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VIRUS REMOVAL BY CHEMICAL COAGULATION

by

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and
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ABSTRACT

VIRUS REMOVAL BY CHEMICAL COAGULATION

Using bacterial viruses (Bacteriophages T4 and MS2 against Escherichia coli) as models and aluminum as the coagulant metal ion, it was shown that removal of viruses from water by chemical coagulation and flocculation with aluminum sulfate consists of a primary reaction step which possibly results in the formation of coordination complexes between aluminum and carboxyl groups on the virus coat protein. The complexed viruses were not inactivated and active viruses could be recovered from the settled floc following their removal from water by coagulation and flocculation.

The process of chemical coagulation and flocculation was found quite effective in removing bacteriophages T4 and MS2 from water. The optimum coagulant dosages and pH values were 40 to 50 mg/l of aluminum sulfate at pH 5.24 for bacteriophage T4 and at pH 6.0 for bacteriophage MS2. The highest removals attained were 98.0 and 99.9 percent, respectively. Presence of bivalent cations like calcium and magnesium up to a concentration of 50 mg/l each did not interfere with the efficiency of the process. Organic matter like albumins, wastewater and wastewater effluent lowered the removal efficiency significantly. Commercially available cationic polyelectrolytes were found effective both as coagulant aids and as prime coagulants.

Engelbrecht, R. S. and Malay Chaudhuri
VIRUS REMOVAL BY CHEMICAL COAGULATION
DESCRIPTORS---*coagulation/*flocculation/bacteriophage/*aluminum/tertiary treatment
IDENTIFIERS---/virus removal/T4/MS2/coordination complex/polyelectrolytes
## CONTENTS

1. INTRODUCTION .................................. 1

2. LITERATURE REVIEW ............................ 2
   A. Removal of Viruses by Coagulation and Flocculation ... 2
   B. Aqueous Chemistry of Aluminum .......................... 6

3. SCOPE OF THE INVESTIGATION ...................... 6

4. MATERIALS AND METHODS .......................... 8
   A. Materials ........................................ 8
       (1) Viruses and preparation ........................ 8
           a. Escherichia coli Bacteriophage T4 ........... 9
           b. Escherichia coli Bacteriophage MS2 .......... 11
       (2) Biological media .............................. 11
       (3) Coagulant and polyelectrolytes (coagulant aids) .... 13
       (4) Water ........................................ 13
       (5) Organic Materials .............................. 13
       (6) Glassware .................................... 14
   B. Chemical and Biological Assay Techniques .......... 14
       (1) Determination of aluminum ..................... 14
       (2) Assay procedure for bacteriophages ............. 14
   C. Experimental Technique and Equipment ............... 14
       (1) Kinetics of adsorption of aluminum by viruses ... 14
       (2) Virus inactivation studies ..................... 17
       (3) Quantitative studies on adsorption of aluminum
           by viruses ..................................... 17
           a. Studies on adsorption of aluminum by viruses .... 17
           b. Aluminum saturation curves .................... 17
       (4) Quantitative studies on virus removal by chemical
           coagulation and flocculation (jar tests) .......... 18
       (5) Virus recovery from settled floc ................ 21

5. RESULTS AND DISCUSSION .......................... 21
   A. Adsorption of Aluminum by Viruses ................. 22
       (1) Kinetics of adsorption ........................ 22
       (2) Quantitative adsorption ....................... 25
   B. Virus Inactivation by Aluminum ..................... 37
   C. Virus Removal by Chemical Coagulation and Flocculation 40
       (1) Optimum pH and aluminum sulfate dosages ......... 40
       (2) Effect of calcium and magnesium on virus removal ... 41
1. INTRODUCTION

The need of processes for the removal or inactivation of water-borne viruses has been demonstrated beyond question by virus transmission in drinking water and polluted streams. Because of the decrease in the available water supplies, the potential health hazard of viral pollution is increasing, and more knowledge on the survival and removal of viruses in water and wastewater treatment processes is needed.

Viruses have been demonstrated to be the causative agents of a wide variety of diseases. Polio, Coxsackie, Infectious Hepatitis, Echo, and Adenoviruses have all been demonstrated in the feces of infected humans (Clarke et al., 1964). Of greater significance is the fact that a great many of these viruses may be found in wastewater treatment plant effluents (Kelly and Sanderson, 1959). Enteric viruses have been found in raw wastewater and treatment plant effluents by a number of investigators (Paul, Trask and Gard, 1940; Paul and Trask, 1942; Clarke et al., 1951; Kelly, Winsser and Winkelstein, 1957; Mack et al., 1958; Carlson, Ridenour and McKann, 1943; and Bancroft, Engelhard and Evans, 1957).

The presence of these organisms in water supplies, then, is certainly possible. Although water is far from being an ideal medium for sustaining viruses, they can exist in it for substantial periods of time. In Paris, France, more than six virus isolations have been made from tap water in at least four different sections of the city (Berg, 1964). Water has been suspected as being the mode of transmission in outbreaks of several virus diseases. At least four epidemics plus numerous lesser outbreaks of viral diseases have been attributed to water-borne viruses (Bancroft, Engelhard and Evans, 1957; Dennis, 1959; Hayward, 1946; Little, 1954; and Standly and Eliassen, 1960). Hudson (1962), in attempting to correlate drinking water quality with the incidence of infectious hepatitis, found that a water supply that met all existing standards could still transmit these diseases. This would indicate that a specific virus quality control or standard should be established for drinking water supplies (Hartung, 1961). Before this can logically be accomplished, more knowledge should be gained on the effectiveness of presently used water and wastewater treatment methods for the removal of viruses and the epidemiology of water-borne viral diseases.

Although the process of chemical coagulation and flocculation has been used for many years in the treatment of water supplies, little is known of the basic physico-chemical principles involved in the removal of dead, as well as living, organic matter from the treated water. Chemical coagulation and flocculation is applied in various forms in a modern water treatment plant to produce a safe and potable water. However, little information is available about the basic mechanisms and kinetics involved in the removal of viruses by this unit process. Virus removals ranging from 25 to 99 percent have been reported by different workers (Senn et al., 1961). Understanding the mechanism by which viruses, which behave as typical proteins in water, are removed from water by the process of chemical coagulation and flocculation is important in optimizing its use.
of virus particles (bacteriophage T2) adsorbed on clay particles when aluminum salt was present in the system instead of calcium or sodium salts. However, no attempt was made to reactivate the virus particles or to assess the toxic effect of the aluminum on the virus or the effect of aluminum on the surface charge of the virus.

Other metal ions may have an important role in the removal of viruses by coagulation and flocculation, e.g., in slowing down the rate of aluminum-virus complex formation. Interfering effects of calcium and magnesium were noticed by Chang et al. (1958b) during virus removal by two-stage flocculation with Ohio River water. They believed that the presence of calcium and magnesium ions in the raw water and the addition of CaO in the second stage interfered with the formation of the coagulant-cation bacterial-virus complex.

Little information is available regarding the influence of organic matter on the removal of viruses by flocculation. Using alum and artificially contaminated river water with coxsackie virus, Frankova, Cervenka and Symon (1964) obtained highest removal of infectivity when the virus was added in the form of a suspension of infected mouse brain and found that the optimum amount of alum for the removal of the virus was dependent on the concentration of organic matter in the water. However, Carlson et al. (1968) observed considerable reduction in percent virus (bacteriophage T2 and type 1 poliovirus) inactivation by clays (Illite, Montmorillonite and Kaolinite in presence of sodium chloride or calcium chloride) when albumin or wastewater was present in the system. This was shown to be due to competition with virus for the adsorption sites on the clay.

In connection with their studies on the movement of viruses in ground water, Eliassen et al. (1966) observed the effects of synthetic polyelectrolytes on virus removal by alum flocculation. Virus removal was improved by the addition of varying concentrations of polyelectrolytes. Of the three species of polyelectrolytes tested, the results indicated that for similar dosages the cationic polyelectrolyte was approximately two orders of magnitude more effective than the anionic and neutral polyelectrolytes. Little virus removal was accomplished when the sand phase was absent in their jar tests. Presumably stirring and settling conditions were not sufficient for the floc to settle in the absence of the sorption effect of sand.

Quite contradictory observations have been reported regarding the effect of pH on coxsackie and bacterial virus removal. Chang, Isaac and Baine (1953) found pH 5.5 to be the optimum and obtained very low removal at pH 7.0. In a later paper lower removal was observed at pH 5.0 (Chang et al., 1958a). Quite different results were obtained with bicarbonate and phosphate buffers. Research conducted at Kings College in England (1966) reported 99 percent removal of bacteriophage with 60 ppm of ferric chloride at pH 6.5.
The present work was undertaken in order to delineate the basic mechanisms involved in the removal of viruses by chemical coagulation. Studies were also conducted to investigate the quantitative and practical aspects of the process. Various parameters, believed to affect the process, were evaluated. It is believed that the information obtained from this investigation, though it may not be quantitatively applicable to a particular water treatment plant, will contribute fundamental knowledge regarding the removal of viruses from water by chemical coagulation and flocculation and the various environmental parameters affecting the process.

2. LITERATURE REVIEW

A. Removal of Viruses by Coagulation and Flocculation

The information on the removal of viruses in water by coagulation and flocculation is extremely sketchy and nonquantitative. This is mainly due to the lack of accurate quantitative assay techniques for animal viruses and a reliable method for concentrating water samples containing viruses. Many investigators have used bacterial viruses as models for studying this problem. When animal viruses were used, biological assays involving laboratory animals were employed instead of quantitative tissue culture technique. Furthermore, it is difficult to correlate the observations of different workers due to the fact that coagulation and flocculation conditions were different. In most of the studies undertaken in this area, attempts were made to arrive at quantitative values like percent virus removal, relationships between virus removal and alum dosage, and settling time, etc. None of these studies was directed toward an understanding of the basic physico-chemical processes involved.

Using the virus of the mouse-adapted strain of human poliomyelitis, Carlson, Ridenour and McKhann (1942) found that flocculation using alum (100 ppm) did not render the water noninfective for mice, and that a rapid sand filter heavily impregnated with alum was somewhat more effective in removing the virus than the former process. Using both alum flocculation and filtration on water containing the virus of a monkey-adapted strain of human poliomyelitis, Kempf et al. (1942) freed the supernatant of the virus in two experiments and freed the effluent (sedimented and filtered) of the virus in one, out of the three experiments. Only by greatly increasing the alum dosage were they able to remove all the viruses. Since the data were measured by a biological assay involving the infecting of monkeys, there is some doubt that 100 percent removal was achieved even then. These authors also centrifuged their flocculated water and claimed that the supernatant could be freed of the virus if a floc sediment of 1.5 mg/l (in a Hopkins tube) was obtained with water flocculated at a dosage of 8 grains/gal of alum; but the virus in the floc sediment was not destroyed. They could recover virus activity in the resuspended sediment when none was present in the supernatant. Working with the virus of infectious hepatitis, Neefe et al. (1947) found that alum flocculation

-2-
and filtration through a diatomaceous earth filter did not completely remove the infective agent from the treated water. Forty percent of the human volunteers developed the disease after ingesting the treated water.

The basic mechanism involved in the removal of viruses by flocculation has been thought to be the formation of a metal cation (coagulant)-protein (virus) complex followed by precipitation and flocculation (Chang et al., 1958b; Chang, Isaac and Baine, 1953; Felix, 1965; and Kabler et al., 1963). This hypothesis seems quite reasonable from the chemical aspects of coagulation. Stumm and Morgan (1962) observed:

"The physical or double-layer theory has been developed in great detail and has, in its various forms of simplification, found wide acceptance. . . This theory has virtually replaced and superseded the older chemical theory. These two coagulation theories are not, however, as mutually exclusive as they might appear to be on first sight, and it is important to call attention to the fact that purely chemical factors must be considered in addition to the theory of the double layer in order to explain, in a more quantitative way, the dependence of the stability of colloids upon the chemical composition of the medium. . . Occasionally, specific chemical equilibria, such as complex formation, may be more important than double layer compaction through counter-ion adsorption. . . Complex formation reactions between aluminum or iron coagulant metal ions and carboxylic, phosphate, sulfate, or aromatic hydroxyl functional groups are important in the destabilization of such naturally occurring colloidal or dissolved impurities as color, proteins, and carbohydrate materials. The marked difference in the response of carboxyl, sulfate and phosphate colloids to coagulation by metal ions is indicative of specific chemical interactions."

By extrapolating the interactions of proteins and salts of metals to that of virus protein and aluminum, Chang, Isaac, and Baine (1953) first postulated the aluminum-virus complex formation. Later, they showed the importance of this complex formation as the first stage in the removal mechanism by using Gum Arabic, a substance which interferes with flocculation (Chang et al., 1958b). Gummy substances form a protective coating on charged particles and are well known for their stabilizing effect on emulsions. Viruses are not destroyed as a result of the complex formation as was evidenced by the recovery of viruses from the floc fraction (Chang, Isaac, and Baine, 1953; and Gilereas and Kelly, 1955).

Chang, Isaac and Baine (1953) using bacterial virus showed that virus removal followed a Freundlich adsorption isotherm and that preformed floc had little effect on virus removal. In concentrating Coxsackie virus suspensions by alum flocculation it was again noticed
that virus recovery was not high when preformed floc was used (Stevenson et al., 1956). A linear relationship was obtained between percent removal/alum dosage and percent virus remaining in suspension for a defined pH, temperature, flocculation and sedimentation time. The percent removal was a function of coagulant dose below the upper limit of the 'zone of flocculation.' Robec, Clarke and Dostal (1962) also noticed that increasing alum dosage increased the virus removal up to 99 percent. Polyelectrolytes were useful when filtration and coagulation were employed together. Furthermore, Chang, Isaac and Baine (1953) obtained different values of u in the relationship $R = uX^n$ (where $R =$ percent removal/alum dose; $u$ and $n$ are constants and $X =$ percent virus remaining in suspension) for two series of experiments conducted under identical experimental conditions. They concluded that some other unknown factor or factors were responsible apart from the cation-virus complex formation. As to the kinetics, they showed that 20 min were required for the first stage (complex formation). For the second step (aluminum-virus precipitate formation) they obtained a linear relationship between the log of virus remaining active and the square of the contact time. The energy of activation was calculated and found to be of the order of magnitude of that of diffusion ($E = 6,770$ calories).

In a recent publication, Berg (1964) reported 96 and 94 percent removal of coxsackie A2 virus by alum and ferric chloride, respectively. Removal was 96.6 percent when both coagulants were used. Temperature in the range 5°C to 25°C did not affect the removal significantly. On the other hand, Chang noticed slightly lower removal in cold months (Senn et al., 1961). Virus removal ranging from 25 to 99 percent were reported by different workers (Senn et al., 1961). It is of interest that high virus removal by Chang's group was achieved with low alum dosages, whereas low efficiency was attained by others who used high dosages. A critical comparison of virus removal efficiencies by flocculation cannot be made unless the conditions under which the experiments were conducted are known. As most of the investigators did not indicate the relative performance of the flocculation process as judged by visual inspection of turbidity removal, the relatively low virus removal reported might suggest that the flocculation process was inadequate.

Using bacterial virus (bacteriophage against M. pyogenes var. albus) Chang, Isaac and Baine (1953) studied the fate of virus particles removed by flocculation. They found that virus particles were temporarily 'inactivated' during the complex formation as a result of the formation of the aluminum salt of protein in the virus and would be 'active' again when dissociated from the medium. They were able to 'reactivate' the virus particles by redispersing the flocculated mixture at pH 7.6 with vigorous stirring. Sixty percent of the removed viruses could be recovered by this technique. Quite contrary to this observation, Puck, Garen and Cline (1951) demonstrated that trivalent cations like Al, Cr, and Fe permanently inactivate bacteriophages and their host cells. Later Yamamoto, Hjatt and Haller (1964) reported that AlCl$_3$ at concentrations between $10^{-3}$ and $2.10^{-3}$ M failed to inactivate bacteriophage MS2. Carlson et al. (1968) reported a significant increase in the percent inactivation
of virus particles (bacteriophage T2) adsorbed on clay particles when aluminum salt was present in the system instead of calcium or sodium salts. However, no attempt was made to reactivate the virus particles or to assess the toxic effect of the aluminum on the virus or the effect of aluminum on the surface charge of the virus.

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B. Aqueous Chemistry of Aluminum

It is apparent from the solubility considerations of aluminum and iron that coagulation in water and wastewater treatment is carried out under conditions of pH and coagulant dosage such that the system is oversaturated with respect to the metal hydroxide (Stumm and O'Melia, 1968). However, it is thought that a brief discussion of the aqueous chemistry of aluminum is warranted here because a great majority of the experiments will involve soluble concentrations of aluminum.

Recently, studies on the hydrolysis of the aluminum ion in dilute aqueous solution have received increased attention. A substantial amount of new information concerning the specific chemical structures of the hydrolysis products of the aluminum ion have been obtained. According to the available literature on the aluminum hydrolysis reactions, solubility curves for aluminum have been calculated and presented (Black and Chen, 1967; Stumm, 1964; and Stumm and O'Melia, 1968). Figure 1 shows the aluminum solubility curve presented by Black and Chen (1967).

Figure 1 is useful for visualizing the specific regions of various aluminum ion species for varying pH values at different total concentrations of aluminum. Within a range of total aluminum ion concentration from $10^{-6}$ M to $10^{-4}$ M, the pH scale from 3 to 10 can be roughly divided into four different regions in which different predominant aluminum ion species are present. In the region below pH 4, the hydrated trivalent aluminum ion is the most active species. In the region between pH 4 and 6, the predominant aluminum ion species are the hydrolyzed polymeric multivalent cation species: $\text{Al}_7(\text{OH})^{14+}$ and $\text{Al}_{13}(\text{OH})^{+5}$. Predominance of other cation species, such as $\text{Al}_6(\text{OH})^{+3}$ and $\text{Al}_9(\text{OH})^{+4}$ have also been proposed in the literature (Black and Chen, 1967). In the pH range roughly from 6 to 8, insoluble aluminum hydroxide, $\text{Al}(\text{OH})_3$, is the predominant species, and the aluminate ion, $\text{Al}(\text{OH})_4^-$, is believed to predominate above pH 8. However, it is interesting to note that, in the region between pH 5 and 8.5, the formation of insoluble aluminum hydroxide colloids or precipitate does not take place up to a total aluminum concentration of about $10^{-4}$ M as evidenced by the Tyndall effect in aluminum sulfate solution (Black and Chen, 1967; and Morgan, 1967).

3. SCOPE OF THE INVESTIGATION

From the preceding discussion, it is evident that there is a great need to obtain more information on the basic mechanisms involved in virus removal by chemical coagulation and flocculation so as to enable the engineer to put the process on a sound scientific basis. The removal of viruses from water supplies is extremely important and, becomes more so, with the potential need for wastewater reuse. Information on the basic mechanisms involved in virus removal by coagulation
FIGURE 1. SOLUBILITY CURVE FOR ALUMINUM HYDROXIDE.
and flocculation should contribute significantly to the solution of the problem and aid in the development of sound design standard for their removal in water treatment facilities.

Most of the investigations so far undertaken in this area have been directed towards quantitative results, i.e. gross removal efficiencies. Very limited attention has been given to the mechanisms of virus removal (Chang et al., 1958b; Chang, Isaac and Baine, 1953; Felix, 1965; and Kabler et al., 1963). Considering the observations of the past researchers in this area and the interaction of proteins with metals ions, the virus removal mechanism may be postulated as a two-stage reaction. The first stage is the formation of a virus-aluminum complex, the second stage is the subsequent precipitation and flocculation of the complex. Basically, the first stage is the interaction of virus with metal ion. The present research was initiated to investigate this interaction. The study was undertaken along the following lines:

a. demonstration of a "complex formation" between viruses and aluminum, and
b. nature of the "complex" and virus inactivation by aluminum.

The second phase of the study was directed towards a quantitative study of virus removal by alum flocculation under controlled laboratory conditions. The effects of the following variables in virus removal by chemical coagulation and flocculation were also investigated:

a. pH and coagulant dose,
b. bivalent metal ions like calcium and magnesium,
c. organic matter, and
d. synthetic polyelectrolytes (coagulant aids).

4. MATERIALS AND METHODS

A. Materials

(1) Viruses and preparation

One of the main criteria in selecting a virus for this study was feasibility of culturing and enumeration. Assay techniques for bacterial viruses are better developed than for animal viruses. Bacterial virus assays require about 12 to 24 hr compared with 5 to 10 days for animal viruses, and culture procedures are simpler for bacterial hosts. Bacterial and animal viruses have many similar physical, chemical and biological properties, i.e. size, net electric charge, protein coating, etc. (Adams, 1959). Thus, it may be assumed that their differences in behavior when subjected to coagulation
and flocculation may not be much greater than the differences in these properties among the animal viruses alone, which could cause significant variations in removal by chemical coagulation and flocculation. Furthermore, there is much more known about the composition and properties of bacterial viruses than is known about the animal viruses. This allows for a more detailed examination of their behavior in chemical coagulation.

Two bacterial viruses, bacteriophages T4 and MS2 against Escherichia coli, were selected as the model viruses for this study. Bacteriophage T4 is a DNA (deoxyribonucleic acid) containing virus whereas bacteriophage MS2 is a RNA (ribonucleic acid) containing virus. Bacteriophage T4 was selected as the principal model virus because of its stability in agitated systems and its greater ease of culturing and enumeration (Cookson, 1966; and Drake, 1967). Bacteriophage MS2 was selected as the second model virus in order to confirm the results with bacteriophage T4. It was thought that use of MS2 along with T4 in some of the major experiments would allow the data to be interpreted in terms of viruses which may be more significant in water supplies. MS2 was selected because of its resemblance in size and shape, and the type of nucleic acid to poliovirus (Kruse, 1968; Hanson, 1969; and Spiegelman, 1969).

a. Escherichia coli Bacteriophage T4

Bacteriophage T4 (Figure 2) and its host E. coli BB were obtained from Dr. John W. Drake, Department of Microbiology, University of Illinois. Bacteriophage T4 has the following properties (Table 1) (Kellenberger, 1962; Stent, 1963; and Putnam, 1953).

<table>
<thead>
<tr>
<th>TABLE 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROPERTIES OF E. coli BACTERIOPHAGE T4</td>
</tr>
<tr>
<td>Size</td>
</tr>
<tr>
<td>head</td>
</tr>
<tr>
<td>tail</td>
</tr>
<tr>
<td>Specific weight</td>
</tr>
<tr>
<td>pH stability range</td>
</tr>
<tr>
<td>Sedimentation constant</td>
</tr>
<tr>
<td>Nucleic acid</td>
</tr>
</tbody>
</table>

The procedure for growing bacteriophage T4 was also obtained.
from Dr. John W. Drake. Stock suspensions were prepared by infecting an early log phage broth culture of *E. coli* BB with bacteriophage T4 at a low multiplicity of infection (approximately 0.02 phages/ml). The infected culture was incubated at 37°C until the lysis was complete as evidenced by visible reduction of turbidity. A few drops of chloroform were then added to the lysed culture and mixed well in a vortex mixer in order to facilitate lysis of the unlysed cells. The suspension was centrifuged at low speed (5,900 ×g for 10 min at 4°C) to remove bacterial cells and cell debris followed by high speed centrifugation (34,800 ×g for one hr at 4°C) to sediment the virus particles. The sedimented pellet was finally resuspended in phage buffer. The final purification step was repeated twice. The purified stock was then stored at 4°C. Stocks prepared in this way usually titered between 10^10 - 10^11 phages/ml.

b. *Escherichia coli* Bacteriophage MS2

A purified stock suspension of Bacteriophage MS2 (Figure 3) and its host *E. coli* A19 were originally obtained from Dr. S. Spiegelman, Department of Microbiology, University of Illinois. Later, purified stock suspension of MS2 in 0.05 M tris buffer (pH 7.6) were purchased from Miles Laboratories, Inc., Elkhart, Indiana. Bacteriophage MS2 has the following properties (Table 2) (Overby et al., 1966).

**TABLE 2**

PROPERTIES OF *E. coli* BACTERIOPHAGE MS2

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle diameter</td>
<td>25 μm</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>3.7 × 10^6 gm</td>
</tr>
<tr>
<td>Isoelectric point (pH)</td>
<td>3.9</td>
</tr>
<tr>
<td>Sedimentation constant</td>
<td>79 Svedberg units (S_{20,w})</td>
</tr>
<tr>
<td>Nucleic acid</td>
<td>RNA (ribonucleic acid)</td>
</tr>
</tbody>
</table>

(2) Biological media

L broth (T4) was as described by Drake (1963). T agar (T4) was L broth (T4) with 1.20 percent Difco agar. Soft agar (T4) overlay consisted of L broth (T4) with 0.65 percent Difco agar. Phage buffer (T4) was 0.01 M tris (pH 7.4), 0.005 M MgSO\textsubscript{4} and 0.1 M NaCl.

Media for *E. coli* bacteriophage MS 2 were L agar (MS2), L broth (MS2), soft agar (MS2) and phage buffer (MS2) as described by Overby et al. (1966).
FIGURE 3. ELECTRON MICROGRAPH OF BACTERIOPHAGE MS2.

Final magnification 100,000 X. Provided by S. Spiegelman, University of Illinois, Urbana, Illinois.
(3) Coagulant and polyelectrolytes (coagulant aids)

The coagulant used in this study was aluminum sulfate \( \text{Al}_2(\text{SO}_4)_3 \cdot 18 \text{H}_2\text{O} \) marketed by Allied Chem. Corp., Morristown, New Jersey.

The polyelectrolytes (coagulant aids) used are listed in Table 3. Two cationic and two anionic polyelectrolytes were selected. Stock and working solutions were prepared according to the instructions given in the product literature.

TABLE 3

POLYELECTROLYTES (COAGULANT AIDS)

<table>
<thead>
<tr>
<th>Name</th>
<th>Type</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primafloc C-7</td>
<td>Cationic (polyamine)</td>
<td>Rohm and Haas</td>
</tr>
<tr>
<td>Catfloc</td>
<td>Cationic</td>
<td>Calgon Corp.</td>
</tr>
<tr>
<td>Primafloc A-10</td>
<td>Anionic (polycarboxylic)</td>
<td>Rohm and Haas</td>
</tr>
<tr>
<td>Coagulant Aid #243</td>
<td>Anionic</td>
<td>Calgon Corp.</td>
</tr>
</tbody>
</table>

(4) Water

Distilled water demineralized through two Illco-Way Research Model Demineralizer (Illinois Water Treatment Co., Rockford, Illinois), and then sterilized by autoclaving at 15 psi for 15 min was used in all soluble aluminum studies. Sterilized laboratory deionized water was used in preparing the raw water for the coagulation and flocculation studies (jar tests).

(5) Organic materials

The following organic materials were used in this study:


d. Wastewater and wastewater effluents. Collected fresh from the Urbana-Champaign Sanitary District waste treatment plant and prepared by filtering through four layers of cheesecloth so as to remove the larger size particles.

(6) Glassware

All glassware used for the soluble aluminum studies were cleaned by soaking overnight in chromic acid followed by rinsing in tap water and deionized water. Glassware used for the coagulation and flocculation studies (jar tests) were cleaned by soaking overnight in Haemo-sol (Meinecke and Co., New York, New York) followed by rinsing in tap water and deionized water, as suggested by Chang, Isaac and Baine (1953). Sterilization was accomplished in a hot air oven at 200°C for one hr or longer.

B. Chemical and Biological Assay Techniques

(1) Determination of aluminum

A simple, rapid and sensitive method for determining soluble aluminum concentrations in the microgram range was required. The eriochrome cyanine R method originally introduced by Knight (1960) and later modified by Shull and Guthan (1967), and Chaudhuri and Engelbrecht (1968) was used. The standard aluminum curve shown in Figure 4 was used to determine the aluminum concentration in unknown samples.

(2) Assay procedure for bacteriophages

The assay procedure for bacteriophage T4 was obtained from Dr. John W. Drake. Before assaying, the sample was diluted in L broth (T4) to yield about 200 plaques per plate. A liquid top-agar mixture was prepared from 2.5 ml of soft agar (T4) at 45°C, 0.25 ml of a log growth phase culture of E. coli BB cells, and 0.1 ml of the diluted virus sample. The top-agar mixture was then poured on solidified bottom agar (T agar) plates and incubated at 37°C for 12 to 24 hr. "Plaques" were counted with the aid of a Quebec colony counter. Triplicate plates were prepared from each sample to increase accuracy.

The assay procedure used for bacteriophage MS2 was very similar to that of bacteriophage T4. E. coli A19 was the indicator bacteria. The top agar was soft agar (MS2) and the bottom agar was L agar (MS2). Plates were incubated at 37°C for 6 to 8 hr before counting.

C. Experimental Technique and Equipment

(1) Kinetics of adsorption of aluminum by viruses

All studies on the kinetics of adsorption of aluminum involved soluble concentrations of aluminum. Concentrations of aluminum were always kept below the solubility at the pH of the test
FIGURE 4. STANDARD CURVE FOR ALUMINUM.
(Figure 1). Experiments were carried out at three different pH values: pH 5.0 (0.2 M acetate buffer), pH 6.0 (0.2 M succinate-sodium hydroxide buffer), and pH 9.0 (0.2 M borate-potassium chloride-sodium hydroxide buffer). All experiments were performed at room temperature (24°C to 25°C).

In the procedure the reaction mixture (total volume 150 ml) was prepared by adding 95 ml of deionized water, 20 ml of buffer solution, 25 ml of aluminum solution (1 ml = 0.25 μg aluminum) to give a final concentration of 1.54 x 10⁻⁶ M, and 10 ml of purified virus stock suspension to yield a final virus concentration of 3-6 x 10⁻⁶ /ml. A control was prepared to which 10 ml of phage buffer was added instead of the virus stock suspension, and a reagent blank consisting of 120 ml of deionized water, 20 ml of buffer, and 10 ml of phage buffer. Immediately after adding the virus stock suspension, the reaction mixture was mixed well and 20 ml samples were withdrawn periodically and immediately filtered through a 0.45μm membrane filter (Millipore Corp., Bedford, Mass.). The first sample was withdrawn immediately after mixing. Subsequent samples were withdrawn at 2, 5, 10 and 15 min. In the filtration procedure, the first 5 ml of the filtrate were wasted; the remaining 15 ml were saved for the determination of aluminum. Aluminum concentrations in these filtrate samples were determined by the eriochrome cyanine R method using a filtered reagent blank. Aluminum concentrations were also determined in duplicate 15 ml filtered samples from the control. The difference in aluminum concentrations between this and that in a filtered sample of the reaction mixture was used to compute the quantity of aluminum adsorbed by the virus particles. The average of duplicate aluminum determinations on the unfiltered 15 ml samples from the control flask was denoted as "available aluminum." This available aluminum concentration was always found to be slightly lower than the calculated concentration added. This indicated that some loss of aluminum from the liquid phase was taking place. It is believed that this was due to the adsorption on glassware. A typical experimental protocol with accompanying calculations is shown in Table 4.

**TABLE 4**

**EXPERIMENTAL PROTOCOL FOR KINETIC STUDIES**

<table>
<thead>
<tr>
<th>Deionized Water (ml)</th>
<th>pH Buffer (ml)</th>
<th>Aluminum Solution (ml)</th>
<th>Virus Suspension (ml)</th>
<th>Phage Buffer (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent Blank</td>
<td>120</td>
<td>20</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>Reaction Mixture</td>
<td>95</td>
<td>20</td>
<td>25</td>
<td>10</td>
</tr>
<tr>
<td>Control</td>
<td>95</td>
<td>20</td>
<td>25</td>
<td>-</td>
</tr>
</tbody>
</table>

Aluminum adsorbed by the virus particles = (Aluminum concentration in filtered sample from the control) - (Aluminum concentration in filtered sample of the reaction mixture)

Available aluminum = Aluminum concentration in unfiltered sample from the control
Filtration through a 0.45μ Millipore membrane filter was used as a means for rapid physical separation of the virus particles from the reaction mixture. Viruses are known to adsorb on 0.45μ Millipore membrane filters (Oliver, 1966; and Wallis and Melnick, 1967a, 1967b). Further, it has been demonstrated that E. coli bacteriophages adsorb on 0.45μ Millipore membranes (Hoff and Jakubowski, 1965; and Loehr and Schwegler, 1965). Preliminary tests indicated that under the experimental conditions used in this study, not more than 9 to 10 percent of the virus particles were passing through the membrane filters.

(2) Virus inactivation studies

Experiments to evaluate inactivation of bacteriophages T4 and MS2 in the presence of soluble concentrations of aluminum were carried out at pH 5 (0.2 M acetate buffer) and at room temperature (24°C to 25°C). Experimental tubes were prepared by adding 3.3 ml of buffer solution, 1.0, 5.0 or 7.0 ml of aluminum solution (1 ml = 0.25 μg aluminum), 0.5 ml of a suitable dilution of the virus stock suspension to yield a final virus concentration of 10⁶ - 10⁷/ml, and deionized water to make up to a final volume of 25 ml. A control tube was also prepared without any aluminum. Samples for virus assay (0.1 ml) were withdrawn at 0, 3, 6, 12, and 24 hr and the virus titer determined by the standard technique. Duplicate experimental tubes were also prepared in which 0.5 ml of phage buffer was added instead of the virus dilution. Samples were withdrawn from each of these tubes for determination of "available aluminum."

(3) Quantitative studies on adsorption of aluminum by viruses

a. Studies on adsorption of aluminum by viruses

These studies were carried out at pH 5 (0.2 M acetate buffer) and at room temperature (24°C to 25°C). A procedure similar to that used in the kinetic studies was followed for preparation of reaction mixtures, blanks, and control flasks. Virus concentrations were varied from 2 x 10⁻⁶/ml to 1.8 x 10⁻⁵/ml. Since it was evident from the kinetic studies that adsorption of aluminum by the virus particles was very rapid and took place within the first 30 to 40 sec, samples from the reaction flasks were withdrawn and filtered 5 to 10 min after the start of the reaction. The quantity of aluminum adsorbed by virus particles and "available aluminum" were computed in the same manner as before.

b. Aluminum saturation curves

Experiments were also carried out to obtain saturation curves for aluminum adsorption by different concentrations of bacteriophage T4. Studies were made at pH values of 5.0 (0.2 M acetate buffer), 6.0 (0.2 M succinate-sodium hydroxide buffer), and 9.0 (0.2 M borate-potassium chloride-sodium hydroxide buffer), and at room temperature (24°C to 25°C). Using a procedure similar to the one used
in the studies on adsorption of aluminum by viruses, quantities of aluminum adsorbed by a particular concentration of bacteriophage T4 particles at varying "available aluminum" concentrations were computed and plotted.

(4) Quantitative studies on virus removal by chemical coagulation and flocculation (jar tests)

A bench-scale apparatus employing a carbonic acid-bicarbonate buffer system for pH control was set up in the laboratory for coagulation and flocculation studies (Figure 5). Bicarbonate buffer was used because this is the main buffering system present in natural surface waters (Chang et al., 1958a). All experiments were performed at room temperature (24°C to 25°C).

Mixing was provided with a six-place multiple stirrer (Phipps and Byrd, Richmond, Va.). The tachometer readings were found to agree with paddle rpm values. Specially constructed paddles (Figure 6), made from 0.25 in. stainless steel rod, were used in an attempt to provide more uniform velocity gradients than is thought to occur with the conventional 1 x 3 in. paddles. The paddles were cleaned and sterilized before each experiment using the standard procedure for cleaning glassware.

Control of pH, over the range of 8.3 to 5, was obtained by introducing various mixtures of air and CO2 into the partially confined atmosphere above each beaker. By measuring the individual gas-flow rates prior to blending, various mixtures of the two gases could be obtained. The blended gas was distributed from a central manifold to the individual beakers. Each experiment was initiated when an equilibrium pH was achieved; this usually required 0.5 to one hr.

Values of pH were determined in individual beakers before the addition of coagulant and after the settling period. A Beckman Electromate pH meter was used. It was standardized daily using commercial buffer solutions of pH 4, 7, and 9.

The "raw" water was made up in 10 liter batches in a covered polyethylene vat. The raw water for all experiments contained 150 mg/l of sodium bicarbonate, 120 mg/l of Montmorillonite (Wyoming Bentonite), and 4-5 x 10^3/ml of bacteriophage T4 or MS2. The clay was supplied by Ward's Natural Science Establishments, Inc., Rochester, New York. Montmorillonite was selected because it has been shown to be present in natural waters (Packham, 1962).

Samples for turbidity measurement, 30 ml each, were taken with a 4 mm bore pipet, 1.5 in. below the air-water interface and delivered to 50 ml beakers. All turbidity measurements were made after the samples had been in the instrument (Model 1860, Hach Chemical Co., Ames, Iowa) for two min.
FIGURE 5. SCHEMATIC DRAWING OF APPARATUS FOR COAGULATION AND FLOCCULATION STUDIES (JAR TESTS).
FIGURE 6. SCALE DRAWING OF REACTION VESSEL FOR COAGULATION AND FLOCCULATION STUDIES (JAR TESTS).
Samples for virus enumeration, 0.1 ml each, were taken with 0.1 ml serological pipets, 1.5 in. below the air-water interface and immediately diluted in L broth for subsequent assay by the standard assay procedure for bacteriophages.

The following method was used in performing an experiment: (1) six 975 ml aliquots of the "raw" water were placed in individual beakers situated on the mixing apparatus, (2) the six beakers were mixed simultaneously as pH was adjusted, (3) a calculated quantity of water was added to each beaker to give a final total volume of one liter after the addition of chemicals, (4) while mixing rapidly at 100 rpm, a stoichiometric amount of sodium bicarbonate was added to each experimental beaker to neutralize the acidity due to aluminum sulfate added in the next step and then chemicals (aluminum sulfate, polyelectrolytes, etc.) were added into all but one beaker which served as a blank, and (5) mixing was continued at 100 rpm for one min followed by 30 min of slow mixing at 20 rpm. Mixing was stopped gradually over a 40 sec period and the mixing paddles were left in place during the settling period. Samples for virus assay and turbidity measurements were withdrawn after 30 min of quiescent settling. The pH of each system was determined also. In experiments using coagulant aids, rapid mixing was extended for a period of one min after the polyelectrolyte addition, as recommended by the manufacturer.

(5) Virus recovery from settled floc

After performing a jar test at the optimum pH and aluminum sulfate dosage for the particular virus, contents of both the blank and the experimental beakers were stirred at a high speed for 15 min using a magnetic stirrer so as to disperse the floc completely. While being stirred, duplicate 5 ml aliquots were withdrawn from each beaker with a broken-tip pipet and poured into screw-cap tubes containing 15 ml of an elutant. Deionized water, 3 percent beef extract, one percent bovine serum albumine, 0.1 M tris buffer (ph 8.0), and 0.2 M phosphate buffer (pH 8.0) were used as elutants. Tubes were kept at 4°C with the contents mixed every 15 min in a vortex mixer. After 6 hr the content of each tube was centrifuged at 5,900 xg for 10 min and the supernatant assayed for the virus titer. Recoveries were calculated on the basis of the virus titer of the supernatant from the blank tube.

5. RESULTS AND DISCUSSION

Results of all experiments are presented in graphical or tabular form. In order to facilitate the presentation, a discussion of the results follows each phase of the experimental work. For the most part, only typical results are shown and discussed although all results were substantiated by two or more replicate experiments.

The terms "coagulation" and "flocculation" often denote different meanings. Recently there has been shown an awareness of the long existing general confusion in the literature over the meaning of these
two terms. It should be recognized that in the overall process of coagulation and flocculation there is a cause-and-effect relationship. For reactions of the type which are encountered in water treatment practice, destabilization and particle collision opportunity can be viewed as "causes." Aggregation of the destabilized, colliding particle is then an "effect." In order for a conventional water treatment plant to operate effectively both destabilization (accomplished by chemical addition) and particle collisions (accomplished primarily by mixing) must be provided. In presenting the results of this study, "coagulation" will refer to destabilization and "flocculation" will refer to aggregation. When used together, these two terms will denote destabilization and aggregation as provided in a water treatment plant and will be referred to as "the process of coagulation and flocculation."

A. Adsorption of Aluminum by Viruses

(1) Kinetics of adsorption

Kinetics of adsorption of aluminum at pH values 5, 6, and 9 by bacteriophages T4 and MS2 are shown in Figures 7 and 8, respectively. Attempts were made to keep the virus concentrations constant for a particular bacteriophage in each system. However, it is to be borne in mind that the virus concentrations reported here are as determined by the plaque count method and do not indicate the total number of actual virus particles in the sample. The latter can be determined only by an electron micrographic count (Luria, Williams and Backus, 1951). Although specific information is lacking, it is generally accepted that the precision of the plaque count method is on the order of 5 to 10 percent (Drake, 1967). Since processing of the sample took approximately 30 to 40 sec before the virus particles were physically separated from the reaction mixture, the data for the samples withdrawn from the reaction mixture immediately after mixing were plotted against 30 sec.

It is evident from Figures 7 and 8 that adsorption of aluminum by virus particles is very rapid and takes place at least within the first 30 to 40 sec. Maximum adsorption is also attained at this time. For all practical purposes it may be regarded that adsorption of aluminum by virus particles is instantaneous. However, Chang, Isaac, and Baine (1953) estimated that 20 min would be required for aluminum virus precipitate formation. It is to be noted that their experimental conditions were entirely different and that they attempted to separate the first-stage reaction (aluminum-virus complex formation) from the second-stage (precipitation and flocculation) by assuming that the second-stage reaction would not start in the absence of SiO₂ which was found to be required for the formation of good flocs under their experimental conditions. Time required for the first-stage reaction was assumed to be the longest contact time between aluminum and virus particles at which there were no significant differences in percentages of recovery of the virus between the tests performed in the presence of SiO₂ and those in the absence of SiO₂.
Available Aluminum: 33 µg/l

**Figure 7. Kinetics of Adsorption of Aluminum by Bacteriophage T4.**
FIGURE 8. KINETICS OF ADSORPTION OF ALUMINUM BY BACTERIOPHAGE MS2.
It is also seen from Figure 8 that the amount of aluminum adsorbed by the MS2 particles does not change appreciably with pH in the range of 5.0 to 9.0 and the variations are well within the limits of experimental error. However, the variations are more pronounced in the case of the T4 particles (Figure 7). It is not possible to provide a completely satisfactory explanation based upon the ionization of the charged groups on the coat protein of the bacteriophage T4. Thus, the differences may be due to the variations in the amount of aluminum adsorbed on the surfaces of the glassware.

(2) Quantitative adsorption

Figures 9 and 10 show the quantitative adsorption of aluminum by bacteriophages T4 and MS2, respectively. For studies with bacteriophage T4, three different virus stock suspensions and two different aluminum concentrations were used. The three virus stocks were prepared at different times and were stored for varying periods of time ranging from 1 to 6 months at 4°C.

These figures show that a linear relationship exists between virus concentration and the amount of aluminum adsorbed. In other words, aluminum is reacting stoichiometrically with the virus particles. It can also be concluded from observations with bacteriophage T4 that the amount of aluminum adsorbed is not affected by the period and conditions of storage of the virus particles used. The amount of aluminum available also does not exert an effect providing the amount available is greater than the limiting concentration.

To substantiate the reliability of the Millipore membrane technique used so far in these studies, a comparison was made between this method and another method involving high speed centrifugation. Virus particles along with adsorbed aluminum were separated from the reaction mixture by centrifuging at 34,800 xg for one hr. The difference in aluminum concentrations in supernatants of the control and the reaction mixture (Table 4) was used to compute the quantity of aluminum adsorbed. Results agreed very well. For a bacteriophage T4 concentration of \(7 \times 10^{10}\) /ml, the amount of aluminum adsorbed was 20 μg/l. Using the phosphate modification method (Shull and Guthan, 1967) concentration of aluminum in the sedimented pellet was also determined for a mass balance of aluminum. The pellet was eluted with 0.2 M tris buffer (pH 8.0), digested in 1 N sulfuric acid at 15 psi for 30 min, neutralized to pH 4.5 with 1 N sodium hydroxide and the concentration of aluminum determined (Table 5).

In this example (Table 5), the initial concentration of aluminum in the control and in the reaction mixture was 37.5 μg/l. The concentration of aluminum in the control after centrifugation was 27.5 μg/l. This was the amount of aluminum available for reaction with the T4 particles. The 26.7 percent loss is assumed to be due to adsorption on the glassware and on the wall of the centrifuge tube. A total recovery of 30.0 μg/l of aluminum was made from the supernatant and the sedimented
FIGURE 9. QUANTITATIVE ADSORPTION OF ALUMINUM BY BACTERIOPHAGE T4 AT pH 5.0.
FIGURE 10. QUANTITATIVE ADSORPTION OF ALUMINUM BY BACTERIOPHAGE MS2 AT pH 5.0.


**TABLE 5**

MASS BALANCE ON ALUMINUM BY CENTRIFUGATION METHOD

Virus concentration = $7 \times 10^{10}$/ml; pH = 5.0 (0.2M Acetate Buffer)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial conc.</td>
<td>37.5 pg/l</td>
</tr>
<tr>
<td>Conc. after</td>
<td>27.5 pg/l</td>
</tr>
<tr>
<td>Percent loss</td>
<td>$\frac{37.5 - 27.5}{37.5} \times 100 = 26.7$</td>
</tr>
</tbody>
</table>

**Reaction Mixture**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>7.5 pg/l</td>
</tr>
<tr>
<td>Concentration</td>
<td>22.5 pg/l</td>
</tr>
<tr>
<td>Total conc.</td>
<td>$7.5 + 22.5 = 30.0$ pg/l</td>
</tr>
</tbody>
</table>

pellet from the reaction mixture. This is within 9 percent of the amount of aluminum available (27.5 pg/l). Hence, excellent mass balance was obtained.

In order to further investigate the quantitative nature of aluminum adsorption, saturation curves for aluminum adsorption for different bacteriophage T4 concentrations at pH values 5.0, 6.0, and 9.0 were obtained. These curves are shown in Figures 11, 12, and 13.

The saturation curves follow the same pattern at pH values 5.0, 6.0, and 9.0. It can be seen that when the concentration of available aluminum is less than the limiting concentration, almost complete adsorption of aluminum from the solution takes place. The plateau regions of the saturation curves indicate complete saturation of the adsorption sites on the virus coat protein. Consequently, the quantity of aluminum that can be adsorbed on the virus coat protein surface is constant.

These adsorption data were then plotted according to the linear form of the Langmuir adsorption equation. This was done using the least squares method of curve fitting to facilitate the calculation of the amounts of aluminum adsorbed by a T4 particle at different pH values. The basic assumptions involved are that (1) all adsorbent sites are identical and that (2) no interaction takes place between molecules adsorbed on adjacent sites (Graham, 1959). The Langmuir equation can
ALUMINUM ADSORBED (μg/l)

AVAILABILITY ALUMINUM (μg/l)

FIGURE 11. SATURATION CURVES FOR ALUMINUM ADSORPTION BY BACTERIOPHAGE T4 AT pH 5.0.
FIGURE 12. SATURATION CURVES FOR ALUMINUM ADSORPTION BY BACTERIOPHAGE T4 AT pH 6.0.

FIGURE 13. SATURATION CURVES FOR ALUMINUM ADSORPTION BY BACTERIOPHAGE T4 AT pH 9.0.
be expressed by

\[ \frac{C}{q} = \frac{1}{KZ} + \frac{C}{Z} \]

where

- \( C \) = concentration of aluminum in the solution at equilibrium, \( \mu g/l \)
- \( q \) = aluminum adsorbed per unit of adsorbent at equilibrium, \( \mu g/particle \)
- \( Z \) = saturation ratio, \( \mu g/particle \)
- \( K \) = constant, \( particle/l \)

Adsorption data of Figures 11, 12, and 13 fit well to the linear form of the isotherm (Figures 14, 15, and 16). The amounts of aluminum adsorbed by a T4 particle at different pH values were calculated from these plots. These data are given in Table 6.

**TABLE 6**

**AMOUNTS OF ALUMINUM ADSORBED BY A BACTERIOPHAGE T4 PARTICLE AT DIFFERENT pH VALUES**

<table>
<thead>
<tr>
<th>pH</th>
<th>Aluminum adsorbed</th>
<th>( \mu g/particle )</th>
<th>atoms/particle</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>3.31 x 10^{-13}</td>
<td>7.37 x 10^{3}</td>
<td></td>
</tr>
<tr>
<td>6.0</td>
<td>2.79 x 10^{-13}</td>
<td>6.21 x 10^{3}</td>
<td></td>
</tr>
<tr>
<td>9.0</td>
<td>2.83 x 10^{-13}</td>
<td>6.30 x 10^{3}</td>
<td></td>
</tr>
</tbody>
</table>

Even though saturation curves were not obtained for the bacteriophage MS2 - aluminum system, an estimate can be made of the amount of aluminum adsorbed by a MS2 particle at pH 5.0. Taking values from Figure 10, this appears to be 2.05 x 10^{-13} \( \mu g/particle \) or 4.6 x 10^{3} atoms/particle. Due to the smaller size of a MS2 particle compared to a T4 particle, lesser amount of aluminum is adsorbed.

It is evident that the amounts of aluminum adsorbed by a T4 particle at pH values 6.0 and 9.0 are the same and the variations reported are well within the limits of experimental error. However, it is not possible to provide a completely satisfactory explanation for the
FIGURE 14. McBAIN - BRITTON PLOT OF ADSORPTION DATA OF BACTERIOPHAGE T4 - ALUMINUM SYSTEM (pH 5.0) TO FIT LANGMUIR ADSORPTION EQUATION.

\[ \frac{c}{q} = 1.425 \times 10^{13} + 3.02 \times 10^{12}c \]

- ○ Virus Conc: \(1.1 \times 10^{11}/\text{ml}\)
- □ Virus Conc: \(6.3 \times 10^{10}/\text{ml}\)
- △ Virus Conc: \(3.5 \times 10^{10}/\text{ml}\)
Equilibrium aluminum concentration, $C$ (mg/l)

$C/q = 1.15 \times 10^{-3} + 3.59 \times 10^{-12} C$

Virus Conc.: $5.67 \times 10^{10}$ ml

Virus Conc.: $3.67 \times 10^{10}$ ml

Figure 15. McBain Britton plot of adsorption data of bacteriophage T4 - Aluminum system (pH 6.0) to fit Langmuir adsorption equation.
\[ \frac{C}{q} = 1.1 \times 10^{13} + 3.8 \times 10^{12}C \]

FIGURE 16. McBAIN-BRITTON PLOT OF ADSORPTION DATA OF BACTERIOPHAGE T4 - ALUMINUM SYSTEM (pH 9.0) TO FIT LANGMUIR ADSORPTION EQUATION.
The difference in the amounts of aluminum adsorbed at pH values 5.0 and 6.0. The ionization of the charged groups on the bacteriophage T4 coat protein may not be appreciably different at these two pH values. The fact that a stoichiometric amount of aluminum is adsorbed by a T4 particle at pH values between 5.0 and 9.0 leads to the likelihood of a reaction of the "complex formation" type. Coordination complexes between aluminum and some ionic group(s) on the coat protein of bacteriophage is quite likely. Physical adsorption involving electrostatic attraction is ruled out because of the excellent stoichiometry between aluminum and T4 particles in spite of the fact that the predominant aqueous aluminum species at pH values 5.0 and 6.0 are positively charged whereas the species at pH 9.0 is negatively charged (Figure 1). The positively charged species are either $\text{Al}_7\text{(OH)}_7^{+4}$ and $\text{Al}_{13}\text{(OH)}_{17}^{+5}$ or $\text{Al}_6\text{(OH)}_{34}^{+3}$ and $\text{Al}_8\text{(OH)}_{15}^{+4}$. The negatively charged species is $\text{Al(OH)}_7^-$. It seems quite likely that aluminum bound in these hydroxo complexes is complexing with the ionic group(s) on the coat protein of the bacteriophage.

By considering the protein coat of bacteriophage T4, it is possible to make some approximate calculations as to the availability of ionic groups for the formation of coordination complexes with aluminum. Detailed amino acid composition of the T4 coat protein is available in the literature (Table 7). The head of the phage particle is made up of about 300 identical protein subunits (Edgar and Epstein, 1965), which is the major protein constituent of the phage (Stent, 1963). A large protein generally consists of subunits which are the minimal physical units. Assuming about 200 amino acid units in a protein subunit, the total number of amino acids units in the head protein are about $6 \times 10^4$. This estimate and the mean amino acid composition of the T4 coat protein lead to the following approximate calculations:

**Number of carboxyl groups**

$$= (\text{Number of terminal carboxyl groups}) + \left(\text{Total number of } w\text{-carboxyl groups due to aspartate and glutamate residues} - \text{number of } w\text{-carboxyl groups combined with ammonia in amide linkage}\right)$$

$$= 300 + \left[ (11.10 + 10.15) \times \left(\frac{100 - 46.6}{100}\right) \right] \times \frac{6 \times 10^4}{100}$$

$$= 7,120$$

**Number of hydroxyl groups**

$$= \text{Number of phenolic hydroxyl groups of tyrosine}$$

$$= \frac{2.30}{100} \times 6 \times 10^4$$

$$= 1,980$$
<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Fitch and Susman (1965)</th>
<th>Polson and Wyckoff (1948)</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysin</td>
<td>6.4</td>
<td>7.1</td>
<td>6.75</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.0</td>
<td>2.0</td>
<td>1.00</td>
</tr>
<tr>
<td>Arginine</td>
<td>4.1</td>
<td>4.5</td>
<td>4.30</td>
</tr>
<tr>
<td>Aspartate and Aspargin</td>
<td>11.1</td>
<td>11.1</td>
<td>11.10</td>
</tr>
<tr>
<td>Threonine</td>
<td>7.0</td>
<td>7.1</td>
<td>7.05</td>
</tr>
<tr>
<td>Serine</td>
<td>5.9</td>
<td>5.5</td>
<td>5.70</td>
</tr>
<tr>
<td>Glutamate and glutamine</td>
<td>10.4</td>
<td>9.9</td>
<td>10.15</td>
</tr>
<tr>
<td>Proline</td>
<td>4.1</td>
<td>5.3</td>
<td>4.70</td>
</tr>
<tr>
<td>Glycine</td>
<td>9.4</td>
<td>11.8</td>
<td>10.60</td>
</tr>
<tr>
<td>Alanine</td>
<td>11.8</td>
<td>12.8</td>
<td>12.30</td>
</tr>
<tr>
<td>Valine</td>
<td>6.9</td>
<td>6.8</td>
<td>6.85</td>
</tr>
<tr>
<td>Methionine</td>
<td>2.1</td>
<td>1.1</td>
<td>1.60</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>6.5</td>
<td>3.6</td>
<td>5.05</td>
</tr>
<tr>
<td>Leucine</td>
<td>6.0</td>
<td>6.0</td>
<td>6.00</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>4.1</td>
<td>2.5</td>
<td>3.30</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>4.3</td>
<td>3.1</td>
<td>3.70</td>
</tr>
<tr>
<td>Amide Content</td>
<td>46.6*</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Mole percent of total dicarboxylic acids.*
Number of ammonium and guanidinium groups

\[ \text{Number of ammonium and guanidinium groups} = (\text{Number of terminal ammonium groups}) + (\text{Number of } \epsilon\text{-ammonium groups of Lysine}) + (\text{Number of guanidinium groups of arginine}) \]

\[ = 300 + \left( \frac{6.75 \times 6 \times 10^4}{100} \right) + \left( \frac{4.3 \times 6 \times 10^4}{100} \right) \]

\[ = 6,930 \]

The amount of aluminum adsorbed by a T4 particle is in the range of 6,210 to 7,370 atoms of aluminum (Table 6). From a comparison of these calculations and the data in Table 6, it seems quite possible from a consideration of the head protein only that adsorption of aluminum is due to the formation of coordination complexes between aluminum and either the carboxyl or ammonium groups. However, it is more probable that the carboxyl groups are involved because these groups have been postulated to be responsible for metal ion binding by proteins (Froton and Simmonds, 1953; and Stumm and Morgan, 1962). On the basis of pH-metric and viscometric observations, carboxyl groups have also been shown to be involved in binding of aluminum by casein, gelatin, etc. (Schulman and Dogan, 1952; Salahuddin and Malik, 1962, 1964; Salahuddin, 1964; and Malik and Muzaferuddin, 1965).

B. Virus Inactivation by Aluminum

In order to study the condition of the "complexed" viruses, inactivation of the bacteriophages was studied in the presence of soluble aluminum. Figures 17 and 18 show the inactivation of bacteriophages T4 and MS2, respectively, at pH 5.0 in the presence of soluble aluminum. Inactivation of the bacteriophages at pH 6.0 and 9.0 was not studied because it was thought that the inactivation studies at pH 5.0 would reveal the entire picture due to the fact that the kinetics and the quantity of adsorption of aluminum by these bacteriophages are identical at pH values 5.0, 6.0, and 9.0.

It is seen that inactivation rates of both the bacteriophages in the presence of various soluble aluminum concentrations are the same as those of the control without any aluminum. Since bacteriophage assay involves adsorption of bacteriophage on the surface of the host bacterium, it may be concluded that different sets of sites or ionic groups are involved in the electrostatic interaction between the bacteriophage and the host bacterium preceding an infection and in the interaction between aluminum and the bacteriophage. Furthermore, complexing of aluminum does not in any manner interfere with the capacity of the phage particle to infect its host.

From the preceding discussion it is quite logical to assume that virus particles removed from a water supply by chemical coagulation and flocculation could remain "viable" in the settled sludge. In order
FIGURE 17. INACTIVATION OF BACTERIOPHAGE T4 IN THE PRESENCE OF SOLUBLE ALUMINUM AT pH 5.0.
FIGURE 18. INACTIVATION OF BACTERIOPHAGE MS2 IN THE PRESENCE OF SOLUBLE ALUMINUM AT pH 5.0.
to study this aspect, attempts were made to recover the bacteriophage particles from the settled floc following their removal from water by chemical coagulation and flocculation (jar tests). Table 8 shows the extent to which bacteriophages T4 and MS2 could be recovered from the settled floc by different elutants. Recoveries greater than 53.75 percent were never obtained. This is presumably due to the fact that the elutants used were not able to completely dissociate the bacteriophage particles from the floc resulting in infection of a host bacterial cell by more than one bacteriophage.

**TABLE 8**

**RECOVERY OF BACTERIOPHAGES FROM SETTLED FLOC FOLLOWING CHEMICAL COAGULATION AND FLOCCULATION**

<table>
<thead>
<tr>
<th>Elutant</th>
<th>Percent Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bacteriophage T4</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>28.50</td>
</tr>
<tr>
<td>3% Beef Extract</td>
<td>52.27</td>
</tr>
<tr>
<td>1% Bovine Serum Albumine</td>
<td>53.75</td>
</tr>
<tr>
<td>0.1 M Tris Buffer (pH 8.0)</td>
<td>42.05</td>
</tr>
<tr>
<td>0.2 M Phosphate Buffer (pH 8.0)</td>
<td>42.95</td>
</tr>
</tbody>
</table>

|                                | Bacteriophage MS2  |
|                                | 52.85              |
|                                | 52.85              |
|                                | 53.75              |
|                                | -                  |
|                                | 45.25              |

Chang, Isaac, and Baine (1953) were able to recover 60 percent of the removed viruses by redispensing the flocculated mixture at pH 7.6 with vigorous stirring. It is evident from these observations that disposal of water treatment plant sludges treating water containing pathogenic viruses might constitute a public health hazard. Especially in the case of land disposal of water treatment plant sludges, this might constitute a ground water contamination problem.

C. Virus Removal by Chemical Coagulation and Flocculation (Jar Tests)

(1) Optimum pH and aluminum sulfate dosages

In presenting the results of the coagulation and flocculation studies (jar tests), virus removals are reported as percent removals. It should be noted that for an initial input virus concentration of \(4 \times 10^5/\text{ml}\), a removal of 99 percent would mean a reduction in the virus filter from \(4 \times 10^5/\text{ml}\) to \(4 \times 10^3/\text{ml}\) in the supernatant. Correspondingly a removal of 98 percent would mean a reduction in the
virus titer from $4 \times 10^5$/ ml to $8 \times 10^3$/ ml and so on.

Figure 19 shows the removal of bacteriophage T4 and clay turbidity at different pH values and coagulant dosages. It is interesting to note that virus removal closely parallels turbidity removal. It can be seen from this figure that based on both virus and turbidity removal, the optimum aluminum sulfate dosage and pH for bacteriophage T4 removal was 40 to 50 mg/l ($1.2 \times 10^{-4}$ M to $1.5 \times 10^{-4}$ M as aluminum) and the lowest pH studied (5.24). The highest removal obtained was 98 percent. All subsequent experiments with bacteriophage T4 were performed at pH values ranging from 5.2 to 5.3 with 50 mg/l of aluminum sulfate.

Removal of bacteriophage MS2 is shown in Figure 20. The optimum aluminum sulfate dosage and pH was 40 to 50 mg/l ($1.2 \times 10^{-4}$ M to $1.5 \times 10^{-4}$ M as aluminum) at pH 6.0. Removal was 99.9 percent. Turbidity removal curves for MS2 were similar to those shown in Figure 19. Removals higher than 99.3 percent were never obtained at pH 5.10 even with higher aluminum sulfate dosage. However, all subsequent experiments with bacteriophage MS2 were performed at pH 6.0 with 50 mg/l of aluminum sulfate.

(2) Effect of calcium and magnesium on virus removal

Chang et al. (1958b) believed that the presence of calcium and magnesium ions in raw water interfered with virus removal by reducing the rate of coagulant-cation bacterial-virus complex formation. Experiments were performed to investigate this phenomenon in further detail. Figure 21 shows the kinetics of adsorption of aluminum by bacteriophage T4 at pH values 5.0 and 9.0 in the presence of 50 mg/l of each of the cations calcium and magnesium. Effect of these two cations on the removal of bacteriophage T4 by coagulation and flocculation (jar tests) is shown in Table 9. It was not possible to conduct jar tests at pH 9.0 due to the limitation of the carbonic acid-bicarbonate buffer system, the upper limit of pH for this system being 8.3.

<table>
<thead>
<tr>
<th>Calcium mg/l</th>
<th>Magnesium mg/l</th>
<th>Percent Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>97.86</td>
</tr>
<tr>
<td>25</td>
<td>25</td>
<td>98.02</td>
</tr>
<tr>
<td>50</td>
<td>50</td>
<td>97.91</td>
</tr>
</tbody>
</table>

TABLE 9

REMOVAL OF BACTERIOPHAGE T4 IN THE PRESENCE OF CALCIUM AND MAGNESIUM

Average Input Virus Concentrations: $4.05 \times 10^5$/ ml; pH range: 5.1-5.4

Aluminum Sulfate: 50 mg/l; Average Turbidity: 12.5 JTU
Figure 19. Removal of bacteriophage T4 and clay turbidity by coagulation and flocculation.

Average input virus conc: $4.5 \times 10^5$/ml

Aluminum sulfate dose (mg/l)
Average Input Virus Conc: $3.9 \times 10^5$/ml

**FIGURE 20. REMOVAL OF BACTERIOPHAGE MS2 BY COAGULATION AND FLOCCULATION.**
FIGURE 21. KINETICS OF ADSORPTION OF ALUMINUM BY BACTERIOPHAGE T4 IN THE PRESENCE OF CALCIUM AND MAGNESIUM.
It is evident that presence of calcium and magnesium ions up to a concentration of 50 mg/l does not interfere with kinetics of adsorption of aluminum or with removal of bacteriophage T4 by coagulation and flocculation. The fact that presence of bivalent cations like calcium and magnesium does not change the kinetics and stoichiometry of aluminum virus interaction is further evidence for the formation of coordination complexes between aluminum and virus coat protein.

(3) Effect of organic matter on virus removal

It is logical to assume that the presence of extraneous organic matter of proteinaceous character will interfere with virus removal by coagulation and flocculation. This may occur because of the competitive action of the organic matter in question with the virus particles in the coagulation and flocculation reaction. To study this effect, virus removal in the presence of albumin, wastewater and wastewater effluent was studied. Table 10 shows the removal of bacteriophage T4 in the presence of egg albumin, bovine serum albumin, settled wastewater and wastewater effluent. Removal of bacteriophage MS2 in the presence of settled wastewater and wastewater effluent is shown in Table 11.

It is seen that egg albumin, bovine serum albumin and settled wastewater interfered with bacteriophage T4 removal in the pH range 5.1-5.4 (Table 10). The albumins also interfered with the flocculation process as evidenced by lower turbidity removals. The effect of wastewater effluent was not very much pronounced either on turbidity removal or on the removal of bacteriophage T4 in the pH range 5.1 to 5.4. However, considerably lower removals were obtained for turbidity and bacteriophage MS2 in the range 5.9-6.1 in the presence of settled wastewater or wastewater effluent (Table 11). These observations indicate that the process of coagulation and flocculation may not be expected to operate with high efficiency if the raw water contains organic matter.

(4) Effect of preformed floc on virus removal

At this stage it is clear from the discussions in the preceding sections that the nature of the interaction between aluminum and virus is most probably that of a coordination complex formation. Consequently it follows that intimate contact between the virus particles and the soluble aluminum species is necessary before the formation of any hydrated aluminum oxide precipitate in a coagulation and flocculation system. Experiments were performed to study this aspect by adding the virus stock suspension to the water at various times after the addition of aluminum sulfate. Table 12 shows the results of this study.

It can be seen that preformed floc was not very effective in removing bacteriophage T4 from the water. Similar observations also have been reported in the literature (Chang, Isaac, and Baine, 1953). These observations demonstrate that intimate contact between the virus particles and aluminum is necessary before their incorporation into the floc masses of hydrated aluminum oxide precipitates and subsequent
TABLE 10
REMOVAL OF BACTERIOPHAGE T4 BY COAGULATION AND FLOCCULATION IN THE PRESENCE OF ORGANIC MATTER

Average Input Virus Concentration: 3.58 $\times$ 10$^5$/ml; pH range: 5.1-5.4
Aluminum Sulfate: 50 mg/l; Average Turbidity: 12.5 JTU

<table>
<thead>
<tr>
<th>Organic Matter</th>
<th>Concentration</th>
<th>Percent Removal</th>
<th>Bacteriophage T4</th>
<th>Turbidity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg Albumin</td>
<td>0 mg/l</td>
<td>98.02</td>
<td>99.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 mg/l</td>
<td>96.89</td>
<td>98.24</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20 mg/l</td>
<td>96.38</td>
<td>98.20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30 mg/l</td>
<td>95.29</td>
<td>98.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50 mg/l</td>
<td>94.83</td>
<td>95.84</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 mg/l</td>
<td>98.65</td>
<td>99.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 mg/l</td>
<td>97.67</td>
<td>98.80</td>
<td></td>
</tr>
<tr>
<td>Bovine Serum Albumin</td>
<td>20 mg/l</td>
<td>97.63</td>
<td>98.60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30 mg/l</td>
<td>96.85</td>
<td>98.40</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50 mg/l</td>
<td>96.00</td>
<td>95.44</td>
<td></td>
</tr>
<tr>
<td>Settled Wastewater**</td>
<td>0 ml/l</td>
<td>97.40</td>
<td>98.37</td>
<td></td>
</tr>
<tr>
<td></td>
<td>200 ml/l</td>
<td>95.71</td>
<td>98.63</td>
<td></td>
</tr>
<tr>
<td>Wastewater Effluent**</td>
<td>0 ml/l</td>
<td>97.40</td>
<td>98.37</td>
<td></td>
</tr>
<tr>
<td></td>
<td>200 ml/l</td>
<td>97.03</td>
<td>98.77</td>
<td></td>
</tr>
</tbody>
</table>

*Initial turbidity values ranged from 18 to 32 JTU when settled wastewater was added.

**Characteristics:

<table>
<thead>
<tr>
<th>Raw Wastewater</th>
<th>Wastewater Effluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-day BOD (mg/l)</td>
<td>445</td>
</tr>
<tr>
<td>Total Suspended Solids (mg/l)</td>
<td>210</td>
</tr>
<tr>
<td>Volatile Suspended Solids (mg/l)</td>
<td>194</td>
</tr>
</tbody>
</table>
TABLE 11

REMOVAL OF BACTERIOPHAGE MS2 BY COAGULATION AND FLOCCULATION IN THE PRESENCE OF WASTEWATER AND WASTEWATER EFFLUENT

Average Input Virus Concentration: \(3.87 \times 10^5\) /ml; pH range: 5.9-6.1

Aluminum Sulfate: 50 mg/l; Average Turbidity: 12.5 JTU

<table>
<thead>
<tr>
<th>Percent Removal</th>
<th>Bacteriophage MS2</th>
<th>Turbidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Settled Wastewater or Wastewater Effluent</td>
<td>99.82</td>
<td>98.10</td>
</tr>
<tr>
<td>200 ml/l Settled Wastewater***</td>
<td>89.60</td>
<td>92.10</td>
</tr>
<tr>
<td>200 ml/l Wastewater Effluent***</td>
<td>94.00</td>
<td>93.60</td>
</tr>
</tbody>
</table>

*Initial turbidity was 19.0 JTU when settled wastewater was added.

***Characteristics:

<table>
<thead>
<tr>
<th></th>
<th>Raw Wastewater</th>
<th>Wastewater Effluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-day BOD (mg/l)</td>
<td>181</td>
<td>50</td>
</tr>
<tr>
<td>Total Suspended Solids (mg/l)</td>
<td>253</td>
<td>10</td>
</tr>
<tr>
<td>Volatile Suspended Solids (mg/l)</td>
<td>178</td>
<td>9</td>
</tr>
</tbody>
</table>
TABLE 12

REMOVAL OF BACTERIOPHAGE T4 BY PREFORMED FLOCS

Average Input Virus Concentration: 4.0 x 10^5/ml; pH range: 5.1-5.4
Aluminum Sulfate: 50 mg/l; Average Turbidity: 12.5 JTU

<table>
<thead>
<tr>
<th>Bacteriophage T4 added (minutes after aluminum sulfate addition)</th>
<th>Percent Removal</th>
<th>Turbidity</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>98.04</td>
<td>99.17</td>
<td>Control</td>
</tr>
<tr>
<td>-</td>
<td>80.60</td>
<td>97.0</td>
<td>Preformed floc not in situ. Bacteriophage T4 added one min after addition of preformed floc.</td>
</tr>
<tr>
<td>1</td>
<td>76.40</td>
<td>99.0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>60.50</td>
<td>99.04</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>37.25</td>
<td>99.17</td>
<td></td>
</tr>
</tbody>
</table>
removal by settling. In contrast, removal of clay turbidity by coag- 
ulation and flocculation with aluminum sulfate is due to a physical inter- 
action between aluminum and the clay particles resulting in adsorption of 
polymeric aluminum hydrolysis species onto the clay particles and con- 
sequent aggregation by interparticle bridging involving particle trans- 
port and chemical interaction (Stumm and O'Melia, 1968).

(5) Effect of polyelectrolytes on virus removal

Commercially available synthetic polyelectrolytes 
(coagulant aids) are being used extensively in the water treatment 
industry for better coagulation and flocculation and longer filter 
runs. It was thought appropriate to study the effect of these polyelec- 
trolytes on virus removal by chemical coagulation and flocculation 
(jar tests). Inactivation of bacteriophages T4 and MS2 in the presence 
of the polyelectrolytes in deionized water was studied in order to be 
able to interpret the results of the jar tests using the polyelectro- 
lytes. Samples for bacteriophage assay were withdrawn after a contact 
time of 1 hr. Table 13 shows the results of this study. Tables 14 to 
19 show the results of the jar tests performed at pH values of approxi- 
mately 5.2 and 6.0 for bacteriophages T4 and MS2, respectively. These 
were the best pH values for their removal in jar tests.

It is evident from Table 13 that both the cationic polyelec- 
trolytes, Primafloc C-7 and Catfloc, inactivated bacteriophages T4 and 
MS2. This was presumably due to adsorption of the phage particles to 
the cationic sites on the polyelectrolyte molecules, which resulted in 
subsequent infection of one host bacterium by more than one phage par- 
ticle during phage assay. However, no attempt was made to reanimate 
or to free the phage particles from the polyelectrolyte. The anionic 
polyelectrolytes, Primafloc A-10 and Coagulant Aid #243, did not in- 
activate T4 and MS2 particles. This was due to the absence of cationic 
adsorption sites on the polyelectrolyte molecules.

It is seen from Table 14 that both the cationic polyelectro- 
lytes used were quite effective as coagulant aids for bacteriophage T4 
removal in the dosage range 0.5 to 1.0 mg/l. Turbidity removal was 
less efficient at higher dosages even though virus removal was higher. 
This was presumably due to virus inactivation by the polyelectrolyte 
per se. No improvement was noticed in the removal of bacteriophage 
MS2 (Table 15). Both the cationic polyelectrolytes were quite effec- 
tive as prime coagulants (Tables 16 and 17). Primafloc C-7 and Catfloc 
in the dosage ranges 5.0 to 7.5 mg/l and 10.0 to 12.5 mg/l, respectively, 
gave the best results from the viewpoint of both virus and turbidity re- 
moval. Neither of the anionic polyelectrolytes used were effective as 
coagulant aids (Tables 18 and 19). This was presumably due to the ab- 
sence of a sufficient concentration of calcium ions in the system which 
has been thought to be necessary for the action of anionic polyelectro- 
lytes (Packham, 1967). This was also the reason for not using anionic 
polyelectrolytes as prime coagulants in this study.
TABLE 13

INACTIVATION OF BACTERIOPHAGES T4 AND MS2
IN THE PRESENCE OF POLYELECTROLYTES (COAGULANT AIDS)

Average Input Virus Concentration: 3.44 x 10^5/ml

<table>
<thead>
<tr>
<th>Polyelectrolyte</th>
<th>Concentration mg/l</th>
<th>pH</th>
<th>Bacteriophage</th>
<th>Percent Inactivation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primafloc C-7</td>
<td>0.5-1.5</td>
<td>5.2</td>
<td>T4</td>
<td>81*</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>6.0</td>
<td>MS2</td>
<td>85</td>
</tr>
<tr>
<td>Catfloc</td>
<td>0.5-10.0</td>
<td>5.2</td>
<td>T4</td>
<td>77*</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>6.0</td>
<td>MS2</td>
<td>82</td>
</tr>
<tr>
<td>Primafloc A-10</td>
<td>1.0-5.0</td>
<td>5.2</td>
<td>T4</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>6.0</td>
<td>MS2</td>
<td>None</td>
</tr>
<tr>
<td>Coagulant Aid #243</td>
<td>1.0-5.0</td>
<td>5.2</td>
<td>T4</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>6.0</td>
<td>MS2</td>
<td>None</td>
</tr>
</tbody>
</table>

*Average value for the concentration range of polyelectrolyte
### TABLE 16

**REMOVAL OF BACTERIOPHAGE T4 BY COAGULATION AND FLOCCULATION WITH CATIONIC POLYELECTROLYTES AS PRIME COAGULANTS**

Average Input Virus Concentration: $4.98 \times 10^5$/ml

pH range: 5.2-5.5; Average Turbidity: 12.5 JTU

<table>
<thead>
<tr>
<th>Cationic Polyelectrolyte</th>
<th>Concentration mg/l</th>
<th>Percent Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Bacteriophage T4</td>
</tr>
<tr>
<td>Primafloc C-7</td>
<td>2.5</td>
<td>84.40</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>99.27</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>99.93</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>99.98</td>
</tr>
<tr>
<td></td>
<td>20.0</td>
<td>99.99</td>
</tr>
<tr>
<td>Catfloc</td>
<td>1.0</td>
<td>17.00</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>52.40</td>
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<td></td>
<td>5.0</td>
<td>85.70</td>
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<td></td>
<td>7.5</td>
<td>97.22</td>
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<td>10.0</td>
<td>99.04</td>
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<td>12.5</td>
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</tr>
<tr>
<td></td>
<td>15.0</td>
<td>99.84</td>
</tr>
<tr>
<td></td>
<td>20.0</td>
<td>99.93</td>
</tr>
<tr>
<td></td>
<td>25.0</td>
<td>99.94</td>
</tr>
</tbody>
</table>
TABLE 17

REMOVAL OF BACTERIOPHAGE MS2 BY COAGULATION AND FLOCCULATION
WITH CATIONIC POLYEOLECTROLYTES AS PRIME COAGULANTS

Average Input Virus Concentration: $2.8 \times 10^5$/ml

pH range: 5.8-5.9; Average Turbidity: 12.5 JTU

<table>
<thead>
<tr>
<th>Cationic Polyelectrolyte</th>
<th>Concentration mg/l</th>
<th>Percent Removal Bacteriophage MS2</th>
<th>Turbidity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primafloc C-7</td>
<td>2.5</td>
<td>93.05</td>
<td>89.25</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>98.72</td>
<td>97.84</td>
</tr>
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<td></td>
<td>7.5</td>
<td>99.15</td>
<td>97.84</td>
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<td>10.0</td>
<td>97.30</td>
<td>80.00</td>
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<td></td>
<td>20.0</td>
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<td></td>
<td>5.0</td>
<td>98.02</td>
<td>86.65</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>99.57</td>
<td>97.08</td>
</tr>
<tr>
<td>Catfloc</td>
<td>12.5</td>
<td>99.21</td>
<td>96.75</td>
</tr>
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<td></td>
<td>15.0</td>
<td>98.74</td>
<td>96.30</td>
</tr>
<tr>
<td></td>
<td>20.0</td>
<td>98.76</td>
<td>76.70</td>
</tr>
</tbody>
</table>
TABLE 17

REMOVAL OF BACTERIOPHAGE MS2 BY COAGULATION AND FLOCCULATION
WITH CATIONIC POLYELECTROLYTES AS PRIME COAGULANTS

Average Input Virus Concentration: \(2.8 \times 10^5/\text{ml}\)

pH range: 5.8-5.9; Average Turbidity: 12.5 JTU

<table>
<thead>
<tr>
<th>Cationic Polyelectrolyte</th>
<th>Concentration mg/l</th>
<th>Percent Removal (%)</th>
<th>Bacteriophage MS2</th>
<th>Turbidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primafloc C-7</td>
<td>2.5</td>
<td>93.05</td>
<td>89.25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>98.72</td>
<td>97.84</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>99.15</td>
<td>97.84</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>97.30</td>
<td>80.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20.0</td>
<td>95.40</td>
<td>15.00</td>
<td></td>
</tr>
<tr>
<td>Catfloc</td>
<td>5.0</td>
<td>98.02</td>
<td>86.65</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>99.57</td>
<td>97.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12.5</td>
<td>99.21</td>
<td>96.75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15.0</td>
<td>98.74</td>
<td>96.30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20.0</td>
<td>98.76</td>
<td>76.70</td>
<td></td>
</tr>
</tbody>
</table>
TABLE 18

REMOVAL OF BACTERIOPHAGE T4 BY COAGULATION AND FLOCCULATION
WITH ALUMINUM SULFATE AND ANIONIC POLYELECTROLYTES AS COAGULANT AIDS

Average Input Virus Concentration: $2.78 \times 10^5$/ml; pH range: 5.0-5.4

Aluminum Sulfate: 50 mg/l; Average Turbidity: 12.5 JTU

<table>
<thead>
<tr>
<th>Anionic Polyelectrolyte</th>
<th>Concentration mg/l</th>
<th>Percent Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Bacteriophage T4</td>
</tr>
<tr>
<td></td>
<td>0.0</td>
<td>97.13</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>97.20</td>
</tr>
<tr>
<td>Primafloc A-10</td>
<td>1.0</td>
<td>97.20</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>97.33</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>97.88</td>
</tr>
<tr>
<td></td>
<td>0.0</td>
<td>98.03</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>97.80</td>
</tr>
<tr>
<td>Coagulant Aid #243</td>
<td>2.0</td>
<td>97.22</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>96.93</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>96.60</td>
</tr>
</tbody>
</table>
TABLE 19
REMOVAL OF BACTERIOPHAGE MS2 BY COAGULATION AND FLOCCULATION
WITH ALUMINUM SULFATE AND ANIONIC POLYELECTROLYTES AS COAGULANT AIDS

Average Input Virus Concentration: $2.76 \times 10^5 / \text{ml}$; pH range: 5.9-6.0

Aluminum Sulfate: 50 mg/l; Average Turbidity: 12.5 JTU

<table>
<thead>
<tr>
<th>Anionic Polyelectrolyte</th>
<th>Concentration mg/l</th>
<th>Percent Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Bacteriophage MS2</td>
</tr>
<tr>
<td>Primafloc A-10</td>
<td>0.0</td>
<td>99.78</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>99.70</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>99.34</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>98.75</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>97.68</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>99.61</td>
</tr>
<tr>
<td>Coagulant Aid #243</td>
<td>2.0</td>
<td>97.53</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>98.36</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>95.22</td>
</tr>
</tbody>
</table>
D. Qualitative Description of Virus Removal by Chemical Coagulation and Flocculation

For purposes of attaining a better understanding of the removal of viruses from water by chemical coagulation and flocculation, it seems appropriate to present a qualitative description of the process. On the basis of the results obtained in this study and the information available in the literature, it is possible to visualize the entire process.

In a natural surface water, depending on the concentrations of cations like sodium and calcium, a certain fraction of the virus particles present remain reversibly adsorbed to the clay particles constituting turbidity due to the formation of a clay-cation-virus bridge (Carlson et al., 1968). The other fraction may be assumed to be free. Addition of a coagulant like aluminum sulfate to this water immediately results in the formation of certain hydrolyzed polymeric multivalent aluminum species depending on the pH of the water (Black and Chen, 1967). Interaction between aluminum species and viruses, other organic matter, and clay particles proceeds immediately. The interaction between aluminum and viruses and other organic matter is a very rapid one and presumably results in the formation of coordination complexes. On the other hand, the interaction between aluminum and the clay particles constituting turbidity results in adsorption of polynuclear aluminum hydrolysis species to the clay particles and consequent aggregation of the destabilized particles by interparticle bridging involving particle transport and chemical interaction (Stumm and O'Melia, 1968). Furthermore, precipitation of hydrated aluminum oxide species proceeds simultaneously, incorporating the complexed virus particles and the aggregating clay particles which then grow into "flocs" and ultimately settle down resulting in a clear supernatant. Presence of organic matter in the water can have considerable effect on the overall efficiency of the process by interfering with virus removal. This may occur because of the competitive action of the organic matter with the virus particles in the coagulation and flocculation reaction.

6. SUMMARY AND CONCLUSIONS

It has been shown that removal of bacteriophages T4 and MS2 from water by chemical coagulation and flocculation with aluminum sulfate consists of a primary reaction step which involves interaction between aluminum and virus coat protein. The reaction was found to be instantaneous and proceeded according to a definite stoichiometry. The kinetics and the stoichiometry of the reaction were not affected by the pH, period and condition of storage of the virus particles, the quantity of available aluminum or the presence of bivalent cations like calcium and magnesium as studied in this investigation.

Aluminum adsorption data were found to fit Langmuir adsorption equation. Amounts of aluminum adsorbed by a single virus particle at pH values 5.0, 6.0, and 9.0 were calculated and found to be comparable.
Considering the aqueous chemistry of aluminum, amino acid composition of the virus coat protein and the evidences of an aluminum-protein interaction as reported in the literature, it was concluded that the interaction between aluminum and virus possibly resulted in the formation of coordination complexes between aluminum and the carboxyl groups associated with the virus coat protein. The complexed viruses were not inactivated and active viruses could be recovered from the settled floc following their removal from water by coagulation and flocculation.

The process of chemical coagulation and flocculation was found quite effective in removing bacteriophages T4 and MS2 from water. The optimum coagulant dosages and pH values were 40 to 50 mg/l of aluminum sulfate at pH 5.24 for bacteriophage T4 and at pH 6.0 for bacteriophage MS2. The highest removals attained were 98.0 and 99.9 percent, respectively. Presence of bivalent cations like calcium and magnesium up to a concentration of 50 mg/l each did not interfere with the efficiency of the process. Organic matter like albumins and that associated with wastewater lowered the removal efficiency considerably. Commercially available cationic polyelectrolytes were found effective both as coagulant aids and as prime coagulants.

Based on the findings of this investigation, the following conclusions may be drawn:

(i) Removal of viruses by chemical coagulation and flocculation with aluminum sulfate comprises of a primary instantaneous reaction step which results possibly in the formation of coordination complexes between aluminum and the carboxyl groups of the virus coat protein.

(ii) Virus particles are not inactivated as a result of this interaction between aluminum and the virus particles and remain active in the settled sludge following their removal from water by coagulation and flocculation with aluminum sulfate.

(iii) Chemical coagulation and flocculation is an effective process in removing viruses from water. Removals in the range 98.0 to 99.9 percent can be expected.

(iv) Presence of bivalent cations like calcium and magnesium up to a concentration of 50 mg/l each does not interfere with the efficiency of the process.

(v) The efficiency of virus removal is reduced when the raw water contains organic matter.

(vi) Intelligent use of commercially available cationic polyelectrolytes with or without hydrolyzed metal ions may markedly increase the efficiency of the coagulation and flocculation process.

It seems appropriate to note the recent observations made by Stumm and O'Melia (1968):
"It is important to reemphasize that coagulation phenomena in natural systems are quite specific. This specificity arises from the fact that colloid stability is affected by colloid-solvent, coagulant-solvent, and colloid-coagulant interactions . . . Overemphasis on electrostatic phenomena in studies of coagulation in natural systems can produce results that are inefficient, uneconomical, or both."

7. ENGINEERING SIGNIFICANCE

The most significant result of this study is that a more complete understanding of the removal of viruses from water by chemical coagulation and flocculation has been attained. This is very pertinent in view of the more stringent water quality standards which are foreseen in the near future. Considering the rapid population growth and accompanying urbanization, there is going to be a greater demand for water for public consumption and other uses which, in turn, will require more water reuse. Thus, the removal of viruses from water supplies becomes extremely important. A good understanding of the basic mechanisms involved in the removal of viruses from water by chemical coagulation and flocculation and the role of various other parameters affecting the process should aid in developing design standards for water treatment facilities. It will enable such standards to be developed on a sound, realistic and rational basis.

From the experimental results it is possible to extrapolate some generalizations which are of practical significance. The foremost among the generalizations is the interpretation of T4 and MS2 removal data in terms of viruses which may be more significant in water supplies, viz., human enteric viruses. Confirmation of T4 removal data by MS2 which is very similar to picornaviruses (enteroviruses of man and other animals) in shape, size and the nucleic acid contained, shows that the process of chemical coagulation and flocculation may be quite effective in removing enteroviruses from water. From the results of jar tests using polyelectrolytes it is apparent that intelligent use of commercially available cationic polyelectrolytes with or without hydrolyzed metal ions may markedly increase the efficiency of the process. However, from economic considerations, the use of the cationic polyelectrolytes as coagulant aids with hydrolyzed metal ions seem more favorable. Based on today's market, polyelectrolytes are rather expensive.

From the results of virus inactivation studies and virus recovery from settled floc it is possible to extrapolate some useful information. The observation that viruses are not inactivated as a result of the complex formation and remain viable in the settled sludge immediately leads to the potential hazard for ground water contamination during land disposal of sludges from water treatment plants treating water contaminated with pathogenic viruses. However, more information should be gained in this area such as the fate, including the survival, of the viable viruses in sludge during land disposal before any definite conclusion can be reached.
On the basis of the findings of Chang, Isaac and Baine (1953) that approximately 20 min would be required for the completion of the first-stage reaction (aluminum-virus complex formation) it may be speculated that the incremental addition of the coagulant may be advantageous in optimizing the process for virus removal. In such a process the procedure might be to first add aluminum sulfate to the raw water in an amount equivalent to or less than its solubility, taking into account the pH of the system; second, provide sufficient contact time (20 min) for the formation of the aluminum-virus complex; and, third, add sufficient aluminum sulfate to bring the total amount added up to the predetermined optimum dosage for coagulation and flocculation. Following the second addition of coagulant, the usual period of flocculation and sedimentation would be included. However, the findings of the current study that the interaction between the aluminum and the virus is instantaneous immediately rules out such a speculation.

The observation that the presence of organic matter interferes with virus removal by coagulation and flocculation leads to another generalization. For virus removal, the process may be more reliable as practiced at a water treatment plant than at a wastewater renovation plant because of the presence of higher concentration of organic matter in a wastewater effluent.

The findings of this study indicate that virus removal by coagulation and flocculation parallel turbidity removal. Robeck, Clarke and Dostal (1962) observed that effective coagulation and flocculation was an essential prerequisite for effective virus removal by rapid sand filtration. They also observed that any breakthrough in turbidity through the filter was accompanied by a breakthrough in virus. Consequently, it may be suggested that in a water treatment plant care should be taken to produce a high quality effluent in terms of turbidity. Any breakthrough in turbidity should serve as a warning to the operator. This is particularly important during heavy pollution of the raw water and may become more critical when marginal chlorination is practiced.

Finally, the results of this study suggest that the process of chemical coagulation and flocculation as it is practiced today can be quite effective in removing pathogenic viruses from water if proper care is taken to control pH and other parameters which affect the process. It should be noted that pH was not found to be an important variable with respect to the formation of the aluminum-virus complex; however, such is not the case when both coagulation and flocculation are considered.
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