenolic groups respectively, and only 2 percent contain imidazole groups. From the information in Table 5-32 and the weight of a virus particle, the number of groups that can dissociate can be approximately calculated. This procedure, which assumes that all side chains are free to dissociate, yields a value of 3×10^5 ionizable groups per virus particle (Table 5-33). About 80 percent of these ionizable groups are

TABLE 5-32

Amino Acid Composition of T_2 Bacteriophage*

Amino Acid	Amino Acid in gm per 100 gm dry Virus (8 determinations)
Alanine	3.5 ± 0.14
Arginine	2.3 ± 0.21
Aspartic acid and Asparagine	5.3 ± 0.11
Glutamic acid and Glutamine	5.4 ± 0.11
Glycine	4.3
Histidine	0.4 ± 0.02
Isoleucine	3.0 ± 0.04
Leucine	2.7 ± 0.10
Lysine	2.9 ± 0.16
Methionine	1.0 ± 0.04
Phenylalanine	2.5 ± 0.11
Proline	1.8 ± 0.09
Serine	2.4
Threonine	2.7
Tyrosine	2.9
Valine	2.7 ± 0.12
Total	45.7

* From Stent (147)

TABLE 5-33

Calculation of Number of Dissociable Groups per Virus Particle Furnished by Amino Acid Side Chains

Amino Acid	gm Amino Acid per virus particle $\times 10^{18}$	Weight of One Molecule of Amino Acid $\times 10^{23}$ gm	Number of Amino Acid Units per Virus Particle
Aspartic acid Asparagine	17.5	22.2	7.9×10^4
Glutamic acid Glutamine	17.8	24.5	7.2×10^4
Lysine	9.6	24.4	4.0×10^4
Serine	7.9	17.5	4.5×10^4
Arginine	7.6	29.0	2.6×10^4
Histidine	1.3	25.9	0.5×10^4
Tyrosine	9.6	30.2	3.2×10^4
			29.9×10^4

amino and carboxyl groups. This comparison indicates the expected significance of amino and carboxyl groups over others in explaining the pH effect.

The adsorption rate falls to zero at pH values beyond the limit at which the amino and carboxyl groups ionize when incorporated in protein. This pH dependence also rules out the possibility of participation of ionized forms of sulfhydryl (pK = 9), guanidino (pK = 12.5), and phenolic (pK = 10.1) groups. The pK values are incompatible with points of maximum pH effect. Although the imidazolyl group has a more compatible pK of 6, it only makes up 2 percent of the amino acid-side chains.

The pH effect on adsorption of bacteriophage T_4 to activated carbon is similar to interactions between carboxyl and amino groups. As discussed in Chapter 2, these groups are important in the adsorption of bacterial viruses to host cells. Amino groups of viruses attach to carboxyl groups on host cells. Adsorption is decreased at low pH levels since the carboxyl groups on host cells are not ionized. At high pH levels adsorption also decreases owing to repulsive forces between like charges. At high pH values the carboxyl groups on host cells are negative and the net charge on the virus is negative. This condition is represented schematically below. At about pH 7 positively charged amino groups on the virus are available to interact with negatively charged carboxyl groups on the host cell. Cells are known to contain carboxyl groups, but the active groups on activated carbon have not been investigated extensively.

	Low pH	Neutral	High pH
Cell	$R\text{-}COOH$	$R\text{-}COO^-$	$R\text{-}COO^-$
Virus	$^+H_3N\text{-}\underset{\displaystyle R}{\underset{\vert}{C}}H\text{-}COOH$	$^+H_3N\text{-}\underset{\displaystyle R}{\underset{\vert}{C}}H\text{-}COO^-$	$H_2N\text{-}\underset{\displaystyle R}{\underset{\vert}{C}}H\text{-}COO^-$

In most studies of amino-acid adsorption on carbon no effort has been made to control pH, since the ampholytes tend to maintain pH values near their isolectric points. Tiselius (144) found that adsorption of mono-amino-dicarboxylic acids and the di-amino-mono-carboxylic acids to carbon was strongly dependent on pH. Greatest adsorption occurred between pH 6.9 and 11.0. Adsorption to activated carbon by other substances that contain no amino or carboxyl groups indicates that different phenomena must be involved. For example, the adsorption rate of benzene-sulfonate to activated carbon steadily increases over the pH range 8.73 to 1.91 (124).

The explanation of the interaction of carboxyl and amino groups, must not be considered as the only possible mechanism leading to an optimum pH for adsorption rate. Equally significant and also dependent on ionization of certain side groups is the configuration of the tail fibers on the phage. If carboxyl groups are indeed present on activated carbon, then tail fibers of the phage would be the part expected to adsorb. This mechanism is in direct analogy with phage adsorption to host cells.

T-even bacteriophages have been noted to have two distinct sedimentation rates in the ultracentrifuge. A rate of 1000 X 10^{-13} sec occurs at pH 5.5 and goes through a transition to 700 X 10^{-13} sec at pH 6.02 to 6.55 (151). This dual sedimentation rate is also experienced with changes in the ionic composition of the solution (138)(151)(152). There has been speculation on the formation of virus aggregates and orientation during settling, but recently the phenomenon has been shown to result from the position of the tail fibers. At pH values from 6.0 to 9.5, tail fibers are extended as shown in Figure 3-3. At pH 5, however, they are not extended but adsorb to the tail sheath (152)(153)(154). The pH effect, therefore, results not only from an electrostatic interaction between phage and the adsorbent, but also between tail fibers and tail sheath. At pH levels where appropriate groups are dissociated to aid attachment to host cells, the fibers and tail sheath have like charges that cause their repulsion. At low pH values the repulsive forces between fibers and tail sheath decrease, allowing interaction. As discussed in section 5-6-1, tail fibers are the units causing adsorption to bacteria cells. The ionic strength effect (to be discussed in section 5-11) indicates that tail fibers also adsorb to activated carbon.

5-10 Treatment of Activated Carbon with Carboxyl-Blocking Reagents

As discussed in Chapter 3, carbon activated in an atmosphere containing oxygen becomes covered with carbon-oxygen complexes. These complexes form acidic groups that can be titrated with alkali. The existence of carboxyl groups on activated carbon can reasonably be expected.*

*A large fraction of the acidic groups on carbon react with diazomethane and have been described as carboxyl and phenolic hydroxyl groups (155). Clauss (156) oxidized suspensions of graphite with potassium chlorate in a mixture of concentrated sulfuric and nitric acid. He found that carboxyl groups were formed. Investigations have also demonstrated the existence of acid surface groups on carbon that behave like carboxyl groups by forming ammonium salts and amids (155)(157).

The existence of carboxyl groups on activated carbon is also supported by infrared absorption. Infrared spectra indicate the presence of hydroxyl, carboxyl, and possibly ketone groups (156)(158). Absorption bands at 1600 cm^{-1} and 1760 cm^{-1} have been found for activated carbon. Origin of the 1600 cm^{-1} band has been attributed to the aromatic -C=C- bonds or carboxyl groups chelated to phenolic hydroxyl groups. The 1760 cm^{-1} band undoubtedly arises from the presence of a carboxyl group (158) (159).

Titration curves of activated carbon indicate that the carboxyl groups are not of normal strength. Clauss (156) explains this phenomenon by the influence that nearby carboxyl groups have on each other. Garten and Weiss (158) suggest that the weak nature of the carboxyl group may indicate that it is in the form of a lactone.

X-rays of graphite oxide and carbon blacks reacted with oxygen are interpreted in terms of a condensed aromatic layer varying in size from 7 to 16 Å. X-ray studies on the intercrystalline swelling as a result of methylation of acid oxides indicate that hydroxyl groups are situated primarily between the aromatic layers while the carboxyl groups are primarily on the edges (156)(160). Hydrogen is found to be distributed through the carbon particle and oxygen is on the surface or within 10-20 Å of the surface.

If carboxyl groups on carbon are the active units in virus adsorption, the adsorption capacity of carbon should be greatly reduced by esterification of the carboxyl group. As described in section 4-10, carbon was treated with solutions of acid-alcohol, concentrated propylene oxide, and an acid solution of 80 percent propylene oxide.

Adsorption of bacteriophage T_4 to treated carbon showed a marked reduction in carbon capacity (Table 5-34, Figure 5-36). The most pronounced effect occurred with activated carbon treated with the acid-alcohol solution (test 4-10.1). Virus concentration only decreased 2.8 percent in 7 hours (3.6×10^7 to 3.5×10^7 virus particles per ml). The change noted in virus concentration over the 7 hours is almost equivalent to natural inactivation. The rate of inactivation for a pH of 6.9 and ionic strength of 0.10 is 0.004/hr (Figure 5-11). The first-order rate for the virus decrease in this test is 0.008/hr.

The change in virus concentration as expected for 50 mg/l of untreated carbon is plotted in Figure 5-34 for comparison. This expected curve was obtained from the reversible second-order equation for a rate constant of 8.4×10^{-13} ml/virus particles-sec as obtained in tests 4-5.1 through 4-5.7 for untreated carbon (Table 5-23).

An equilibrium condition was obtained with the acid-alcohol treated carbon in only 4 hours. At equilibrium the carbon contained only 2.0×10^7 virus particles per mg. At an equilibrium concentration of 3.5×10^7 virus particles/ml (Table 5-34) the isotherm for untreated carbon gave a value of 1.5×10^9 virus particles/mg of carbon (Figure 5-17). These values indicate that the treatment of carbon with acid-alcohol reduced the number of sites by approximately 99 percent.

TABLE 5-34

Adsorption of T_4 Phage to Activated Carbon Treated With Carboxyl-Blocking Reagents

Time Hr.	Virus Remaining in Solution virus particles/ml		
	Test 4-10.1 (acid-alcohol)	Test 4-10.2 (propylene oxide)	Test 4-10.3 (acid solution of propylene oxide)
0	3.6×10^7	3.7×10^7	3.6×10^7
1.0	3.8×10^7	3.5×10^7	3.5×10^7
2.5	3.6×10^7	3.8×10^7	--
3.0	--	--	3.3×10^7
4.0	3.5×10^7	3.1×10^7	--
6.0	--	--	3.2×10^7
7.0	3.5×10^7	3.1×10^7	--
8.0	--	--	3.1×10^7
	Amount of Virus Adsorbed per milligram of Carbon virus particles/mg		
7.0	2.0×10^7	1.2×10^8	--
8.0	--	--	1.0×10^8

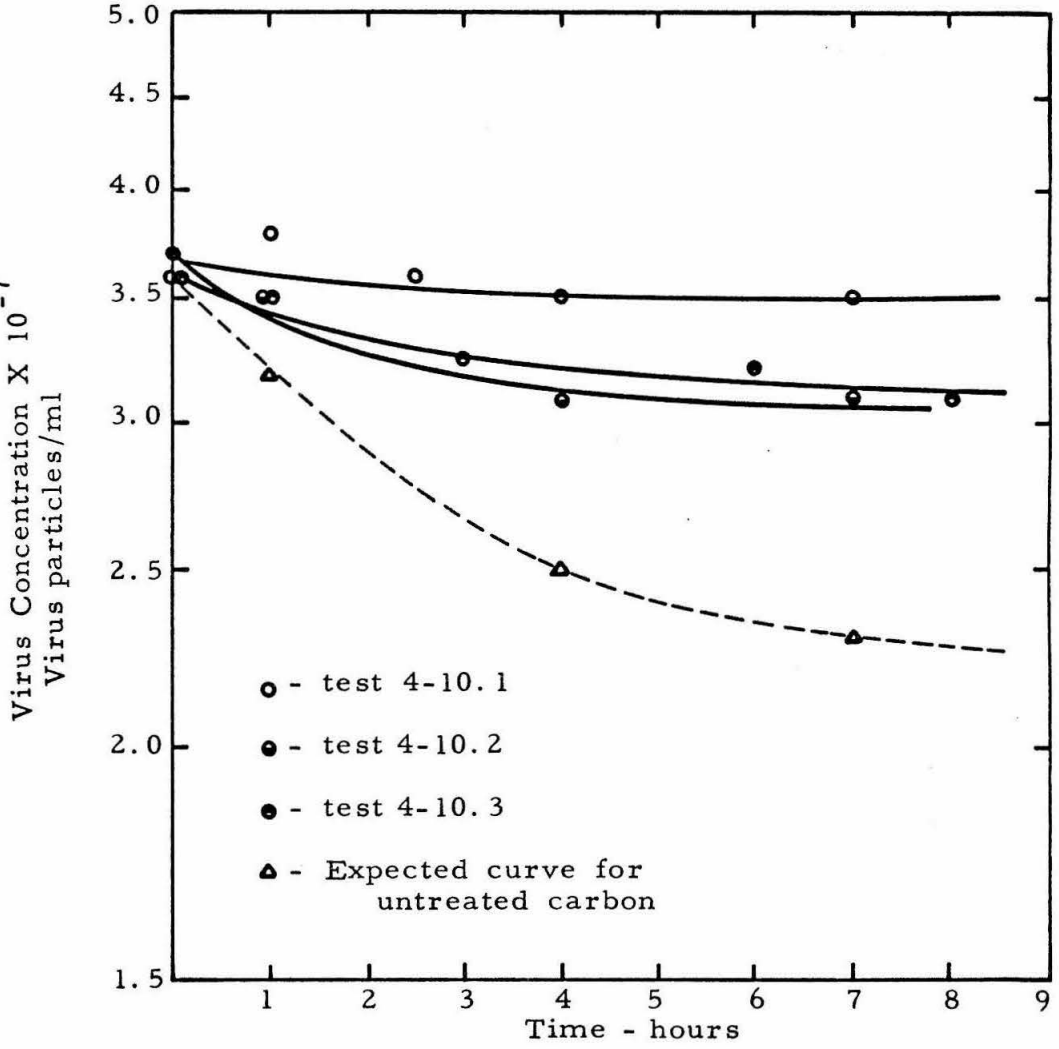


Figure 5-36. Adsorption of Bacteriophage T_4 to Carbon Treated with Carboxyl-Blocking Reagents

Treatment of activated carbon with concentrated propylene oxide (test 4-10.2) also reduced carbon sites substantially. It was not as effective, however, as the acid-alcohol. At equilibrium the carbon contained 1.2×10^8 virus particles/mg. The expected value for untreated carbon with an equilibrium concentration of 3.1×10^7 virus particles/ml would be 1.5×10^9 virus particles/mg (Figure 5-17). These values indicated a 92 percent reduction in sites.

Tolmach and Puck (101) successfully used propylene oxide without an acid catalyst to block adsorption of bacteriophages T_1 and T_2 to host cells. Test 4-10.2 was likewise performed without an acid catalyst. The procedure was repeated in test 4-10.3 with an acid catalyst to see if a greater degree of blocking could be achieved. The results are almost identical to those of test 4-10.2. At equilibrium 1.0×10^8 virus particles/mg of carbon were adsorbed. Reduction in number of sites compared to untreated carbon was 93 percent. This value is only one percent greater than that obtained for treatment without the acid catalyst. Apparently the acidic groups on carbon can supply the necessary protons to cleave the epoxide ring of propylene oxide.

In summary, these tests support the presence of carboxyl groups on activated carbon and demonstrate that they are the active groups in adsorption of viruses. The adsorption of viruses to host cells also involves carboxyl groups. These findings indicate that there is no dependence upon specific sterochemical configurations of the adsorbent. The very similar characteristics between adsorption of viruses to carbon

and to host cells supports the hypothesis that no enzymatic process is necessary for the initial attachment to host cells. The attachment is strictly due to electrostatic forces.

5-11 Effects of Ionic Strength on Adsorption

Preliminary tests were made with T_4 phage and 26/35 carbon. In test 4-9.4 appreciable virus was inactivated by the highest ionic strength (Table 5-35). The rate of inactivation was determined and the assays in test 4-9.1 were correspondingly corrected (Figure 5-37, Table 5-36). Inactivation at lower ionic strengths was much less and corrections were not made.

Changes in adsorption rate occurred with the change in buffer molarity (Figure 5-38). Test 4-9.2 gave a pseudo-first-order rate of 1.30/hr. This rate compares very well with the rate 1.48/hr that was obtained in a similar test (test 4-8.3, discussed in section 5-9). Tests 4-9.1 and 4-9.3 with ionic strengths of 0.83 and 0.02 gave rates of 0.88 and 1.78/hr respectively. These changes were considered significant.

To establish the effect of ionic strength on adsorption, a series of experiments was made with ionic strengths between 0.003 and 0.20. Four control tests (4-9.18 through 4-9.21) were made over the ionic strength range tested. Inactivation of virus in each control was insignificant for the testing period (Table 5-37).

TABLE 5-35

Preliminary Testing of Buffer Influence on Adsorption of T_4 Phage to Carbon

Time	Test No. buffer molarity ionic strength	Viruses Remaining in Solution virus particles per ml 4-9.1 5×10^{-1} 0.83	4-9.2 5×10^{-2} 0.09	4-9.3 5×10^{-3} 0.02
0		2.6×10^8	2.4×10^8	2.4×10^8
10 min.		2.0×10^8	1.8×10^8	1.9×10^8
20 min.		1.9×10^8	1.4×10^8	1.8×10^8
30 min.		2.1×10^8	1.0×10^8	1.8×10^8
45 min.		1.9×10^8	6.1×10^7	1.5×10^8
1.0 hr.		2.0×10^8	4.7×10^7	1.1×10^8
1.5 hr.		2.0×10^8	2.0×10^7	4.0×10^7
2.0 hr.		1.4×10^8	1.3×10^7	1.7×10^7
3.0 hr.		2.1×10^7	2.5×10^6	1.2×10^6
5.0 hr.		5.3×10^5	4.2×10^5	8.4×10^4
8.0 hr.		4.6×10^4	3.0×10^4	6.5×10^3
	Controls	4-9.4		4-9.5
0		2.5×10^8		2.3×10^8
20 min.		2.6×10^8		2.3×10^8
1.5 hr.		2.0×10^8		2.4×10^8
5.0 hr.		1.6×10^8		2.0×10^8

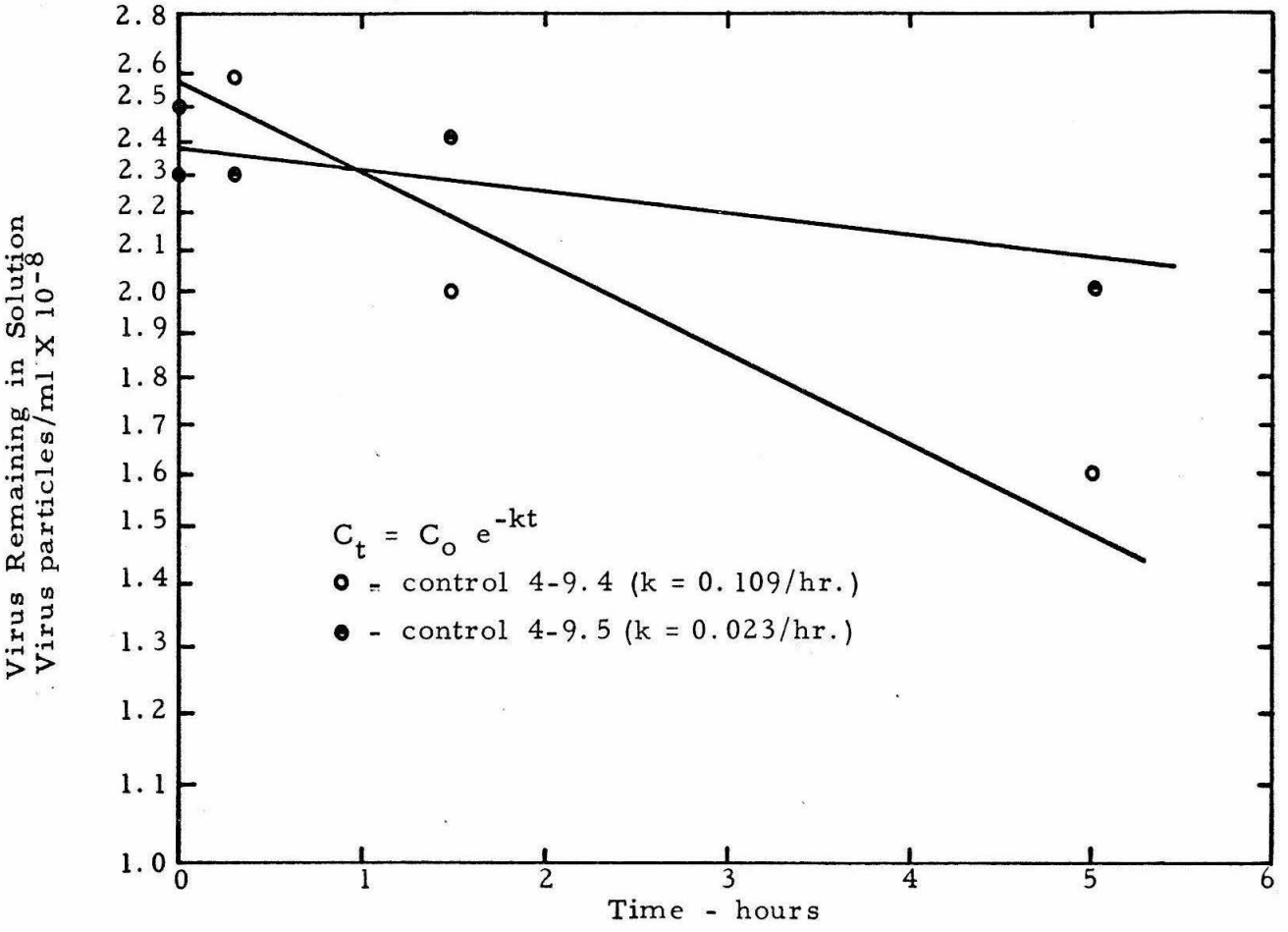


Figure 5-37. Inactivation of Bacteriophage T_4 in tests 4-9.4 and 4-9.5 at Ionic Strengths of 0.83 and 0.02 respectively

TABLE 5-36

Results of Buffer Influence Corrected for Inactivation of Virus in Reaction Solution of 5×10^{-1} Buffer Molarity (test 4-9.1)

Time	Virus Remaining in Solution Corrected for Inactivation virus particles/ml
0	2.6×10^8
10 min.	2.4×10^8
20 min.	2.0×10^8
30 min.	2.1×10^8
45 min.	1.9×10^8
1.0 hr.	2.0×10^8
1.5 hr.	2.0×10^8
2.0 hr.	1.5×10^7
3.0 hr.	3.6×10^7
5.0 hr.	6.5×10^6
8.0 hr.	3.0×10^5

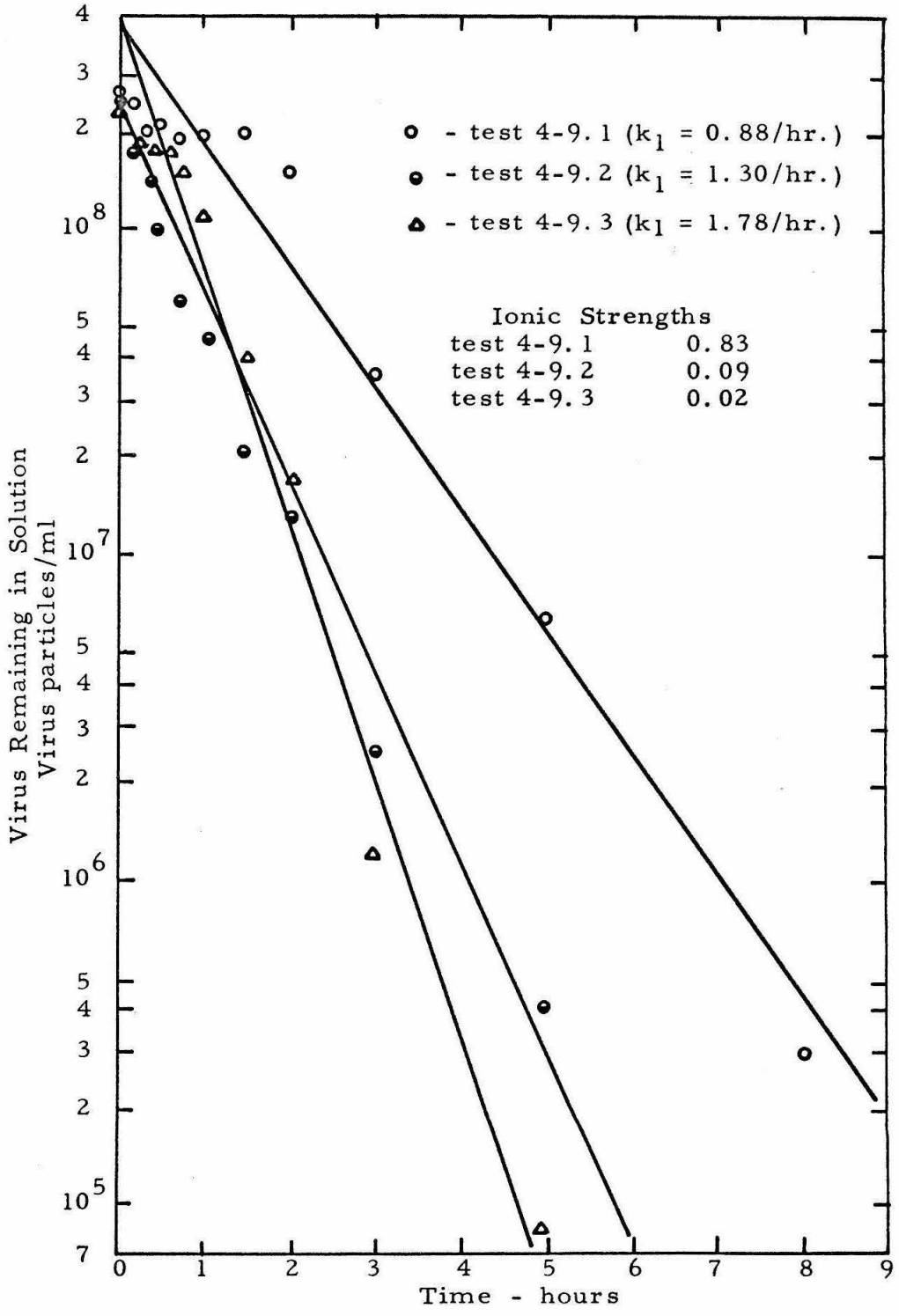


Figure 5-38. Preliminary tests of Buffer Effect on Adsorption; Least Squares Analysis

TABLE 5-37

Influence of Ionic Strength on
Adsorption of T_4 Phage to Carbon

Time hr.	Test No. Ionic Strength	Virus Remaining In Solution virus particles/ml X 10^{-7}					
		4-9.6 0.007	4-9.7 0.003	4-9.8 0.004	4-9.9 0.09	4-9.10 0.04	4-9.11 0.02
0		48.0	47.0	73.0	60.0	40.0	42.0
0.25		--	--	63.0	58.5	24.0	--
0.50		--	42.5	69.0	62.0	--	39.0
0.75		--	--	62.0	49.0	--	--
1.0		39.0	40.0	68.0	44.5	--	36.0
1.5		--	--	61.0	--	--	--
2.0		38.0	40.0	63.0	39.0	14.0	28.0
3.0		35.5	43.0	59.0	31.5	9.2	20.0
4.0		--	--	--	--	--	16.0
4.5		--	--	--	--	5.8	--
5.0		34.0	41.0	56.0	21.0	--	--
6.0		--	--	--	--	3.9	--
6.5		--	--	--	--	--	11.0
7.0		32.0	44.0	--	--	--	--
10.0		--	40.0	--	--	--	--

TABLE 5-37 (Cont'd)

Time Hr.	Test No / Ionic Strength	Virus Remaining In Solution virus particles/ml X 10^{-7}					
		4-9.12 0.12	4-9.13 0.20	4-9.14 0.01	4-9.15 0.015	4-9.16 0.02	4-9.17 0.20
0		49.0	42.0	40.0	72.0	11.0	11.0
0.25		--	--	--	60.0	--	--
0.50		37.0	--	37.5	60.0	7.7	6.5
1.0		28.0	32.0	33.5	43.0	--	5.0
1.5		--	--	36.0	--	6.5	--
2.0		13.0	31.0	29.5	23.0	4.3	--
2.5		--	--	--	--	2.9	2.2
3.0		13.0	26.0	--	44.0	--	--
3.5		--	--	24.5	--	3.3	--
4.0		10.0	--	--	--	--	--
4.5		--	--	--	--	2.9	1.3
5.0		--	--	21.5	42.0	--	--
6.0		7.7	--	--	--	--	--
6.5		--	13.0	--	--	--	--
7.0		--	--	17.5	--	1.8	--
	Controls: Test No. / Ionic Strength			4-9.18 0.20	4-9.19 0.02	4-9.20 0.007	4-9.21 0.09
0				45.0	45.0	49.0	64.0
5.0				46.0	--	--	65.0
6.5				--	43.0	--	--
7.0				--	--	48.0	--

To obtain the effect of ionic strength on the adsorption rate kinetic plots were made of the data according to the reversible second-order equation. Values of 1.9×10^{9} sites per mg of carbon and 4.0×10^{-7} ml/virus particle were used for adsorbent capacity and the equilibrium constant, respectively (Table 5-38). In all tests the data plotted as a straight line in compliance with reversible second-order kinetics (Figures 5-39 through 5-44). Adsorption rates were determined from the slope of the fitted line in each kinetic plot (Table 5-39).

Forward rate constants versus the corresponding ionic strengths were plotted in Figure 5-45. Results from previous tests (4-5.1 through 4-5.7, first and second steps of test 4-3.1, and test 4-3.2) were included in Figure 5-45. The rate constant was maximum at an ionic strength of 0.08.

Test 4-9.6, which used a bicarbonate buffer system, gave an adsorption rate comparable with tests using phosphate buffer of the same ionic strength. A definite correlation between adsorption rate and ionic strength appears to exist. The only exception to this relation was test 4-9.9. An ionic strength of 0.09 yielded a forward rate constant of 1.95×10^{-13} ml/ virus particles-sec. The expected value from Figure 5-45 is 8.3×10^{-13}. No explanation is available except that the test is presumed to be in error. A mistake in recording the amount of carbon or the ionic strength of the reaction solution might have been made.

5-11-1 Discussion of Results at Ionic Strengths Less Than Optimum

In studying adsorption of bacteriophages to host cells, Puck, Garen, and Cline (94) attributed the effect of salt concentrations to the cations (see Chapter 2). Maximum adsorption occurred at the same salt

TABLE 5-38

Interpretation of Adsorption Results at Various Ionic Strengths by Reversible Second-order Kinetics

Time t hr.	Virus Adsorbed X virus particles/ml	$\frac{0.5(a_o+b_o+K^{-1}+m)-X}{0.5(a_o+b_o+K^{-1}-m)-X}$	$\frac{a_o+b_o+K^{-1}-m}{a_o+b_o+K^{-1}+m}$
test 4-9.6, μ = 0.007 (a_o = 4.8 X 10^8, b_o = 4.7 X 10^8, m = 6.9 X 10^7)			
1.0	9.0 X 10^7	1.03	
2.0	10.0 X 10^7	1.04	
3.0	12.5 X 10^7	1.05	
5.0	14.0 X 10^7	1.06	
7.0	16.0 X 10^7	1.07	
test 4-9.7, μ = 0.003 (a_o = 4.7 X 10^8, b_o = 4.7 X 10^8, m = 6.9 X 10^7)			
0.5	4.5 X 10^7	1.02	
1.0	7.0 X 10^7	1.04	
2.0	7.0 X 10^7	1.04	
3.0	4.0 X 10^7	1.01	
5.0	6.0 X 10^7	1.02	
7.0	3.0 X 10^7	1.01	
10.0	7.0 X 10^7	1.04	

μ is the designation for ionic strength

TABLE 5-38 (Cont'd)

Time t hr.	Virus Adsorbed X virus particles/ml	$\frac{0.5(a_o+b_o+K^{-1}+m)-X}{0.5(a_o+b_o+K^{-1}-m)-X}$	$\frac{a_o+b_o+K^{-1}-m}{a_o+b_o+K^{-1}+m}$
test 4-9.8, μ = 0.004 (a_o = 7.3 X 10^8, b_o = 4.5 X 10^7, m = 2.9 X 10^8)			
0.25	10 X 10^7	1.12	
0.50	4 X 10^7	1.04	
0.75	11 X 10^7	1.13	
1.0	5 X 10^7	1.05	
1.50	12 X 10^7	1.17	
2.0	10 X 10^7	1.12	
3.0	14 X 10^7	1.18	
5.0	17 X 10^7	1.24	
test 4-9.9, μ = 0.09 (a_o = 6.0 X 10^8, b_o = 4.5 X 10^8, m = 1.7 X 10^8)			
0.25	1.5 X 10^7	1.01	
0.50	0.0 X 10^7	0.00	
0.75	11.0 X 10^7	1.09	
1.0	15.5 X 10^7	1.15	
2.0	21.0 X 10^7	1.25	
3.0	28.5 X 10^7	1.50	
5.0	39.0 X 10^7	3.05	

TABLE 5-38 (Cont'd)

Time t hr.	Virus Adsorbed X virus particles/ml	$\frac{0.5(a_o+b_o+K^{-1}+m)-X}{0.5(a_o+b_o+K^{-1}-m)-X}$	$\frac{a_o+b_o+K^{-1}-m}{a_o+b_o+K^{-1}+m}$
test 4-9.10, μ = 0.04 (a_o = 4.0 X 10^8, b_o = 4.7 X 10^8, m = 9.6 X 10^7)			
0.25	16.0 X 10^7	1.42	
2.0	26.0 X 10^7	1.75	
3.0	30.8 X 10^7	2.20	
4.5	34.2 X 10^7	3.09	
6.0	36.1 X 10^7	4.56	
test 4-9.11, μ = 0.02 (a_o = 4.2 X 10^8, b_o = 4.7 X 10^8, m = 8.4 X 10^7			
0.5	3.0 X 10^7	1.02	
1.0	6.0 X 10^7	1.03	
2.0	14.0 X 10^7	1.09	
3.0	22.0 X 10^7	1.20	
4.0	26.0 X 10^7	1.35	
6.5	31.0 X 10^7	1.50	
test 4-9.12, μ = 0.12 (a_o = 4.9 X 10^8, b_o = 4.7 X 10^8, m = 6.5 X 10^7			
0.5	12.0 X 10^7	1.04	
1.0	21.0 X 10^7	1.11	
2.0	36.0 X 10^7	1.52	
3.0	36.0 X 10^7	1.52	
4.0	39.0 X 10^7	1.84	
6.0	41.3 X 10^7	2.47	

TABLE 5-38 (Cont'd)

Time t hr.	Virus Adsorbed X virus particles/ml	$\frac{0.5(a_o+b_o+K^{-1}+m)-X}{0.5(a_o+b_o+K^{-1}-m)-X}$	$\frac{a_o+b_o+K^{-1}-m}{a_o+b_o+K^{-1}+m}$
test 4-9.13, μ = 0.20 (a_o = 4.2 X 10^8, b_o = 4.7 X 10^8, m = 8.4 X 10^7)			
1.0	10 X 10^7	1.05	
2.0	11 X 10^7	1.06	
3.0	16 X 10^7	1.11	
6.5	29 X 10^7	1.44	
test 4-9.14, μ = 0.01 (a_o = 4.0 X 10^8, b_o = 4.7 X 10^8, m = 9.6 X 10^7)			
0.5	2.5 X 10^7	1.01	
1.0	6.5 X 10^7	1.04	
1.5	4.0 X 10^7	1.02	
2.0	10.5 X 10^7	1.08	
3.5	15.5 X 10^7	1.13	
5.0	18.5 X 10^7	1.18	
7.0	22.5 X 10^7	1.27	
test 4-9.15, μ = 0.015 (a_o = 7.2 X 10^8, b_o = 4.7 X 10^8, m = 2.6 X 10^8)			
0.25	12 X 10^7	1.11	
0.50	12 X 10^7	1.11	
1.0	29 X 10^7*	1.59	
2.0	49 X 10^7*	--	
3.0	28 X 10^7	1.53	
5.0	30 X 10^7	1.65	

* These values for the virus concentration are apparently in error.

TABLE 5-38 (Cont'd)

Time t hr.	Virus Adsorbed X virus particles/ml	$\frac{0.5(a_o+b_o+K^{-1}+m)-X}{0.5(a_o+b_o+K^{-1}-m)-X}$	$\frac{a_o+b_o+K^{-1}-m}{a_o+b_o+K^{-1}+m}$
test 4-9.16, μ = 0.02 (a_o = 1.1 X 10^8, b_o = 4.8 X 10^8, m = 3.7 X 10^8)			
0.5	3.3 X 10^7	1.35	
1.0	4.5 X 10^7	1.55	
2.0	6.7 X 10^7	2.24	
2.5	7.1 X 10^7	2.44	
3.5	7.7 X 10^7	2.86	
4.5	8.1 X 10^7	4.32	
7.0	9.2 X 10^7	5.20	
test 4-9.17, μ = 0.20 (a_o = 1.1 X 10^8, b_o = 4.7 X 10^8, m = 3.6 X 10^8)			
0.5	4.5 X 10^7	1.54	
1.0	6.0 X 10^7	1.94	
2.5	8.8 X 10^7	4.24	
4.5	9.7 X 10^7	7.24	

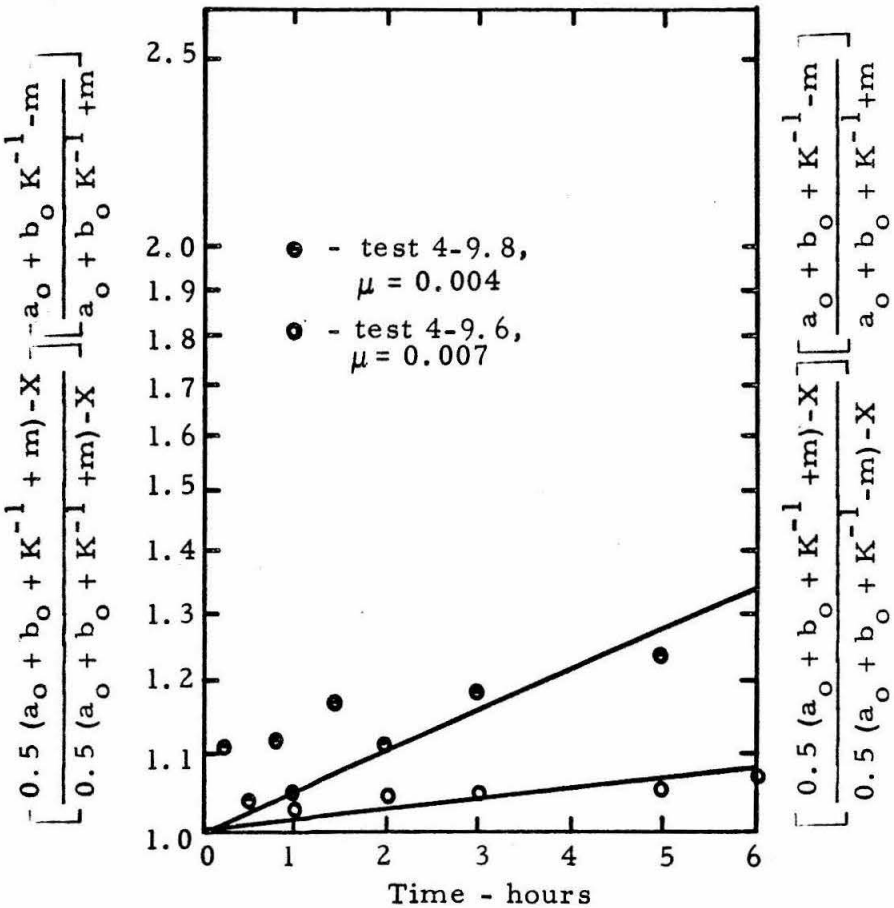


Figure 5-39. Reversible second-order kinetic plot of tests 4-9.6 and 4-9.8; adsorption at ionic strengths of 0.007 and 0.004

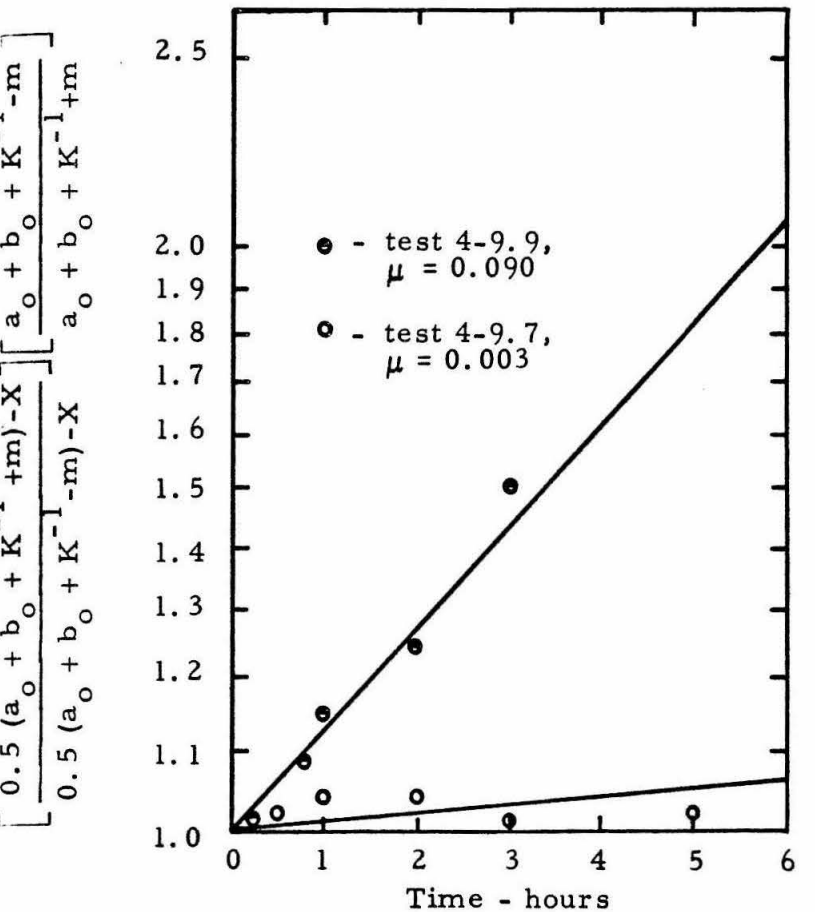


Figure 5-40. Reversible second-order kinetic plot of tests 4-9.7 and 4-9.9; adsorption at ionic strengths of 0.003 and 0.090

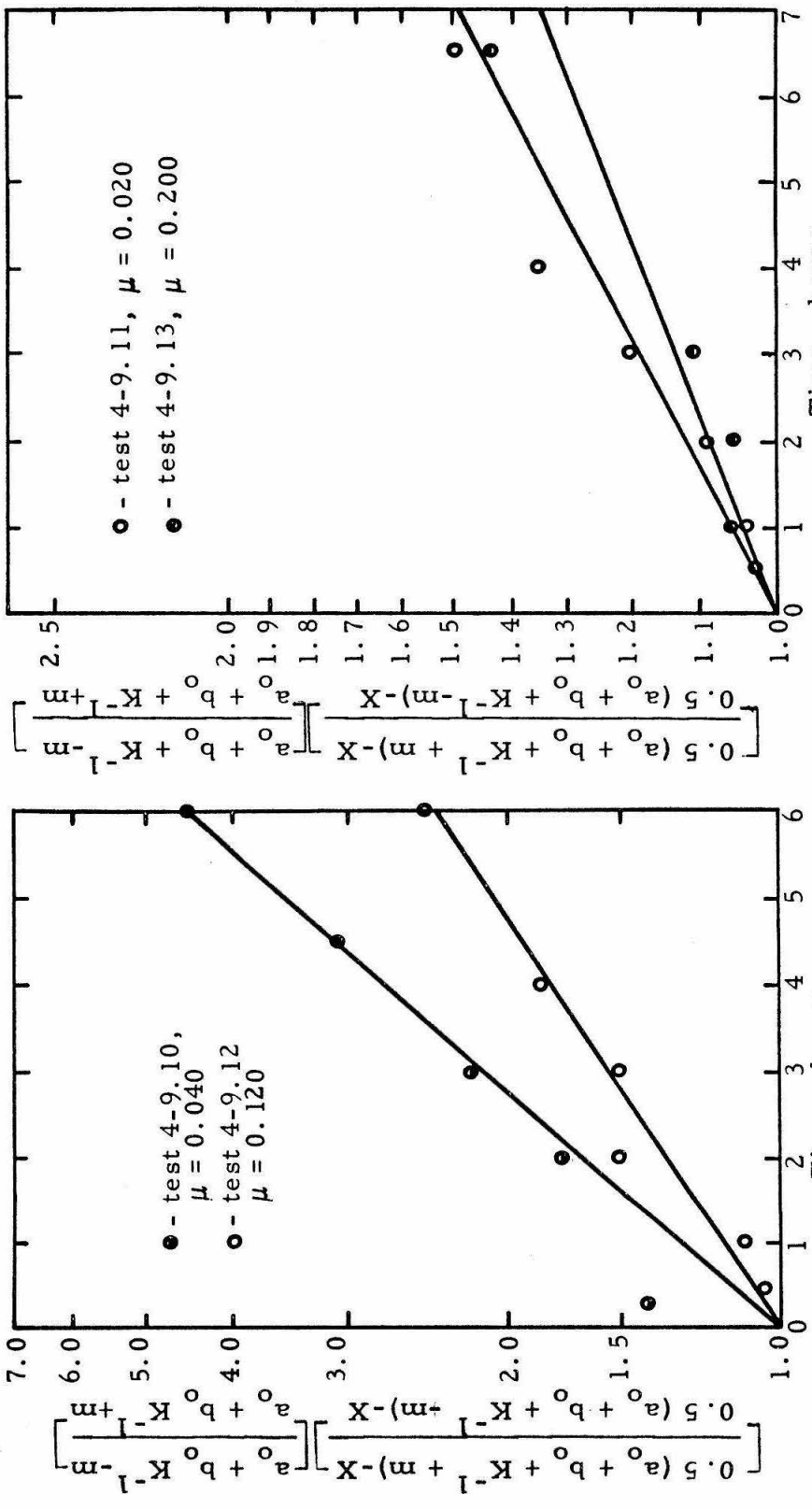


Figure 5-41. Reversible second-order kinetic plot of tests 4-9.10 and 4-9.12; adsorption at ionic strengths of 0.040 and 0.120

Figure 5-42. Reversible second-order kinetic plot of tests 4-9.11 and 4-9.13; adsorption at ionic strengths of 0.020 and 0.200

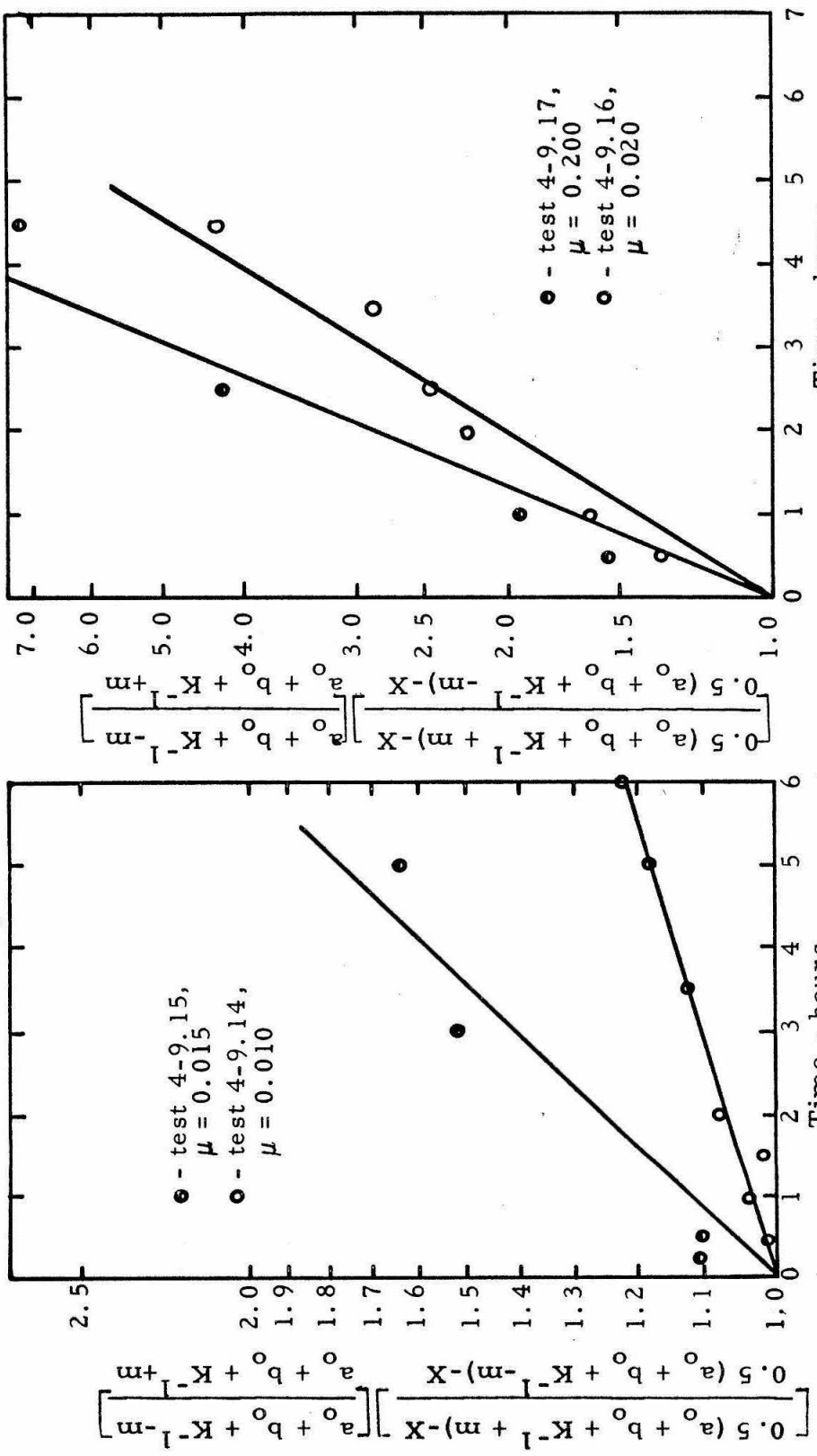


Figure 5-43. Reversible second-order kinetic plot of tests 4-9.14 and 4-9.15; adsorption at ionic strengths of 0.010 and 0.015

Figure 5-44. Reversible second-order kinetic plot of tests 4-9.16 and 4-9.17; adsorption at ionic strengths of 0.020 and 0.200

TABLE 5-39

Forward Rate Constants for Adsorption of Bacteriophage T_4 to Activated Carbon at Different Ionic Strengths

Test No.	Ionic Strength	Figure No.	Slope mk_1 1/hr	Forward Rate Constant $k_1 \times 10^{13}$ $\frac{ml}{virus\ particles\text{-}sec}$
4-9.6	0.007	5-39	0.012	0.50
4-9.7	0.003	5-40	0.010	0.42
4-9.8	0.004	5-39	0.059	0.47
4-9.9	0.090	5-40	0.120	1.95
4-9.10	0.040	5-41	0.250	7.23
4-9.11	0.020	5-42	0.059	1.95
4-9.12	0.120	5-41	0.147	6.40
4-9.13	0.200	5-42	0.042	1.39
4-9.14	0.010	5-43	0.032	0.92
4-9.15	0.015	5-43	0.114	1.22
4-9.16	0.020	5-44	0.370	2.78
4-9.17	0.200	5-44	0.520	4.00

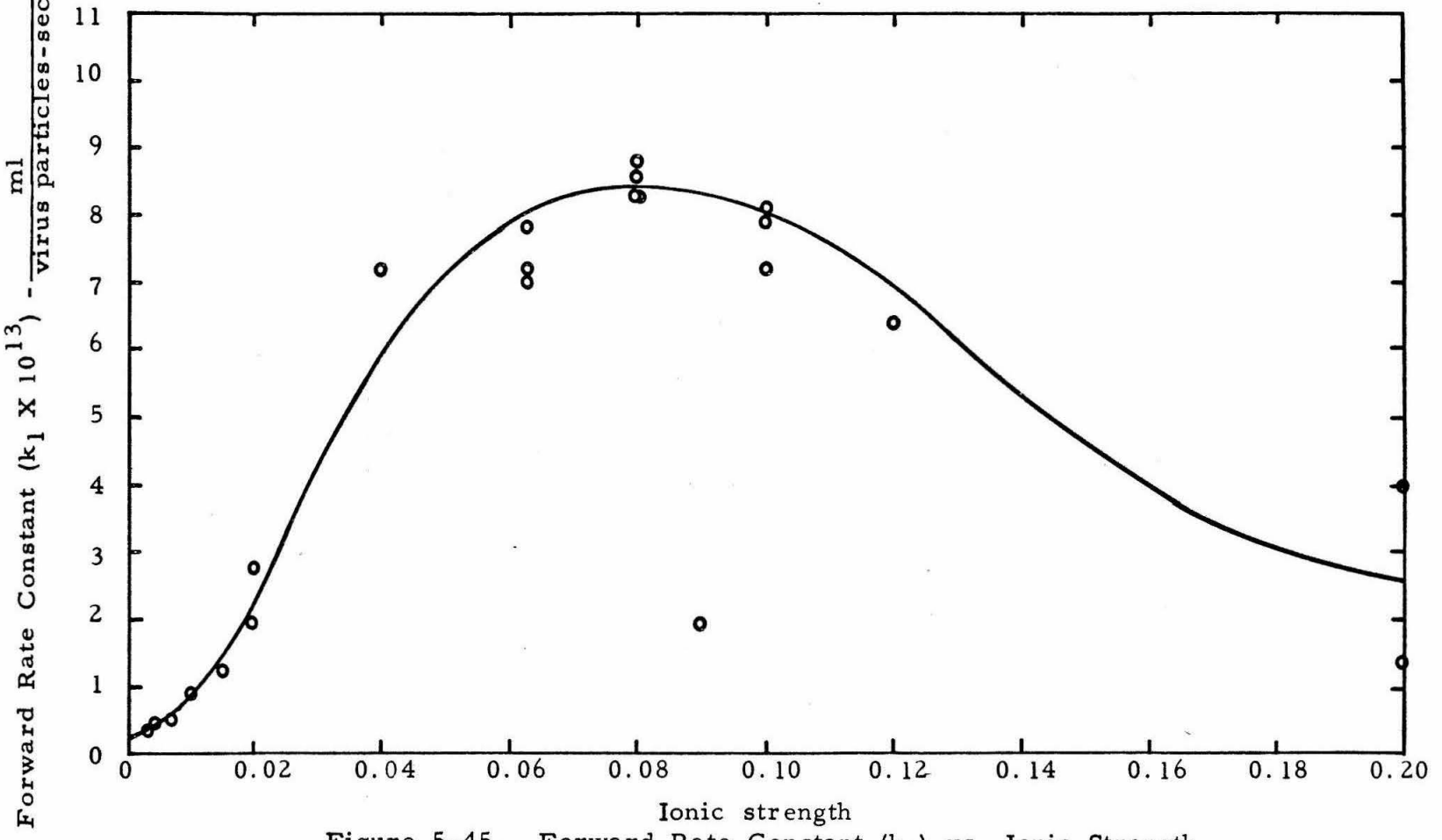


Figure 5-45. Forward Rate Constant (k_1) vs. Ionic Strength for Adsorption of T_4 Phage to Carbon

molarity for all univalent cations. With bivalent cations maximum adsorption occurred at a lower salt molarity. The difference was attributed to cations since the same anion (Cl) was used in all tests. From these experiments they proposed that cations may attach to two different kinds of adsorption sites on host cells. One site was thought to adsorb viruses at all ionic concentrations. For cation concentrations higher than optimum the second site was thought to take up cations and hinder adsorption.

On the basis of their conclusion with respect to the importance of cations a comparison was made between cation molarity and adsorption rate (Table 5-40, Figure 5-46). Adsorption rate versus cation molarity gave a curve similar to Figure 5-45 for ionic strength. This similarity was expected since most of the high ionic strength solutions were obtained with NaCl. The cation molarity and ionic strength of NaCl are the same. Data at low cation concentrations, however, did not correlate cation molarity with adsorption rate as well as did ionic strength (Figure 5-47 and 5-48). In tests 4-9.14 and 4-9.15 the major portion of the ionic strength was contributed by the buffer, in particular the anion $HPO_4^{=}$, and not cations. Correlation of the ionic strengths (0.01 and 0.015) of these two tests with adsorption rate gave a smooth curve (Figure 5-48). When the cation molarities, 0.007 and 0.008 for tests 4-9.14 and 4-9.15 respectively (Table 5-40), were plotted against adsorption rate a rather poor fit to a smooth curve was obtained (Figure 5-47).

From these considerations the adsorption rate appears to vary uniformly with ionic strength. Both cations and anions influenced

TABLE 5-40

Comparison of Ionic Strength with Cation Molarity for tests 4-9.6 through 4-9.17

Test No.	Ionic Strength	Cation Molarity
4-9.6	0.007	0.007
4-9.7	0.003	0.003
4-9.8	0.004	0.004
4-9.10	0.040	0.027
4-9.11	0.020	0.017
4-9.12	0.120	0.094
4-9.13	0.200	0.179
4-9.14	0.010	0.007
4-9.15	0.015	0.008
4-9.16	0.020	0.017
4-9.17	0.200	0.179

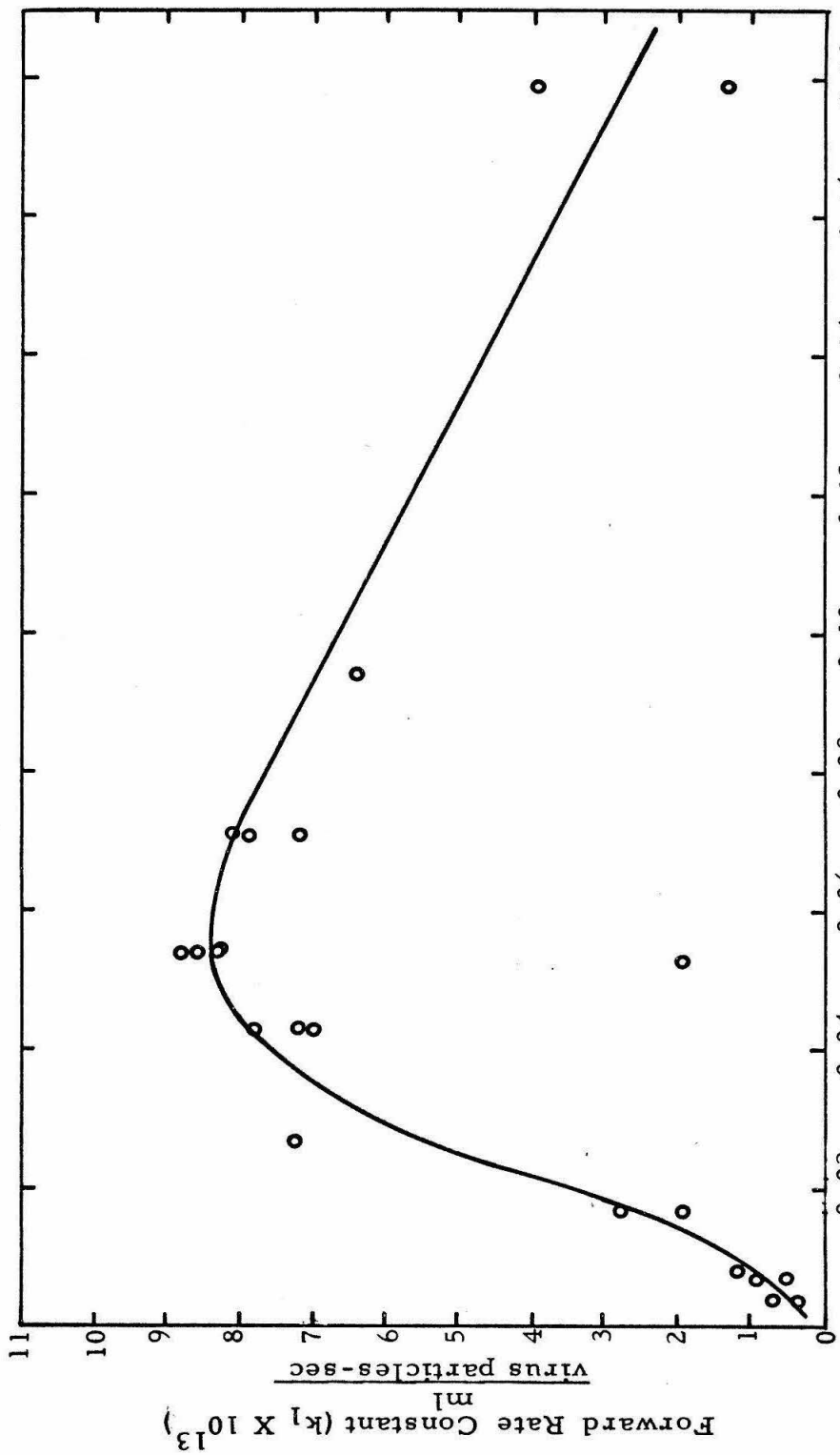


Figure 5-46. Forward Rate Constant (k_1) vs. Molarity of Cations for Adsorption of T_4 Phage to Carbon

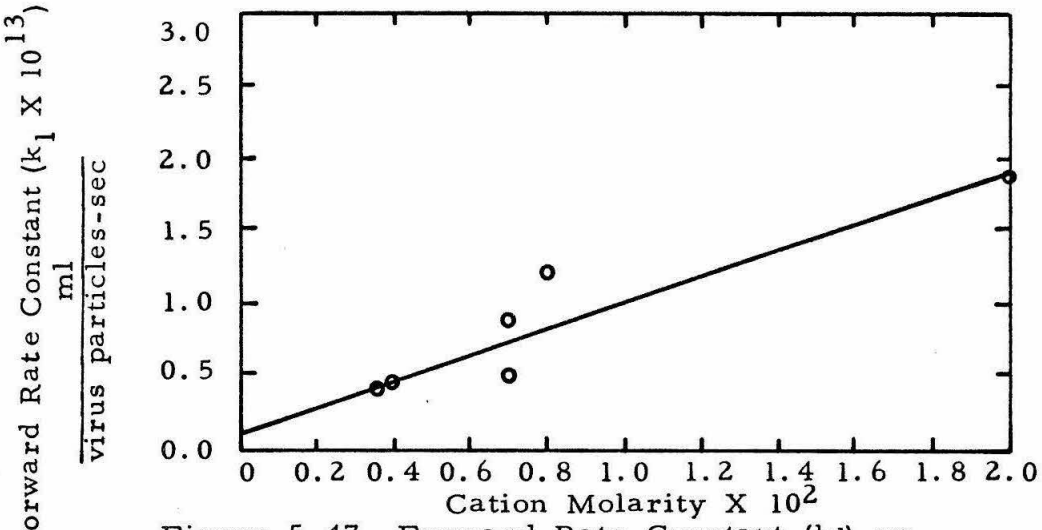


Figure 5-47. Forward Rate Constant (k_1) vs. Molarity of Cations for Adsorption of T_4 Phage to Carbon (Enlarged Scale)

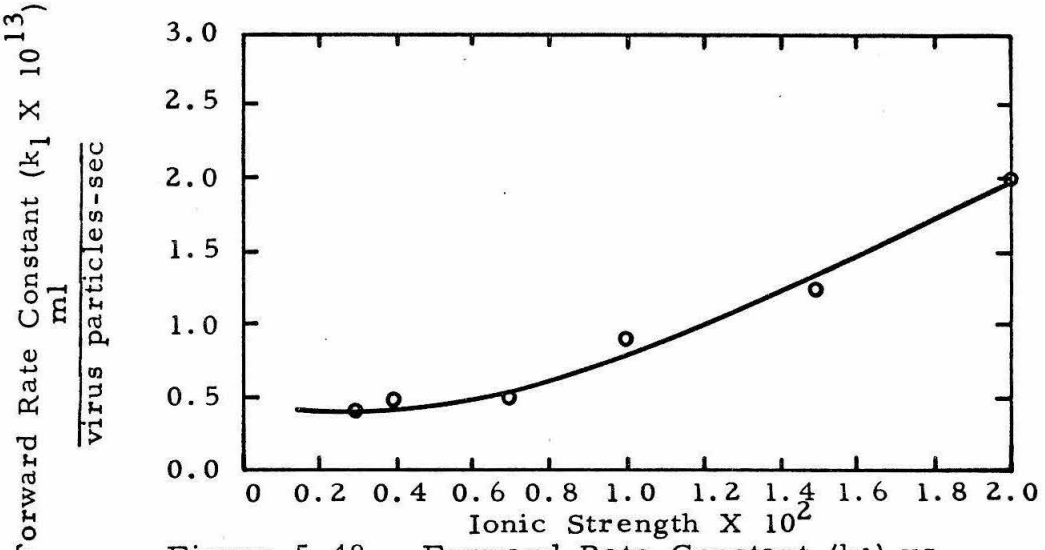


Figure 5-48. Forward Rate Constant (k_1) vs. Ionic Strength for Adsorption of T_4 Phage to Carbon (Enlarged Scale)

adsorption in proportion to the square of their charge. This hypothesis seems simpler and more attractive than postulating that host cells have two sites. One site would adsorb viruses at all cation concentrations. The other site takes up cations to hinder virus adsorption at ion concentrations higher than optimum.

The effect of ions on adsorption probably results from the ion atmosphere surrounding the virus particle and the negative-charged groups on the adsorbent. The ion atmosphere about viruses can be described as a diffused double layer.

In solutions of low ionic strength the net charge on both bacteriophage T_4 and activated carbon is negative. These like charges would tend to repel viruses from the carbon. This effect reduces the collision frequency. In the presence of ions, negative surfaces of a colloid attract a surrounding layer of positive ions. The formation of a diffuse double layer of positive ions about negative colloids would reduce the repelling forces.

Repulsive forces between colloids of like charge can be lowered by decreasing the distance of charge effectiveness. This distance is a function of the ionic strength of the solution. Likewise the interaction of like charges between activated carbon and T_4 phage is weakened by an increase of ionic strength. The adsorption rate, therefore, will increase. This phenomenon, however, will not explain the occurrence of a maximum. The decrease in adsorption rate after an ionic strength of 0.08 must result from another mechanism.

5-11-2 Discussion of Results at Ionic Strengths Greater Than Optimum

One explanation for the drop in adsorption rate above an ionic strength of 0.08 might be agglomeration of virus particles. In the same way that ionic strength enhances collision between virus particles and carbon, it increases collision between virus particles. This reduction of repulsive forces may cause agglomeration of viruses.

Agglomeration was proposed to explain the dual sedimentation rate of bacteriophages in an ultracentrifuge. Electron micrographs, however, have shown no evidence of agglomeration. As discussed in section 5-9, the dual sedimentation rate is due to the configuration of tail fibers on the bacteriophage (152)(154). The fact that agglomeration of bacteriophages is not detected in electron micrographs makes this explanation unlikely.

The occurrence of an optimum ion concentration was also noted in adsorption of bacteriophage to host cells and ion exchange resins (94)(95)(121). Thus, the phenomenon is somewhat more likely to be associated with the virus and not the adsorbent. Garen (95) found that the rate of adsorption of bacteriophage T_1 to host cells increased to a maximum with increasing ion concentration. The equilibrium constant did not change over this range. At ion concentrations above the optimum the adsorption rate and equilibrium constant dropped. Garen (95) explained the decrease in adsorption rate as a competition between ions and the bacteriophage for the same adsorption sites. Since his explanation was proposed, additional information has become available on the properties of bacteriophages, revealing the nature of this phenomenon.

The repulsion and interaction of tail fibers with the tail sheath of bacteriophages have been discussed in relation to pH effects (section 5-9). Tail fibers also change their configuration with ionic strength. Although no direct correlation has been made between ionic strength and the degree of tail fiber extension, it is known to exist from studies on sedimentation rates. Sharp and coauthors (138) found that the two sedimentation rates of 700 X 10^{-13} and 1000 X 10^{-13} sec could also be obtained by changing the ion composition of the solution. These rates are identical with those detected at different pH values (152)(154). The low sedimentation rate occurs when the tail fibers are extended and the high rate when they are bent back around the tail sheath. Kellenberger and coauthors (152) noted that three tail fibers of bacteriophage T_4 are extended at an ionic strength of 0.1. In de-ionized water, however, more fibers were extended. These findings were characteristic interactions of like charges on the fibers and tail sheath. As discussed above, like charges exert their greatestrepulsive forces at low ionic strengths. This condition causes the fibers to be repelled from the tail sheath and exposes their sites for adsorption to the absorbent. As ionic strength is increased, the interaction of the like charges is weakened and this allows the fibers to adsorb to the tail sheath. Their sites, therefore, are not exposed for adsorption to the absorbent.

5-11-3 Equilibrium Condition at Ionic Strengths of 0.02 and 0.20

Four tests (4-9.22 through 4-9.25) were made at an ionic strength of 0.02 (Table 4-12). The pH was maintained at the optimum of 7.0 with mono- and dipotassium phosphate buffer. Previous equilibrium

tests had been made at the optimum ionic strength of 0.08. An ionic strength of 0.02 was known to reduce the adsorption rate greatly and may also have affected the equilibrium point.

Each test was conducted for 22 to 25 hours (Table 5-41). Bacterial contamination developed in the reaction flasks so that samples were not subsequently possible. The reaction solutions had reached an approximate equilibrium after 22 to 25 hours of adsorption. Results for tests 4-9.22 and 4-9.23 were plotted to show the approach to equilibrium (Figure 5-49). At 22 hours the first-order rate of the reaction was approximately 0.016/hr. This value approaches the inactivation rate (0.023/hr) of the virus at an ionic strength of 0.02 (Figure 5-37). After correcting for inactivation, the results (Table 5-42) were plotted in Figure 5-18 for comparison with equilibrium values obtained at an ionic strength of 0.08. The plotted points compare reasonably well with the other data. The low ionic strength of 0.02 does not appear to change the equilibrium condition by any measurable degree.

A significant difference does exist in the time required to reach equilibrium. At an ionic strength of 0.02, equilibrium was not completely established until 22 hours, but tests made at an ionic strength of 0.08 reached equilibrium in half the time. This change in rate of adsorption was discussed in section 5-11-1.

Apparently the number of sites on carbon was not changed significantly by the low ionic strength, but the ability of the virus to get to these sites was lowered. This phenomenon is in agreement with

TABLE 4-41

Effect of Ionic Strength on the Equilibrium Condition of T_4 Phage-Carbon System

Time hr.	Test No.	4-9.22	4-9.23	4-9.24	4-9.25	4-9.26	4-9.27	4-9.28
	Ionic Strength	0.02	0.02	0.02	0.02	0.20	0.20	0.20
	Carbon mg/l	71.8	50.0	31.25	100	31.25	50.0	100
	Virus Remaining in Solution virus particles/ml X 10^{-7}							
0		13.5	11.0	11.0	11.0	12.0	12.0	10.0
1.0		--	--	10.0	7.9	11.6	11.6	10.0
1.5		11.0	9.7	--	--	--	--	--
5.0		--	--	8.3	5.5	10.0	10.8	10.0
10.0		6.0	5.2	--	--	--	--	--
12.0		--	--	6.9	0.9	12.0	10.7	7.3
12.5		6.5	5.6	--	--	--	--	--
22.0		4.8	4.3	--	--	--	--	--
25.0		--	--	4.2	0.5	10.0	9.1	5.4

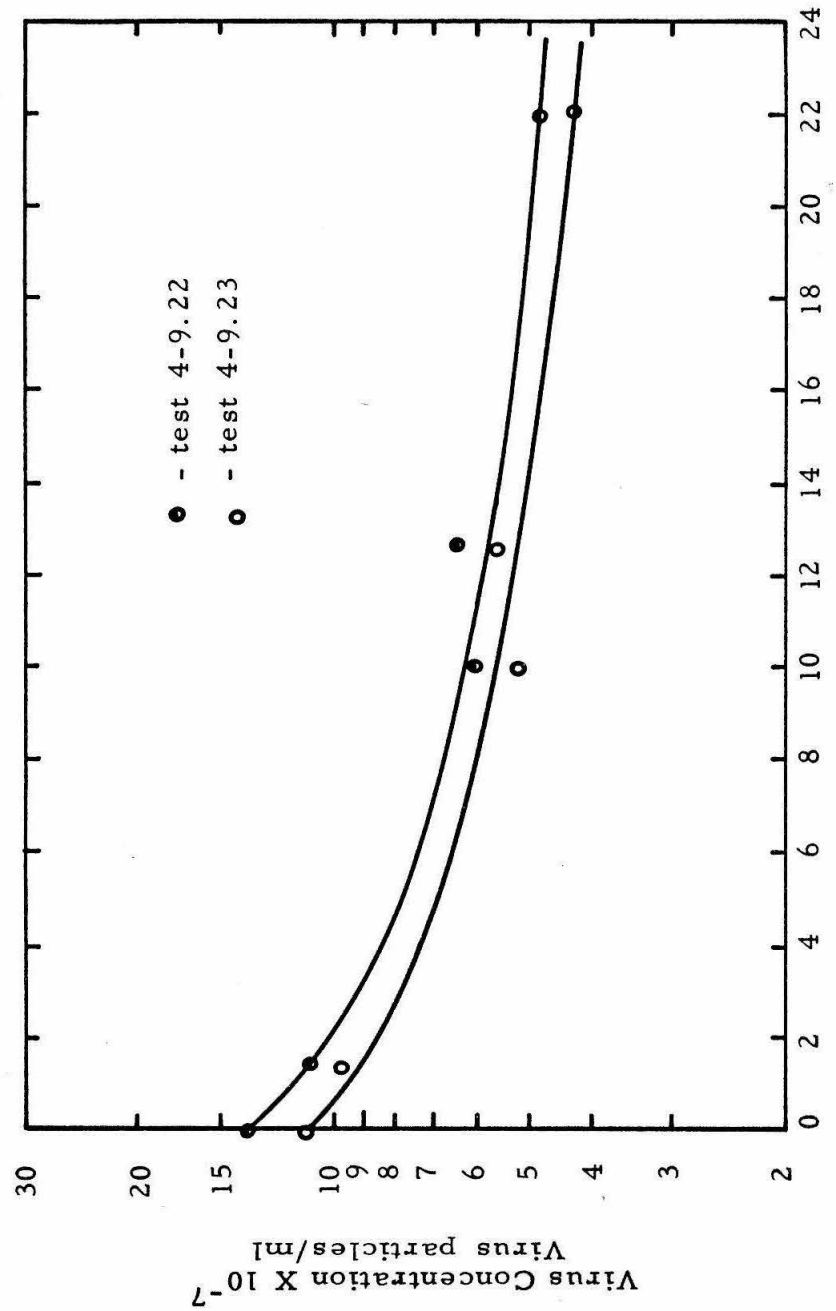


Figure 5-49. Effect of Ionic Strength of 0.02 on Equilibrium Condition of T_4 Phage-Carbon System

TABLE 5-42

Equilibrium Parameters of Langmuir Isotherm for Ionic Strengths of 0.02 and 0.20

Test No.	Ionic strength	Virus concentration at equilibrium (c_e) virus particles/ml	Virus adsorbed per mg of carbon (q) (corrected for natural inactivation of virus)	(c_e/q) virus particles/ml / virus particles/mg
4-9.22	0.02	4.8×10^7	1.2×10^9	0.040
4-9.23	0.02	4.3×10^7	1.3×10^9	0.032
4-9.24	0.02	4.2×10^7	2.0×10^9	0.022
4-9.25	0.02	0.5×10^7	1.1×10^9	0.004
4-9.26	0.20	10.0×10^7	4.5×10^8	0.224
4-9.27	0.20	9.1×10^7	4.2×10^8	0.220
4-9.28	0.20	5.4×10^7	3.9×10^8	0.140

the mechanism described in section 5-11-1. Higher repulsive forces presumably lowered the collision frequency of virus and carbon particles.

Three equilibrium tests (4-9.26 through 4-9.28) were made at an ionic strength of 0.20. The reaction solutions were again buffered at the optimum pH, 7.0 (Table 4-12). The equilibrium achieved at this ionic strength does not fit the Langmuir plot of Figure 5-18 for optimum conditions (Tables 5-41 and 5-42). The results of these three tests were plotted according to the Langmuir equation (Figure 5-50). Although three tests cannot give the accuracy obtained in Figure 5-18, the Langmuir plot does illustrate a significant change in the equilibrium conditions. The slope from Figure 5-50 gave a value of 4.5×10^8 sites per milligram of carbon. A value of 1.6×10^9 sites/mg was obtained for an ionic strength of 0.08 (Figure 5-18). Adsorption data at ionic strengths of 0.20 yielded a value for the equilibrium constant of 1×10^{-7} ml/virus particle, while at 0.08 a value of 4×10^{-7} ml/virus particle was obtained. Although this is a fourfold difference, only three points were used in plotting Figure 5-50, and it is considered that the values may not be significantly different.

The different equilibrium at an ionic strength of 0.20 can be explained by considering tail fibers as the agents adsorbing to carbon. At ionic strengths greater than the optimum the Langmuir equation does not represent the capacity of the carbon for viruses. Instead, the effect is probably the inability of a large portion of the bacteriophage population to adsorb. This phenomenon results from the interaction of the bacteriophage's tail fibers with its tail sheath. The effective

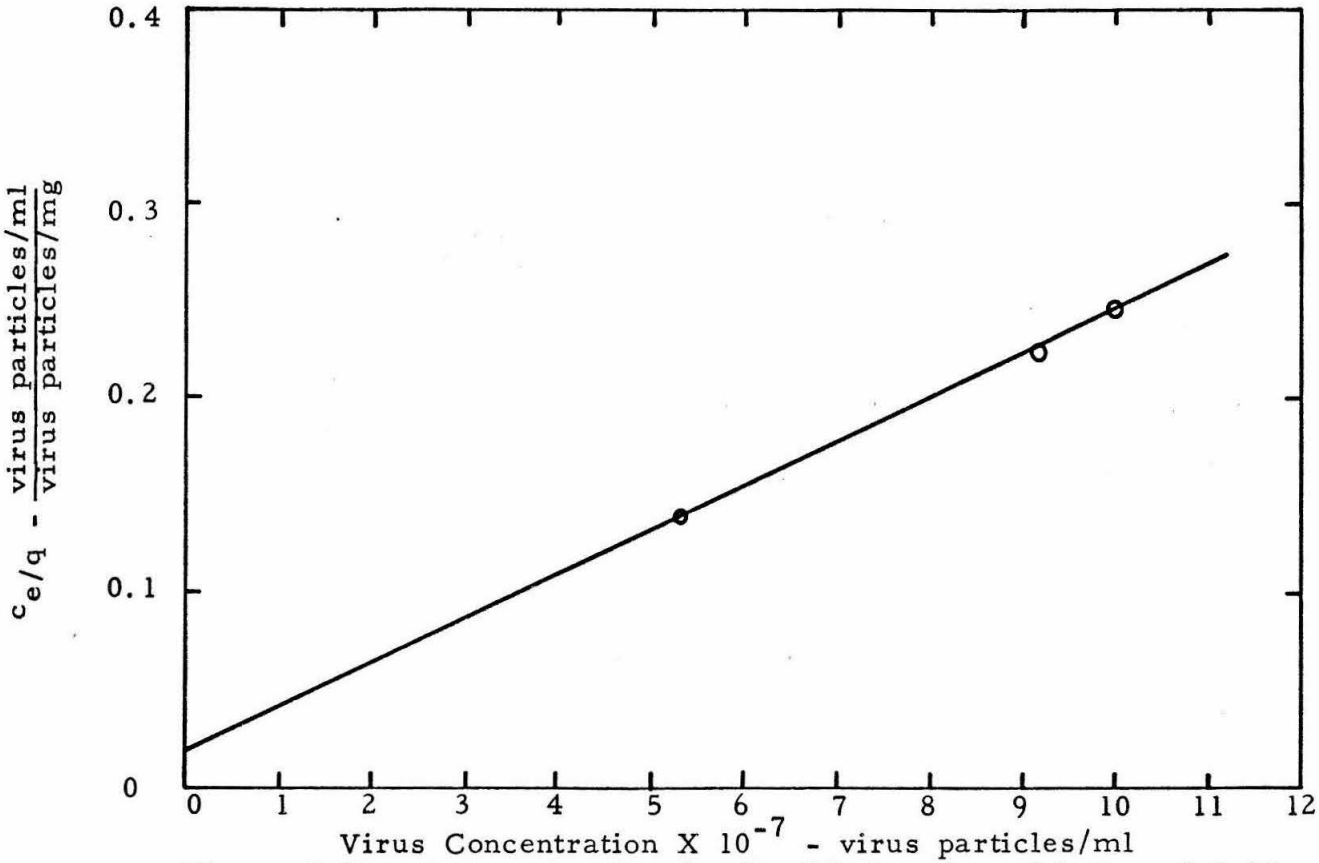


Figure 5-50. Langmuir Plot for Equilibrium in a Solution of 0.20 Ionic Strength and pH 6.9 by Least Squares Analysis

concentration of bacteriophages with respect to adsorption, therefore, would be much less than that given by the assay.

In summary, two mechanisms were proposed to explain the observed effects of ionic strength. At ionic strengths less than the optimum, the adsorption rate increases with increasing ionic strength. This is probably caused by masking of like charges between virus and carbon particles allowing a greater collision frequency. At ionic strengths greater than the optimum the adsorption rate decreases with increasing ionic strength. This was explained as a change in the configuration of tail fibers on bacteriophages. At low ionic strengths the fibers are extended. This position favors adsorption of bacteriophages to carbon. At high ionic strengths like charges are masked on the tail fibers and tail sheath of the virus. Under this condition the fibers withdraw from their extended form and adsorb to the tail sheath, rendering them unavailable for adsorption to the carbon.

CHAPTER 6

INTERPRETATION OF DATA BY THE THEORY OF DIFFUSION-LIMITED PROCESSES

Adsorption of bacteriophage to host cells has been analyzed in terms of the von Smoluchowski theory of diffusion limited processes. Under optimum conditions, adsorption rate is equivalent to the diffusion rate (94)(97)(98). The success in attachment to host cells is almost 100 percent, each collision resulting in adsorption. Host cell adsorption capacity is that of a close-packed, single layer of viruses (95). This calculation assumed that the virus adsorbed by its tail fibers, thus occupying an area equivalent to the diameter of its head.

Equilibrium studies of virus adsorption to activated carbon gave a capacity in the order of 1.6×10^9 to 1.9×10^9 virus particles/mg of carbon (Chapter 5). A surface area of 10 cm^2/mg was obtained for the 120/140 activated carbon (section 3-2-2). Dividing this surface area by the carbon capacity yields 5.7×10^{-9} cm^2 for the area of a virus site. The projected cross-sectional areas for different orientations of the virus are presented in Table 6-1. The smallest and largest areas that one virus particle can occupy are 3.3×10^{-11} and 9.6×10^{-10} cm^2. It appears that at the maximum only 18 percent of the available carbon surface is utilized. This finding supports the model of unimolecular adsorption as predicted by the Langmuir isotherm.

The small surface coverage of viruses probably results from insufficient carboxyl groups on the carbon. Clauss (156) found that of the acid groups on carbon only 10 percent were of carboxyl character. He described these as occurring at the edges of aromatic layers.

TABLE 6-1

Cross-Sectional Areas Utilized by T_4 Phage for Different Orientations on Carbon

Virus Orientation with Respect to Carbon Surface	Considered Cross-sectional Area	Dimensions cm	Area cm^2
Long axis perpendicular	perpendicular to long axis through head	6.5×10^{-6} cm in diameter	3.3×10^{-11}
long axis parallel	parallel to long axis	1.8×10^{-5} cm by 2.0×10^{-6} cm	3.6×10^{-11}
long axis perpendicular with tail attaching	projected area of extended tail fibers	3.5×10^{-5} cm in diameter	9.6×10^{-10}

This condition could explain the unutilized surface area. The close-packed layer of viruses developing on host cells presumably results from the extensive coverage of cells with carboxyl groups. The close-packed layer must produce appreciable overlapping of virus tail fibers. If the tail fibers of the adsorbed virus utilize all carboxyl groups within their circumference, a close-packed layer could not occur. This phenomenon probably does occur on activated carbon and explains the inefficient use of the total surface.

As in the case of adsorption to host cells, virus adsorption by activated carbon may be diffusion limited. Although agitation was provided by the batch adsorption technique, concentration gradients were probably not completely eliminated in the neighborhood of the carbon particles. This condition would manifest itself as a resistance to mass transfer at the carbon's surface. Under these circumstances

the maximum adsorption rate may be determined by the diffusion coefficient of the virus. The amount of virus n_a carried to area A of the carbon can be expressed by Fick's first law. Under this condition adsorption is limited by the diffusion of small virus particles toward large, essentially stationary carbon particles. Microscopic observation of carbon particles indicated that they are approximately spherical in shape.

$$\frac{dn_a}{dt} = -DAN \frac{dn}{dr} \qquad (6\text{-}1)$$

where:

n_a = the concentration of adsorbed virus (virus particles/ml).

n = the concentration of virus in solution (virus particles/ml).

D = the diffusion coefficient (cm^2/sec).

A = the surface area of a spherical carbon particle of radius a (cm^2).

N = the concentration of carbon particles with radius a (number of particles/ml).

t = time (sec).

r = the distance from the carbon particle (cm).

The concentration gradient is expressed by dn/dr. Substituting $4 \pi r^2$ for A and integrating the right side of equation (6-1) between limits of a to ∞ and n_s to n_∞ for r and n, respectively yields:

$$\frac{dn_a}{dt} = -4 \pi a DN(n_\infty - n_s) \qquad (6\text{-}2)$$

where a is the radius of the carbon particle, and n_s and n_∞ are the virus concentrations in solution at the carbon surface and in the bulk of the solution.

The rate of adsorption would be expressed by the constant $k = 4\pi aDN$. The reaction velocity is D/a.

For a diffusion coefficient of 8×10^{-8} cm^2/sec (Table 3-4) and carbon particles with a geometric mean diameter of 2.5 microns (Figure 3-2), the velocity of reaction for a diffusion limiting process is 6.4×10^{-4} cm/sec. Adsorption rates have been calculated using this velocity for diffusion of virus particles to four different site areas (Table 6-2). The four site areas include a value that is derived from the carbon capacity and the three projected cross-sections of the virus presented in Table 6-1.

TABLE 6-2

Adsorption Rates Predicted by the von Smoluchowski Equation for Different Cross-sectional Areas of the Virus

Description	Site Area cm^2	Adsorption Rate $\frac{cm^3}{\text{virus particles-sec}}$
Experimental value as determined from available surface area and carbon capacity for viruses	5.7×10^{-9}	36.4×10^{-13}
Perpendicular to long axis through the head of the virus	3.3×10^{-11}	0.2×10^{-13}
Parallel to long axis of the virus	3.6×10^{-11}	0.2×10^{-13}
Projected area of extended tail fibers	9.6×10^{-10}	6.2×10^{-13}
Experimental maximum adsorption rate as determined from the interpretation of data by reversible second-order kinetics (Chapter 5)		8.8×10^{-13}

The von Smoluchowski equation is derived for a particle diffusing to a stationary sphere. An agitated system was used in the adsorption studies. The experimental adsorption rate, therefore, would be expected to be greater than the predicted value. Three conditions do give a theoretical rate less than the experimental value of 8.8×10^{-13} cm^3/virus particle-sec (Table 6-2). These three rates were obtained by representing the site areas by cross-sectional areas of the virus particle. Since the tail fibers attach to carboxyl groups on host cells, the expected site areas are the head cross section or the extended tail fibers. The first area would occur when there is a great abundance of carboxyl groups on the adsorbent's surface and the latter for a limited number. The head cross-sectional area and the tail-fiber area gave adsorption rates of 0.2×10^{-13} and 6.2×10^{-13} cm^3/virus particle-sec, respectively. These values are approximately 1/40 and 3/4 of the observed experimental adsorption rate. Agitation of the adsorption system may account for this difference between the experimental and predicted values. The adsorption rate obtained from the site area found by dividing available carbon area by its capacity for viruses also gives agreement within less than an order of magnitude. The theoretical value is 4.5 times greater than the experimental value, which is not an unreasonable difference, considering the approximations and assumptions in the calculations.

The above calculations were made for diffusion of a virus to a specific carbon site resulting in adsorption. These calculations provide a very simple representation of adsorption. The results indicate that the maximum rate of adsorption is in the range of a diffusion-

limited process. If diffusion is limiting then the proper mathematical representation would involve a development of the diffusion equation for reversible adsorption--not reversible second-order kinetics.

6-1 Mathematical Representation of Diffusion to a Particle and Adsorption According to the Langmuir Isotherm

The rate of uptake for simple diffusion to a spherical particle is represented by the von Smoluchowski equation. If the adsorption process is diffusion limited, chemical equilibrium exists at the carbon-solution interface. Hence a virus back-pressure is exerted at the surface. This back pressure is proportional to the fraction of sites utilized and increases during adsorption until adsorption ceases. The back pressure can be incorporated into the diffusion equation with the aid of the Langmuir isotherm. As noted above, the diffusion equation is represented by

$$\frac{dn_a}{dt} = -4\pi\, aDN\,(n_\infty - n_s). \qquad (6\text{-}2)$$

The expression for n_s, the virus concentration in solution at the carbon surface, can be obtained from the Langmuir equation. From the Langmuir equation the following relation is obtained (Appendix 3).

$$q = \frac{KZc_e}{1 + Kc_e} \qquad (6\text{-}3)$$

Where q is the number of virus particles adsorbed per milligram of carbon, K is the equilibrium constant, c_e is the virus concentration in solution at equilibrium, and Z is the number of sites per milligram of carbon. At equilibrium c_e is equivalent to n_s. If C_c is the concen-

tration of carbon (mg/ml), then the surface concentration of adsorbed virus particles, n_a, can be expressed as $n_a = q\ C_c$. The initial site concentration, likewise, is $c_s = ZC_c$. Substitution into equation (6-3) and solving for n_s yields:

$$n_s = \frac{n_a}{K(c_s - n_a)} \qquad (6\text{-}4)$$

The virus concentration in the bulk of the solution, n_∞, plus the concentration of adsorbed viruses, n_a, must equal the virus concentration at zero time in the bulk solution.

$$n_\infty + n_a = n_{\infty o} \qquad (6\text{-}5)$$

Substituting expressions (6-4) and (6-5) into equation (6-2) gives the rate of virus adsorption to N carbon particle of radius a as the net result of a driving force and back pressure.

$$\frac{dn_a}{dt} = -4\pi\ DNa \left[n_{\infty o} - n_a - \frac{n_a}{K(c_s - n_a)} \right] \qquad (6\text{-}6)$$

Rearranging and letting $k = -4\pi\ DNa$ the following expression is obtained and integrated as shown below.

$$\frac{K(c_s - n_a)dn_a}{(c_s - n_a)(n_{\infty o} - n_a)K - n_a} = kdt \qquad (6\text{-}7)$$

(6-8)

$$kdt = Kc_s \int_o^{n_a} \frac{dn_a}{(c_s - n_a)(n_{\infty o} - n_a)K - n_a} - K \int_o^{n_a} \frac{n_a\, dn}{(c_s - n_a)(n_{\infty o} - n_a)K - n_a}$$

The denominator is of the quadratic form $\alpha + \beta X + \gamma X^2$, and by reference to a table of integrals, equation (6-8) can be integrated as follows:

$$\int \frac{dx}{\alpha+\beta X+\gamma X} = \frac{1}{\sqrt{p}} \ln\left[\frac{2\gamma X+\beta-\sqrt{p}}{2\gamma X+\beta+\sqrt{p}}\right] \qquad (6\text{-}9)$$

and (6-10)

$$\int \frac{xdx}{\alpha+\beta X+\gamma X^2} = \frac{1}{2\gamma} \ln(\alpha+\beta X+\gamma X^2) - \frac{\beta}{2\gamma}\int \frac{dx}{\alpha+\beta X+\gamma X^2}$$

where $\sqrt{p} = (\beta^2-4\alpha\gamma)^{\frac{1}{2}}$ (6-11)

Quantity p must always be positive. Rearranging the denominator to the quadratic form,

$$c_s n_{\infty_o} K + (-c_s K - n_{\infty_o} K - 1) n_a + K n_a^2 \qquad (6\text{-}12)$$

the following values of α , β , and γ are obtained.

$$\alpha = c_s n_{\infty_o} K$$

$$\beta = (-c_s K - n_{\infty_o} K - 1)$$

$$\gamma = K$$

$$\sqrt{p} = \left[(c_s K - n_{\infty_o} K)^2 + 2 n_{\infty_o} K + 2 c_s K + 1\right]^{\frac{1}{2}} \qquad (6\text{-}13)$$

By substitution, the quantity p is found to be positive under all conditions. This implies that the quadratic expression $\alpha + \beta X + \gamma X^2$ has real roots.

From the integration one obtains:

$$\frac{Kc_s}{\sqrt{p}} \ln\left[\frac{2Kn_a + (-c_s K - n_{\infty_o} K - 1) - \sqrt{p}}{2 K n_a + (-c_s K - n_{\infty_o} K - 1) + \sqrt{p}}\right]$$

$$- 1/2 \ln\left[c_s n_{\infty_o} K + (-c_s K - n_{\infty_o} K - 1) n_a + K n_a^2\right] \qquad (6\text{-}14)$$

$$+\left[\frac{(-c_s K - n_{\infty_o} K - 1)}{2K}\right] \frac{K}{\sqrt{p}} \ln\left[\frac{2 K n_a + (-c_s K - n_{\infty_o} K - 1) - \sqrt{p}}{2 K n_a + (-c_s L = n_{\infty_o} K - 1) + \sqrt{p}}\right]$$

$$= Kt+c$$

Solving for the integration constant C, by means of the condtion that at t = o, n_a = o, one obtains:

$$C = \frac{Kc_s}{\sqrt{p}} \ln\left[\frac{(-c_s K - n_{\infty_o}K - 1)-\sqrt{p}}{(-c_s K - n_{\infty_o}K - 1)+\sqrt{p}}\right] - 1/2 \ln (c_s n_{\infty_o}K) \quad (6\text{-}15)$$

$$+\left[\frac{(-c_s K - n_{\infty_o}K - 1)}{2}\right] \frac{1}{\sqrt{p}} \ln\left[\frac{(-c_s K - n_{\infty_o}K - 1)-\sqrt{p}}{(-c_s K - n_{\infty_o}K - 1)+\sqrt{p}}\right]$$

For simplicity let:

$$R = \frac{c_s K + n_{\infty_o}K + 1 + \sqrt{p}}{2K} \quad (6\text{-}16)$$

and

$$P = \frac{c_s K + n_{\infty_o}K + 1 - \sqrt{p}}{2K} \quad (6\text{-}17)$$

By substituting C, R, and P and rearranging terms one obtains the integrated form of equation (6-8).

$$1/2 (Kc_s - n_{\infty_o}K - 1) \frac{1}{\sqrt{p}}\left[\ln \frac{n_a - R}{n_a - P} - \ln \frac{R}{P}\right] \quad (6\text{-}18)$$

$$+ 1/2 \ln \frac{c_s n_{\infty_o}K}{c_s n_{\infty_o}K - (c_s K + n_{\infty_o}K + 1) n_a + K n_a^2} = kt$$

Equation (6-18) represents adsorption to a stationary spherical particle where the maximum rate is limited by diffusion of the virus to the adsorbent's surface. Adsorption must correspond to the Langmuir isotherm. An equilibrium condition exists where the rate of adsorption, as controlled by diffusion is balanced by desorption as predicted by the Langmuir equation.

6-2 Interpretation of Data by Diffusion Equation for Langmuir Adsorption

The application of this mathematical model to the adsorption data can be tested by plotting terms on the left side of equation (6-18) versus t. For a diffusion-controlled process a straight line should be obtained from which k can be determined. The compatibility of this representation can also be tested by a comparison of the diffusion coefficients obtained from k with the diffusion coefficient for T_4 phage as reported by other investigators.

A diffusion-limited process for a 100 percent adsorption efficiency would only be expected to occur under conditions that gave the maximum adsorption rate. Such conditions occurred at a pH of 7.0 and ionic strength of 0.08 to 0.10 as analyzed by reversible second-order kinetics. Seven adsorption tests (4-5.1 through 4-5.7) were made under optimum conditions (Table 4-5) and results presented in Table 5-13.

The amount of virus adsorbed at the measured times in tests 4-5.1 through 4-5.7 was substituted into equation (6-18) (Tables 6-3, 6-4, and 6-5). A carbon capacity and equilibrium constant of 1.6×10^9 sites/mg of carbon and 4.0×10^{-7} ml/virus particle were used in the calculations. These values were determined from the Langmuir isotherm. In test 4-5.1 a carbon capacity of 1.9×10^9 sites/mg was used. In this particular case a value of 1.6×10^9 gave a lower initial site concentration than adsorbed virus. Terms for equation (6-18) were calculated only over the initial adsorption periods. The data were not sufficiently accurate for use in equation (6-18) in the vicinity of equilibrium, since very small changes occur in the amount of adsorbed virus.

A plot of the calculated terms in Tables 6-3 through 6-5 versus t yields a fairly good linearization of the adsorption data (Figures 6-1, 6-2, and 6-3). In the application of equation (6-18) small errors in data are enlarged. Test 4-5.5 (Figure 6-1) gave the poorest linearization, however, the other tests gave a much better correlation with equation (6-18).

The constant k was determined for each test from the slopes of their graphs (Table 6-6). The value k is equivalent to -4π DNa. The experimental diffusion coefficient D can be calculated from k given a, the radius of the carbon, and N, the number of carbon particles per milliliter of reaction solution. From Figure 3-2 the fifty percent diameter of the carbon is 2.5 microns. One milligram of carbon was found to contain 10.3×10^6 particles of carbon (section 3-2-2). The experimental diffusion coefficient for T_4 phage was determined from these values and the carbon concentration for each test (Table 6-6).

Excellent agreement was obtained between the experimental diffusion coefficient and the values reported in the literature for T_4 phage. The accepted diffusion coefficient is 8×10^{-8} cm^2/sec. Using equation (6-18) an average diffusion coefficient of 12×10^{-8} cm^2/sec was obtained from the adsorption tests (Table 6-6). An experimental diffusion coefficient greater than 8×10^{-8} cm^2/sec is expected since the adsorption system was continuously mixed. Other assumptions involved with equation (6-18) will also influence the value obtained for D.

TABLE 6-3

Substitution of Data for Adsorption
Tests 4-5.1 and 4-5.2 into Equation (6-18)

Time Hr.	Virus adsorbed n_a virus particles/ml	$\frac{K c_s - n_{\infty o} K - 1}{2\sqrt{p}} \left[\ln \frac{n_a - R}{n_a - p} - \ln \frac{R}{p} \right]$	$\frac{1}{2} \ln \frac{\alpha}{\alpha + \beta n_a + \gamma n_a^2}$	kt
Test 4-5.1	($n_{\infty o} = 4.8 \times 10^8$ virus/ml, $c_s = 4.7 \times 10^8$ sites/ml, $K = 4.0 \times 10^{-7}$ ml/virus			
35 min	2.4×10^8	0.01	0.71	0.72
1 hr	2.6×10^8	0.01	0.92	0.93
1 hr, 50 min	3.3×10^8	0.03	1.20	1.23
3 hr	3.7×10^8	0.05	1.56	1.61
4.5 hr	4.2×10^8	0.11	2.36	2.47
6 hr	4.4×10^8	0.31	--	--
Test 4-5.2 ($n_{\infty o} = 10.0 \times 10^7$ virus/ml, $c_s = 8.0 \times 10^7$ sites/ml, $K = 4.0 \times 10^{-7}$ ml/virus)				
1	3.0×10^7	-0.05	0.43	0.38
3	4.3×10^7	-0.07	0.69	0.62

TABLE 6-4

Substitution of Data for Adsorption
Tests 4-5.3, 4-5.4, and 4-5.5 into Equation (6-18)

Time Hr.	Virus adsorbed n_a virus particles/ml	$\frac{Kc_s - n_{\infty_o} K - 1}{2\sqrt{p}} \left[\ln \frac{n_a - R}{n_a - p} - \ln \frac{R}{p} \right]$	$1/2 \ln \frac{\alpha}{\alpha + \beta n_a + \gamma n_a^2}$	kt
Test 4-5.3 (n_{∞_o} = 1.0 X 10^8 virus/ml, c_s = 1.6 X 10^8 sites/ml, K = 4.0^{-7} ml/virus)				
1	5.3 X 10^7	0.17	0.59	0.76
3	8.3 X 10^7	0.44	1.35	1.79
7	9.4 X 10^7	1.18	2.26	3.44
13.5	9.6 X 10^7	1.90	--	--
Test 4-5.4 (n_{∞_o} = 10.5 X 10^7 virus/ml, c_s = 4.0 X 10^7 sites/ml, K = 4.0 X 10^{-7} ml/virus)				
1	1.4 X 10^7	-0.14	0.30	0.16
3	2.5 X 10^7	-0.36	0.95	0.59
Test 4-5.5 (n_{∞_o} = 1.2 X 10^8 virus/ml, c_s = 4.0 X 10^8 sites/ml, K = 4.0 X 10^{-7} ml/virus)				
0.5	8.2 X 10^7	0.46	0.71	1.17
1	9.6 X 10^7	0.67	0.96	1.63
2	10.4 X 10^7	0.87	1.21	2.08
3	11.2 X 10^7	1.23	1.59	2.82

TABLE 6-5

Substitution of Data for Adsorption Tests 4-5.6 and 4-5.7 into Equation (6-18)

Time Hr.	Virus adsorbed n_a virus particles/ml	$\frac{Kc_s - n_{\infty_o}K - 1}{2\sqrt{p}}\left[\ln\frac{n_a - R}{n_a - p} - \ln\frac{R}{p}\right]$	$\frac{1}{2}\ln\frac{\alpha}{\alpha + \beta n_a - \gamma n_a^2}$	kt
Test 4-5.6 (n_{∞_o}= 1.5 X 10^7 virus/ml, c_s = 4.0 X 10^8 sites/ml, K = 4.0 X 10^{-7} ml/virus)				
½	9.6 X 10^6	0.49	0.53	1.02
1	11.4 X 10^6	0.68	0.74	1.42
2	13.7 X 10^6	1.10	1.28	2.38
3	14.5 X 10^6	1.66	1.81	3.47
4	14.7 X 10^6	1.91	2.28	4.19
Test 4-5.7 (n_{∞_o}= 4.7 X 10^8 virus/ml, c_s = 4.0 X 10^8 sites/ml, K = 4.0 X 10^{-7} ml/virus)				
½	2.0 X 10^8	-0.04	1.25	1.21
1	2.5 X 10^8	-0.06	1.76	1.70
3	3.8 X 10^8	-0.29	5.53	5.24

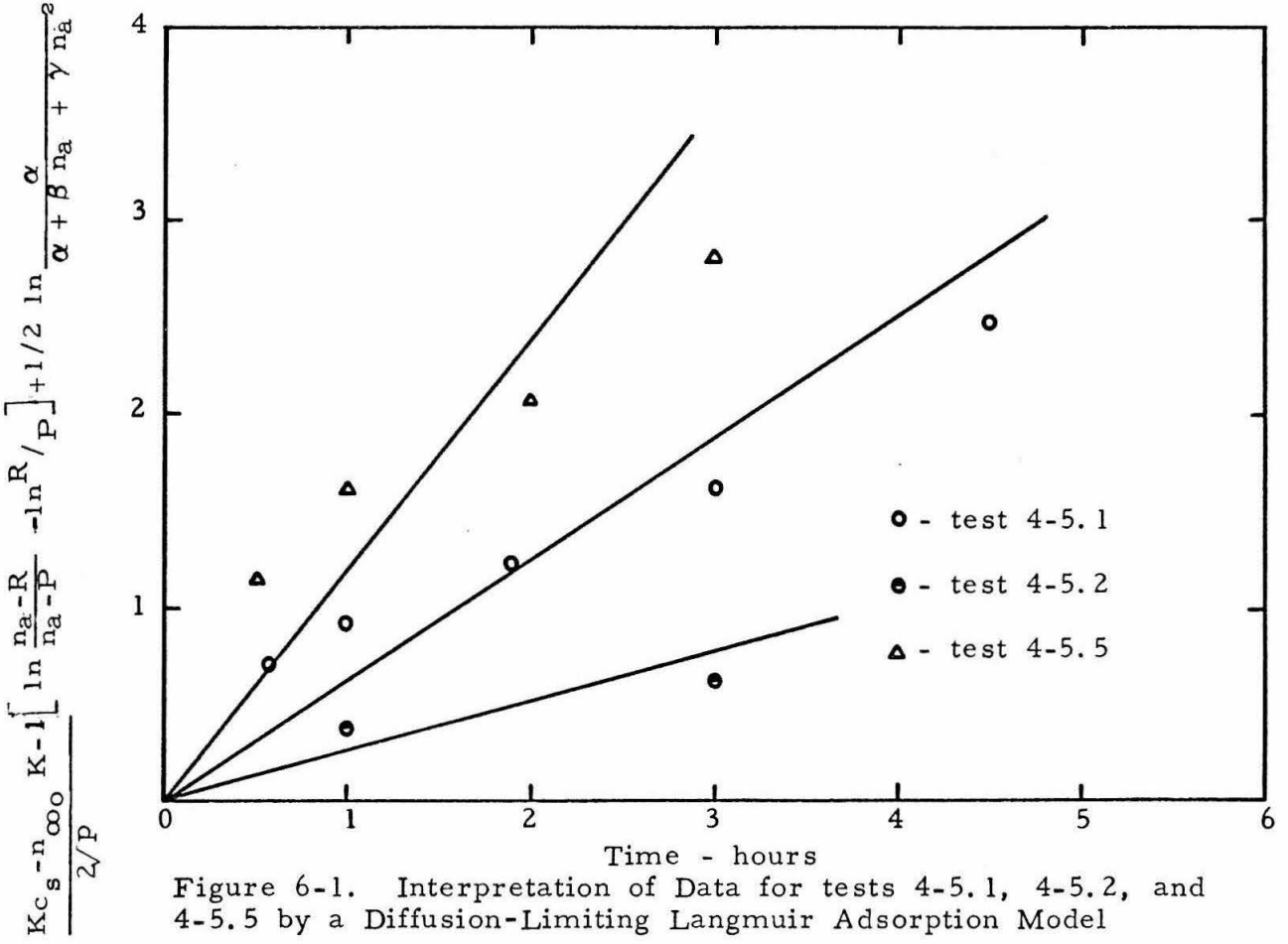


Figure 6-1. Interpretation of Data for tests 4-5.1, 4-5.2, and 4-5.5 by a Diffusion-Limiting Langmuir Adsorption Model

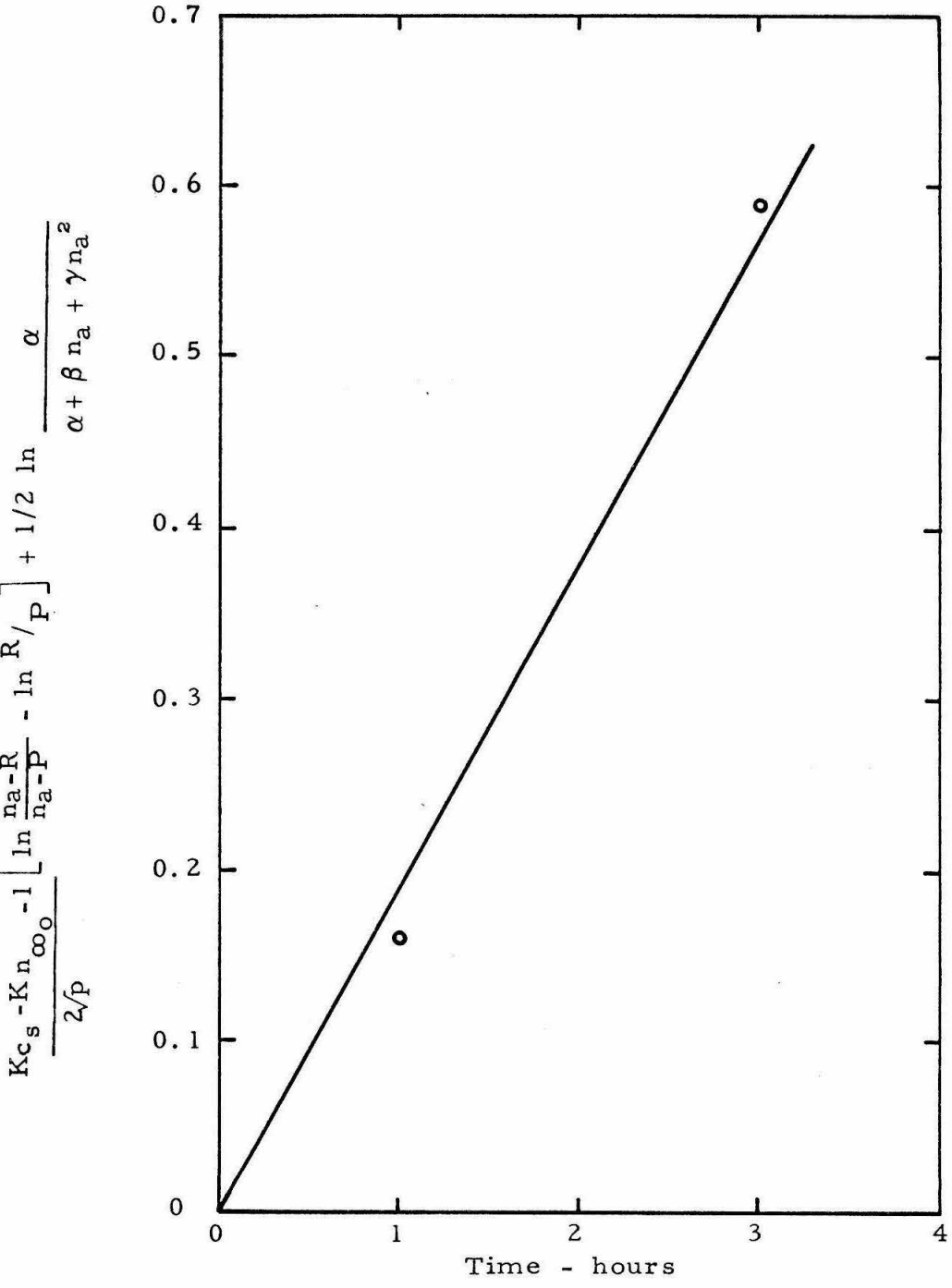


Figure 6-2. Interpretation of Data for test 4-5.4 by a Diffusion-Limiting Langmuir Adsorption Model

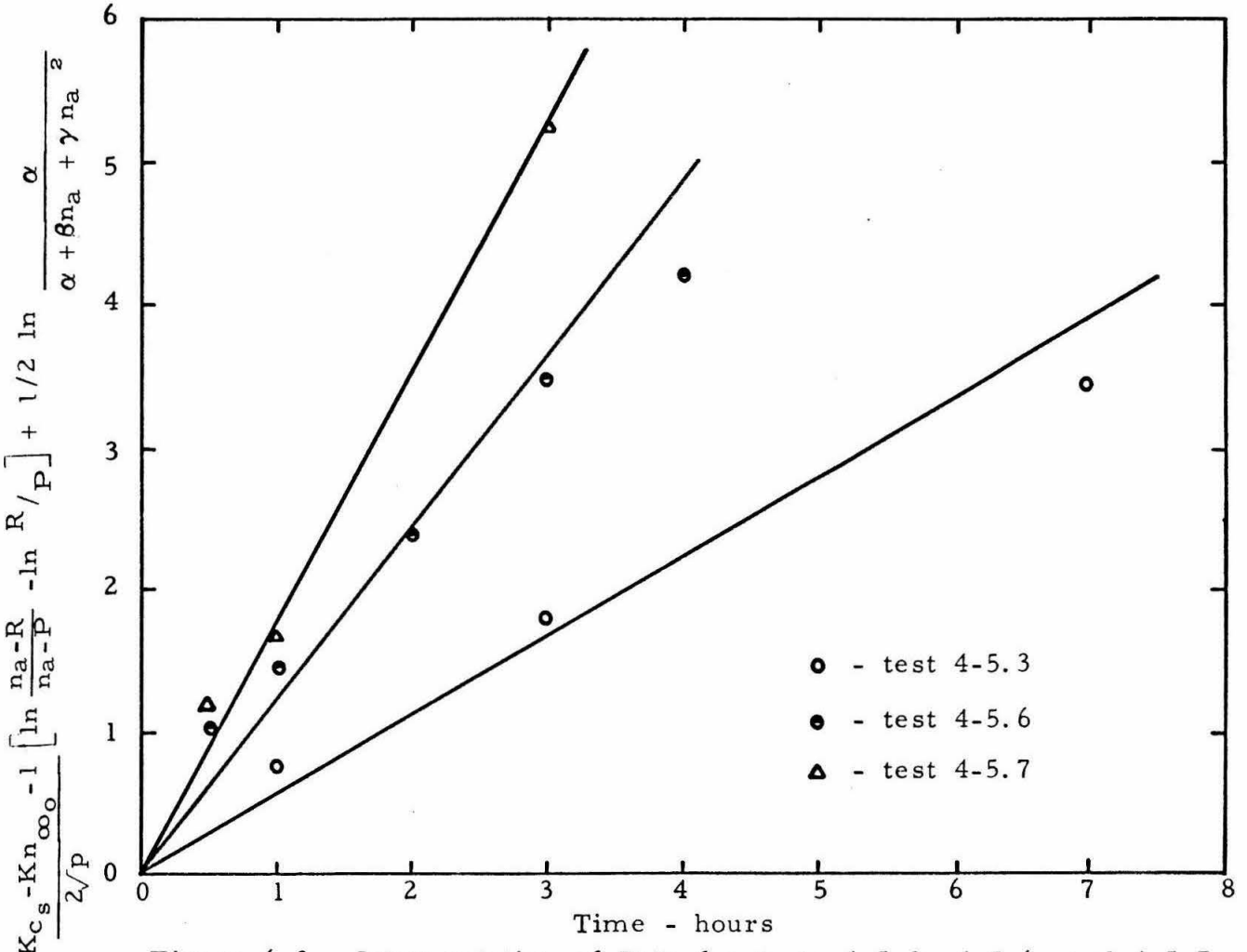


Figure 6-3. Interpretation of Data for tests 4-5.3, 4-5.6, and 4-5.7 by a Diffusion-Limiting Langmuir Adsorption Model

TABLE 6-6

Calculation of Diffusion Coefficients from Adsorption Data at pH 7.0 and Ionic Strengths 0.08 to 0.10 for T_4 Phage Adsorption to Carbon (tests 4-5.1 through 4-5.7)

Test No.	$k = 4 \pi aDN$ as obtained from plot of data 1/sec	Carbon Concentration of reaction solution C_c - mg/l	Number of carbon particles per ml of reaction solution N	Diffusion coefficient obtained from data D cm^2/sec
4-5.1	1.7×10^{-4}	250	2×10^{6}	5.6×10^{-8}
4-5.2	7.3×10^{-5}	50	4×10^{5}	11.6×10^{-8}
4-5.3	1.6×10^{-4}	100	8×10^{5}	13.4×10^{-8}
4-5.4	5.2×10^{-5}	25	2×10^{5}	16.6×10^{-8}
4-5.5	3.3×10^{-4}	250	2×10^{6}	10.6×10^{-8}
4-5.6	3.4×10^{-4}	250	2×10^{6}	10.8×10^{-8}
4-5.7	4.9×10^{-4}	250	2×10^{6}	15.6×10^{-8}

Average diffusion coefficient is 12×10^{-8} cm^2/sec

The most significant assumption concerns the use of the fifty percent diameter for activated carbon. The effective size in the adsorption systems may be several sizes greater or smaller and any means of adjustment would still be an approximation. Other assumptions are those concerned with the Langmuir isotherm as presented previously.

The good agreement between experimental and reported diffusion coefficients for the virus supports the theory of a diffusion-limited adsorption process. The agreement between the data and the combined diffusion and Langmuir isotherm equation is also good. Equation (6-18) probably represents adsorption at the optimum conditions more properly than reversible second-order kinetics.

Diffusion-limited theory may also represent adsorption at conditions other than the optimum pH and ionic strength. Adsorption rates less than the maximal have been discussed in relation to repulsive forces between the virus and carbon, configuration of viral tail fibers, and ionization of the chemical groups involved in attachment. The individual effect of each mechanism on adsorption can be analyzed by a diffusion-limited process. The actual adsorption process, however, involves complex interaction between all three of the above mentioned mechanisms.

Conditions that reduce the collision frequency of virus and carbon particles by repulsive forces can be analyzed as diffusion with a retardation factor. The retardation factor takes into account the interaction between the particles as a result of their potential energy. The potential energy of virus and carbon particles is a function of the

distance between the particles, the number of adsorbed virus particles, and the pH and ionic strength of the suspending medium. Evaluating the potential energy to characterize a retardation factor would be difficult.

The second complicating factor, (adsorption of viral-tail fibers to the tail sheath) decreases the ability of viral-tail fibers to adsorb to carbon. Under such conditions adsorption can be analyzed by the diffusion equation with an adjusted virus concentration, which would represent only that portion capable of adsorbing to carbon. The effective virus population would be a function of pH and ionic strength. In a similar manner, the influence of the ionized groups on the virus and carbon that are involved in attachment can be characterized by an efficiency factor for attachment.

The limiting process of bacteriophage T_4 adsorption to activated carbon might result from diffusion at all adsorption conditions. A clear conclusion concerning the limiting process under all these conditions cannot be made at the present time. The rate of adsorption definitely approaches that predicted by diffusion-limited theory at optimum adsorption conditions.

Intraparticle diffusion is considered the rate limiting step in adsorption to many porous adsorbents. Weber and Morris (161) have described this mechanism for adsorption of alkylbenzenesulfonate to activated carbon. In adsorption of bacteriophage T_4, diffusion to the carbon surface is probably the limiting rate.

Most pores in activated carbon are less than 1200 Å in diameter (132). Average pore diameters are about 20 Å (Table 3-1). Emmett (126) reports that large dye molecules are almost completely excluded from the pores of many charcoals. Bacteriophage T_4 can reach dimensions of 1800 X 3000 Å with tail fibers extended. Without considering tail fibers its dimensions are 650 X 1800 Å. From these considerations the virus is probably completely excluded from the pores of activated carbon. The major portion of the internal area of activated carbon is unavailable to the virus particle.

The very low surface coverage that occurs when the carbon is filled to capacity also supports the hypothesis of little use of pore area. The active sites on activated carbon for virus adsorption are carboxyl groups (section 5-10). By using analytical oxidation and X-rays, Snow and coauthors(160) found the oxygen on carbon to be on the surface or within 10 - 20 Å of the surface. The sites for viruses, therefore, appear to be only at the surface and not in capillary pores.

CHAPTER 7

CONCLUSIONS

The experimental results were presented and analyzed in Chapters 5 and 6 in view of the available information on the absorbent and adsorbate. In this chapter conclusions from the various investigations will be combined to develop a model for adsorption of Escherichia coli bacteriophage T_4 to activated carbon. Before considering the adsorption process, conclusions will be presented on the utility of the viruses and adsorbents for these studies.

7-1 Utility of Viruses for Adsorption Studies

The detailed studies were performed with Escherichia coli bacteriophage T_4 and activated carbon. Although extensive work was conducted in developing an assay procedure for poliovirus Type III, only one adsorption test was made with poliovirus. It indicated that poliovirus is readily adsorbed by activated carbon. Bacteriophage T_4 was used for most of the experiments because it had many advantages over poliovirus for adsorption studies. These advantages included more available information on the virus, better control on host-cell quality, and shorter assay time. The assay for poliovirus was very accurate compared to the usual animal virus assays but was not comparable with that of bacterial viruses.

The efficiency of plaque formation for poliovirus assay was noted to vary with the type of culture bottle, the method of staining host cells with neutral red, and the quality of the monkey kidney cells.

Cells must be of adequate quality to sustain growth through the assay period of six to ten days. This property varied with their metabolic state at initiation of the assay. Plaque formation was much better in plastic bottles than glass bottles. The glass may have had a toxic effect on the cells. Better plaque formation also occurred when cells were stained with neutral red three days after infection with virus. Earlier addition of stain appeared to exert a toxic effect.

Escherichia coli bacteriophage T_4 was very stable under most testing conditions. Greatest stability occurred at a pH of 7.0 and ionic strengths from 0.06 to 0.10. Appreciable inactivation occurred at ionic strengths of 0.02 and 0.20 and was very significant at very low and high ionic strengths of 0.003 and 0.83. The stability of the virus dropped sharply at pH values less than 6 and higher than 9. No change in the adsorption of virus was noted with aging of the stock solutions.

The plaque-forming efficiency of bacteriophage T_4 varied significantly with the metabolic state of the host cells and the time allowed for synthesis of virus progency. Host cells in the log growth phase yielded three times as many plaques as cells in the endogenous growth phase. The greatest number of plaques were found at 10 to 14 hours of incubation. At earlier times plaque formation was not complete. Incubation longer than 14 hours appeared to result in some destruction of plaques by antagonistic properties of the host cell.

7-2 Adsorbent Comparisons

Preliminary adsorption studies were made with diatomaceous earth, Ottawa sand, and coconut charcoal. Diatomaceous earth and Ottawa sand had extremely few, if any, adsorption sites for bacteriophage T_4. It has been proposed above that the adsorption sites on activated carbon are carboxyl groups. The inability of the siliceous materials to adsorb virus was probably due to the lack of carboxyl groups and charges for forming electrostatic bonds with viruses.

Coconut charcoal did adsorb viruses but its capacity was very low. The carbon was not activated or oxidized and would not be expected to have many carboxyl groups.

7-3 Experimental Procedures

Adsorption of bacteriophage T_4 to activated carbon was conducted in a batch mixing process. Adsorption in an activated-carbon column was also investigated, but this process presented difficulties with bacterial contamination and the minute amount of carbon that had to be used to obtain a detectable quantity of virus in the filtrate. Although the batch procedure cannot be defined as well hydraulically as the column process, it offered distinct advantages in working with viruses. The batch procedure was better for studying attachment of viruses to carbon and the effect of this attachment on the virus's infectivity.

In using the batch procedure, carbon was separated from the assay sample by settling or centrifuging. Centrifugation of T_4 phage at 5000 rpm in the presence of carbon caused a significant decrease in virus

titer of the supernatant. Carbon was satisfactorily removed without affecting virus titer by settling for 5 minutes or centrifuging for only 3 minutes.

Preliminary studies with carbon columns indicated complete removal of the virus by the upper layers of carbon. Diatomaceous earth was found to remove some viruses when placed above the carbon in a column arrangement. The mechanism was thought not to be adsorption since diatomaceous earth caused no removal in the batch process.

7-4 Kinetics and Mechanism of Adsorption of T_4 Phage to Activated Carbon

The kinetics of bacteriophage T_4 adsorption to activated carbon can be described by a reversible second-order reaction. The reaction order was first order with respect to both virus concentration and carbon sites. This kinetic representation, however, is probably incorrect at optimum adsorption conditions. At optimum conditions, which occurred at pH 7.0 and ionic strength of 0.08, the rate of adsorption was comparable to a diffusion-limited process.

Adsorption was thermodynamically reversible. The same equilibrium was obtained by either adsorption or desorption. This phenomenon is characteristic of physical adsorption. Physical adsorption was also supported by the lack of temperature effect on adsorption. A variation of 10°C in temperature gave no significant change in adsorption rate. The reversible nature of the adsorption process was also demonstrated by desorption in the presence of a competitive adsorbate. A greater

degree of desorption was obtained in the presence of tryptone. The adsorbed state is favored over that of desorption. In the presence of tryptone the equilibrium was shifted towards a state of greater desorption. Without a competitive adsorbate the equilibrium condition favors adsorption to such a great extent that the data gave close agreement with an irreversible second-order kinetic system.

The ability of virus particles to be desorbed from sites on activated carbon is very significant. A chemical reaction does not take place. This phenomenon indicates that the virus was not inactivated by adsorption. Electrostatic forces are responsible for adsorption to carbon, and they are very similar to those involved in adsorption to host cells. Some investigators have speculated that the injection of viral DNA into host cells resulted from the electrostatic adsorption forces. Ejection of viral DNA from its protein coat did not occur in these adsorption studies with bacteriophage T_4. If ejection occurred reversible adsorption would not have been detected by the assay procedure.

Adsorption of bacteriophage T_4 to activated carbon can be represented by the Langmuir isotherm. Agreement with this isotherm indicates that adsorption forms a unimolecular layer. All carbon sites must also be reasonably equal in their adsorbing ability. Both of these conclusions were supported by other experiments.

Formation of a unimolecular layer rather than a multilayer was indicated by the small surface coverage of carbon by the virus. The application of the Langmuir isotherm and kinetic equations yielded a

carbon capacity of 1.6×10^9 and 1.9×10^9 sites/mg respectively. From the largest cross-sectional area of a virus particle, the maximum surface coverage of the adsorbent is only 18 percent. This small surface coverage not only suggests the lack of multilayer adsorption, but it also shows the likely insignificance of pore area. Bacteriophage T_4 is probably completely excluded from pores owing to its size.

The equal adsorption ability of carbon sites was also illustrated by kinetic studies. Using reversible second-order kinetics the same rate of adsorption was obtained with carbon having all sites available and carbon which had previously reduced its sites 80 percent by adsorbing viruses. If some sites displayed a lower affinity for viruses they would have constituted the major portion of the remaining 20 percent. Adsorption to these sites, therefore, would give a lower second-order rate.

The adsorption sites on activated carbon for bacteriophage T_4 probably exist in the form of a carboxyl group or lactone. Adsorption to carbon can be completely blocked by esterifying these groups. Sites of this nature make the adsorption process similar to adsorption on host cells. Thus, tail fibers of the phage are the likely adsorbing group to activated carbon. This hypothesis was supported by the influence of pH and ionic strength on adsorption. Both pH and ionic strength displayed a maximum adsorption rate.

It is proposed that amino groups on the virus adsorb to carboxyl groups on the carbon by electrostatic attraction. The pH dependence of adsorption rules out the participation of ionized forms of sulfhydryl,

guanidino, and phenolic groups in the electrostatic binding. Ionized imidazolyl groups are probably too few to be significant.

As pH decreases from the optimum of 7.0 to 6.0, adsorption rate decreases, probably from the decrease in ionized carboxyl groups on the carbon. At still lower pH levels a second hindering effect occurs when viral tail fibers start attaching to the tail sheath, making them unavailable for attachment to carbon sites.

At pH values higher than optimum the tail fibers of bacteriophage T_4 are extended. In this case the decrease in adsorption rate presumably resulted from the decrease in the ionized form of the amino groups on the virus. The removal of ionized amino groups prevents electrostatic interactions between viral amino groups and carboxyl sites on carbon. It also gives the virus a greater net negative charge which causes greater repulsive forces towards the negative carbon particle. The collision frequency, therefore, is reduced.

This mechanism for adsorption can be divided into four pH zones as given below. Each zone differs by site characteristics and collision frequency.

Zone A- pH values less than 6.0 (Poor adsorption)

1. Tail fibers are not free to attach to carbon sites.
2. Carboxyl groups are not ionized.
3. Amino groups are ionized.

Zone B- pH values from 6.0 to 7.0 (Fair adsorption)

1. Tail fibers are free to attach to carbon sites.
2. Some carboxyl groups are ionized.
3. Amino groups are ionized.

Zone C- pH value of 7.0 (Maximum adsorption)

1. Tail fibers are free to attach to carbon sites.
2. Both carboxyl and amino groups are ionized.

Zone D- pH values greater than 7.0 (Poor adsorption)

1. Tail fibers are free to attach to carbon sites.
2. Amino groups are not ionized.
3. Carboxyl groups are ionized.
4. Collision frequency is reduced because of greater negative charge on both virus and carbon.

The electrostatic binding of virus and carbon and the designation of viral tail fibers as the adsorbing units were supported by the effect of ionic strength on adsorption. Ionic strength appears to influence adsorption by two mechanisms. One mechanism aids adsorption with increasing ionic strength and the other hinders it. This condition results in an optimum ionic strength of 0.08.

With increasing ionic strength adsorption is aided by the masking of like charges on the virus and carbon particles, reducing the repulsive forces. This condition increases the collision frequency between viral and carbon particles. Ionic strength also reduces repulsive forces between the virus's tail fibers and tail sheath. With increasing ionic strength more and more tail fibers adsorb to the tail sheath. Although high ionic strengths cause a maximum collision frequency between virus and carbon particles, the adsorbing units on the virus (tail fibers) are not available. These two mechanisms were also supported by equilibrium studies at high and low ionic strengths.

At a high ionic strength a significant portion of the virus population could not adsorb. Whereas at low ionic strength they did adsorb, although very slowly owing to a reduced collision frequency.

The proposed mechanism for ionic strength effect on adsorption can be characterized by three zones of ionic strengths.

Zone A- Ionic strengths less than optimum (Poor adsorption)

1. Tail fibers are free to adsorb to carbon sites.
2. Collision frequency is low because of repulsive forces between carbon and virus.

Zone B- Optimum ionic strength (Maximum adsorption)

1. Optimum combination of adequate number of free tail fibers and collision frequency occurs.

Zone C- Ionic strengths greater than optimum (Poor adsorption)

1. Tail fibers are not free to adsorb to carbon sites.
2. Maximum collision frequency occurs.

Adsorption rate at the optimum ionic strength and pH occurs at or very near that of a diffusion-limiting process. At the maximum adsorption rate, data were equally well represented by reversible second-order kinetics or by a diffusion-limited representation for Langmuir adsorption. The diffusion representation yielded an experimental diffusion coefficient of 12×10^{-8} cm^2/sec. The accepted value for bacteriophage T_4 is 8×10^{-8} cm^2/sec. This comparison indicates that the rate of adsorption approaches limitation by diffusion. Thus, reversible second-order kinetics could be an incorrect representation of adsorption. A diffusion-limited process can also occur at adsorption

conditions other than maximal. A clear conclusion concerning the limiting process cannot be made. Intraparticle diffusion in the pores of activated carbon is unlikely. The limiting process would occur at the carbon's surface.

CHAPTER 8

APPENDICES

Appendix 1 - Preparation of Media for Escherichia coli Bacteriophage T_4

Tryptone Bottom Agar:
(Constituents per liter of water)

10.0 gm of dehydrated tryptone
10.0 gm of agar
5.0 gm of NaCl

Tryptone Top Agar:
(Constituents per liter of water)

10.0 gm of dehydrated tryptone
6.5 gm of agar
5.0 gm of NaCl

Tryptone Broth:
(Constituents per liter of water)

10.0 gm of dehydrated tryptone
5.0 gm of NaCl

Appendix 2 - Preparation of Media for Poliovirus Type III

LaYe Medium (Lactalbumin hydrolysate-yeast extract)
(Constituents per liter)

5.0 gm of Lactalbumin hydrolysate
1.0 gm of Yeast extract (Difco)
980.0 ml of Hanks BSS with bicarbonate
20.0 ml of Calf serum
0.2 gm of streptomycin
0.1 gm of kanomycin
50.0 mg of fungizone
0.2 units of penicillin

Hanks Balanced Salt Solution (Hanks BSS)
(Constituents per liter of deionized water)

8.00 gm of NaCl
0.40 gm of KCl
0.20 gm of $MgSO_4 \cdot 7H_2O$
0.06 gm of KH_2PO_4
0.09 gm of $Na_2HPO_4 \cdot 7H_2O$
0.14 gm of $CaCl_2$
1.00 gm of Glucose
0.05 mg of $NaHCO_3$
0.02 gm of Phenol Red
Adjusted to pH 7.5 with bicarbonate

White Agar:

1.3 gm of Difco Nobel agar
55.0 ml of deionized water

Red Agar:

2.6 gm of Difco Nobel agar
11.0 ml of 10 X Neutral Red solution
99.0 ml of deionized water

10 X Neutral Red solution contains
0.3 percent Neutral Red by weight
in deionized water.

Appendix 3 - Derivation of Langmuir Isotherm

The Langmuir isotherm represents an equilibrium condition between adsorption and desorption. The adsorbent may be regarded as providing discrete sites that are considered equal in their ability to adsorb. Each site adsorbs only one molecule, resulting in a unimolecular layer.

The rate of adsorption is proportional to the concentration of available sites and the adsorbate. The rate of desorption is proportional to the concentration of adsorbed molecules. The equilibrium condition can be represented by

$$k_1 (Z - q) c_e = k_2 q.$$

where:

Z = the number of sites/mg of adsorbent

q = the number of adsorbed molecules per mg of adsorbent

c_e = the concentration of solute in solution at equilibrium

k_1 = the forward rate constant

k_2 = the reverse rate constant

Solving for q one obtains:

$$q = \frac{k_1/k_2(c_e\ Z)}{1 + (k_1/k_2)c_e}$$

The ratio of the two rate constants, k_1/k_2, is the equilibrium constant and will be defined by K.

Therefore:

$$q = \frac{K\, c_e\, Z}{1 + K\, c_e}$$

The equation can be plotted as a straight line in the form

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

Appendix 4 - Ratio of Mono- to Dipotassium Phosphate and Corresponding pH in the Presence of 250 mg/l of Activated Carbon and 1 ml of Stock Virus Solution

Both mono- and dipotassium phospate were titrated against each other in a solution containing 250 mg/l of carbon and 1.0 ml of stock virus solution. The carbon and virus concentrations were representative of the test solutions for studying pH effects. The designated pH for a reaction solution was obtained by using the buffer composition given by the titration results (Table 8-1 and Figure 8-1).

Appendix 5 - Derivation of Second-order Reaction Equations

Irreversible Equation:

$$a_o + b_o \xrightarrow{k_1} X$$

Let the initial concentrations of reactants at t = 0 be a_o and b_o. The rate of change of X is

$$\frac{dX}{dt} = k_1\ ab.$$

where k_1 is the rate constant. In this case X is the amount adsorbed. Since $a = a_o - X$ and $b = b_o - X$ we have

$$\frac{dX}{dt} = k_1\ (a_o - X)\ (b_o - X).$$

TABLE 8-1

Ratio of K_1HPO_4 to K_2HPO_4 and Corresponding pH in a Solution Containing 250 mg/l of Carbon and 1.0 ml of Stock Virus Solution

KH_2PO_4 ml	K_2HPO_4 ml	$\frac{KH_2PO_4}{K_2HPO_4}$	pH
10.0	0.0		4.40
10.0	0.2	50.0	5.00
10.0	0.5	20.0	5.40
10.0	1.2	8.3	5.80
10.0	2.3	4.4	6.10
10.0	3.9	2.6	6.30
10.0	7.3	1.4	6.60
10.0	12.5	0.8	6.80
10.0	19.3	0.5	7.00
10.0	25.0	0.4	7.10
0.0	10.0		8.50
0.3	10.0	0.03	8.15
0.4	10.0	0.04	8.00
1.8	10.0	0.18	7.40
3.1	10.0	0.31	7.20
5.1	10.0	0.51	7.00
11.7	10.0	1.17	6.60
20.0	10.0	2.00	6.40
25.0	10.0	2.50	6.30

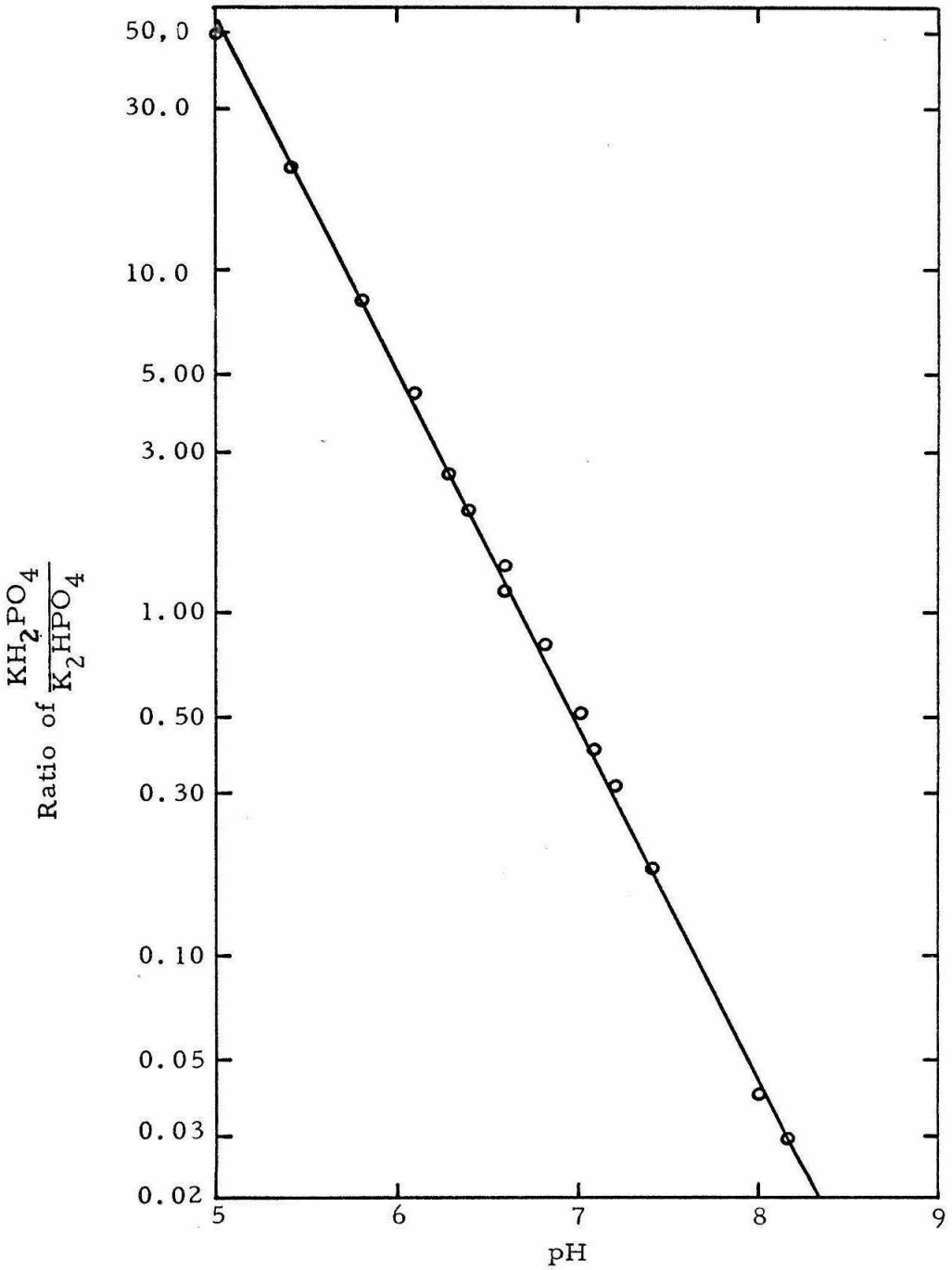


Figure 8-1. Ratio of KH_2PO_4 to K_2HPO_4 in the Presence of 1 ml of Stock Virus Solution and 250 mg/l of Activated Carbon vs. pH

Separating the variable yields

$$\frac{dX}{(a_o - X)(b_o - X)} = k_1 \, dt$$

integration yields

$$\frac{1}{a_o - b_o} \ln \frac{a_o - X}{b_o - X} = k_1 t + \text{constant}$$

When $t = 0$ and $X = 0$, the constant equals $1/a_o - b_o \, (\ln a_o/b_o)$ and the integrated form is

$$\ln \frac{a_o - X}{b_o - X} = (a_o - b_o) \, k_1 t + \ln a_o/b_o$$

Substituting a and b for $a_o - X$ and $b_o - X$ we have

$$\ln a/b = (a_o - b_o) \, k_1 t + \ln a_o/b_o$$

Reversible Equation:

$$a_o + b_o \underset{k_2}{\overset{k_1}{\rightleftharpoons}} X$$

The rate of formation of X, which is the amount of virus adsorbed is

$$\frac{dX}{dt} = k_1 (a_o - X)(b_o - X) - k_2 X \qquad (1)$$

Introducing the equilibrium constant $K = k_1/k_2$ and grouping terms with like powers of X we obtain

$$\frac{dX}{dt} = k_1 a_o b_o - k_1 (a_o + b_o + K^{-1}) X + k_1 X^2$$

Separating the variables, we have

$$\frac{dX}{a_o b_o - (a_o + b_o + K^{-1}) X + X^2} = k_1 \, dt \qquad (2)$$

The denominator is of the quadratic form $\alpha + \beta X + \gamma X^2$, and from a table of integrals the expression can be integrated as follows:

$$\int \frac{dX}{\alpha + \beta X + \gamma X^2} = \frac{1}{\sqrt{p}} \ln \left[\frac{2\gamma X + \beta - \sqrt{p}}{2\gamma X + \beta + \sqrt{p}} \right]$$

where $\sqrt{p} = (\beta^2 - 4\alpha\gamma)^{\frac{1}{2}}$

The quantity p is found to be positive under all conditions.

$$\sqrt{p} = k_1 \left[(a_o - b_o)^2 + K^{-1} (2a_o + 2b_o + K^{-1}) \right]^{\frac{1}{2}} \quad (3)$$

Letting m equal the square root term on the right side, we have

$$\sqrt{p} = k_1 m$$

The integration of equation (2) yields

$$\frac{1}{k_1 m} \ln \frac{2X - (a_o + b_o + K^{-1}) - m}{2X - (a_o + b_o + K^{-1}) + m} = t + \text{constant}$$

when $t = 0$, $X = 0$, and the constant equals

$$\frac{1}{k_1 m} \ln \frac{a_o + b_o + K^{-1} - m}{a_o + b_o + K^{-1} + m}$$

the integrated form is

$$t k_1 m = \ln \left[\frac{0.5(a_o + b_o + K^{-1} + m) - X}{0.5(a_o + b_o + K^{-1} - m) - X} \right] \left[\frac{a_o + b_o + K^{-1} - m}{a_o + b_o + K^{-1} + m} \right]$$

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