APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 1985, p. 91–95
0099-2240/85/010091-05$02.00/0

Vol. 49, No. 1

Adsorption of Viruses to Charge-Modified Silica

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Received 20 April 1984/Accepted 5 October 1984.

The purpose of this study was to provide a clearer understanding of virus adsorption, focusing specifically on the role of electrostatic interactions between virus particles and adsorbent surfaces. The adsorption of poliovirus 1, reovirus types 1 and 3, and coliphages MS-2 and T2 to colloidal silica synthetically modified to carry either positive or negative surface charge was evaluated. Adsorption experiments were performed by combining virus and silica in 0.1-ionic-strength buffers of pH 4.0, 6.4, and 8.5. Samples agitated for specified adsorption periods were centrifuged to pellet adsorbent particles plus adsorbed virus, and the supernatants were assayed for unadsorbed virus. All viruses adsorbed exclusively to negatively charged silica at pH values below their isoelectric points, i.e., under conditions favoring a positive surface charge on the virions. Conversely, all viruses adsorbed exclusively to positively charged silica at pH values above their isoelectric points, i.e., where virus surface charge is negative. Viruses in near-isoelectric state adsorbed to all types of silica, albeit to a lesser degree.

Continued improvements in methods for the successful concentration and detection of viruses from water and wastewater are hindered by a lack of understanding of mechanisms of virus adsorption to the types of filters used in these techniques. Mix (7) presented the first comprehensive discussion of factors affecting virus-filter interactions. He suggested that viruses and adsorbents carried surface charges determined by their chemical composition and that the nature of the surface charges could be controlled by pH and electrolytes. Sobsey and Jones (13) provided further support for this hypothesis by showing that negatively charged viruses adsorbed to positively charged membranes at near-neutral pH without the addition of salts.

The interest in virus adsorption mechanisms is not limited to virus-filter interactions. The practice of applying treated sewage solids and wastewaters to land, as an alternative to their discharge into surface waters, has created the concern that viruses present in soil may migrate downward, eventually resulting in contamination of underlying ground water supplies. Field studies (11) and laboratory investigations using soil columns (2, 5, 6) showed that viruses can migrate through soils, probably through a series of sequential adsorption and elution steps. Although certain factors are known to affect this downward migration (virus type and strain, soil pH, cation-exchange capacity of the soil), a thorough understanding of the mechanisms controlling the virus-soil interactions is lacking.

This study was undertaken to further define the role that electrostatic forces have in governing virus adsorption to solid surfaces. Interpretation of much of the published virus adsorption data is made difficult by the complexity of experimental systems involving many variables. The aim of this project was to examine adsorption under simple, well-defined conditions which would allow clear interpretation of data.

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MATERIALS AND METHODS

Silica. Syloid 266 silica particles were obtained from Davison Chemical Division, W. R. Grace and Co., Baltimore, Md. Surface modifications of the Syloid 266 were made to achieve desired surface properties on the particles. Organosilanes were reacted with the Syloid silica under controlled conditions to favor the reaction scheme shown in Fig. 1. Particles of three types were made, bearing primary amine, quaternary amine, or carboxyl functional groups on the silica surface. Briefly, Syloid 266 particles were washed in concentrated HCl for 30 min, filtered through a Büchner funnel under slow vacuum, and rinsed exhaustively with distilled water. Washed Syloid 266 was dried at 350°F (176.8°C) for 2 h, and then 10 g of the silica was added to 100 ml of a 10% solution of the desired organosilane (Fig. 1) in toluene. The reactants were refluxed overnight, filtered through a Büchner funnel, rinsed well with ethanol, and ovendried at 300°F (149.6°C) for several hours. This procedure was followed for preparation of silica modified to carry either primary or quaternary amine groups. Silica carrying the primary amine groups was further modified by reaction with 1% aqueous succinic anhydride solution adjusted to pH 6.0 with sodium hydroxide, to produce particles bearing carboxyl residues. The reaction mixture was refluxed as before, and the product was washed exhaustively with distilled water and dried.

A Zeta-Meter (Zeta-Meter, Inc., New York, N.Y.) was used to evaluate the surface charges on the modified silica particle at various pH values. Untreated and silane-reacted Syloid 266 samples were dispersed in beakers containing buffer of pH 4.0, 5.0, 6.5, 7.5, or 8.5. Samples were mixed well for several minutes to assure time for ionization of the surface functional groups. The sample to be measured was placed into the electrophoresis cell, electrodes were connected, and a voltage of 50 V was applied across the cell. Velocities of individual particles over a given tracking distance were recorded, as was direction of particle movement. Average velocities were calculated from data on at least 10 individual particles per sample. Electrophoretic mobility (EM) was calculated according to the equation EM = (μ/v)

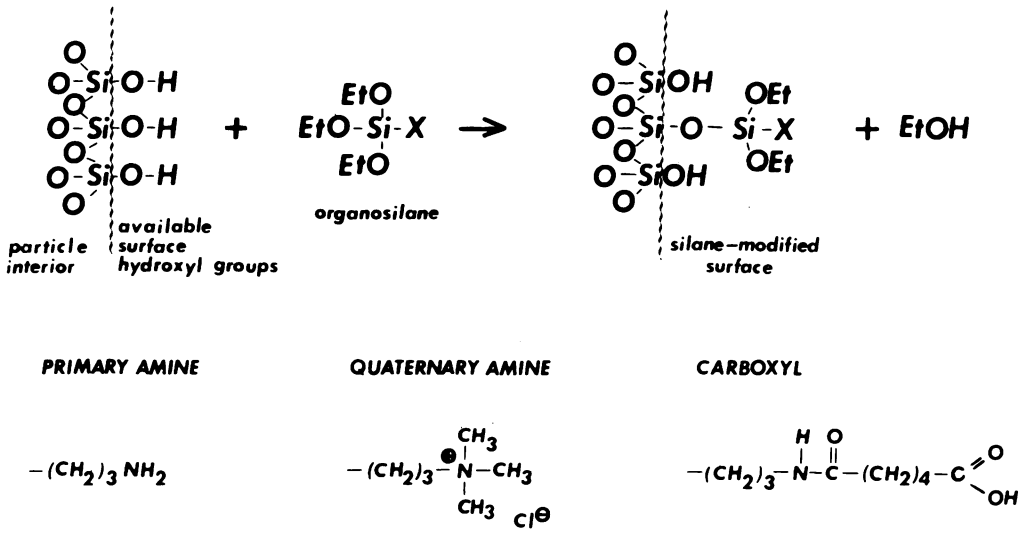

FIG. 1. Silica surface modification. X in the general formula for organosilane as shown in the equation represents one of the three moieties given in the lower portion.

(cm/s), where μ is the distance over which the particle is tracked (micrometers), cm is the distance between ports of the cell (10 cm), v is the voltage applied, and s is the average time in seconds required to track one particle a given distance, μ.

EM results were assigned a positive or negative value depending upon the direction of particle movement. Particles moving toward the anode were assigned negative EM values, whereas those moving toward the cathode were given positive values.

Viruses. Poliovirus 1 strains included strain Brunhilde (ATCC VR-58) and strain LSc (obtained from the culture collection of the Department of Virology and Epidemiology, Baylor College of Medicine, Houston, Tex.) Bacteriophage MS-2 (host *Escherichia coli* B ATCC 15597) and T2 (host *E. coli*) were obtained from G. Schaiberger (University of Miami, Miami, Fla.) Reovirus types 1 (strain Lang) and 3 (strain Dearing) were provided by Robert F. Ramig (Baylor College of Medicine). All viruses were purified by CsCl density gradient ultracentrifugation. Virus-containing gradient fractions were pooled and dialyzed at 4°C for 48 h against frequent changes of appropriate buffer. Virus for adsorption studies was dialyzed against pH 7.0 NaCl-phosphate buffer containing 0.08-ionic-strength PO_4, assayed, and stored at −70°C.

Agarose isoelectrofocusing of viruses. The method developed for this study for determining virus (pI) has been described previously (15). The technique was based upon previously reported methods for focusing proteins in horizontal granulated gel beds (9).

Adsorption studies. A 3-mg portion of silica, bearing carboxyl, primary amine, or quaternary amine surface functional groups, was added to 1.5-ml-capacity microcentrifuge tubes (Denville Scientific, Denville, N.J.) followed by the addition of 0.5 ml of 0.1 M ionic strength buffer of pH 4.0, 6.4, or 8.5. Tubes were vortexed and allowed to stabilize with respect to temperature (4°C) and adsorbent ionization for a minimum of 30 min. At time zero, 5 μl (approximately 10^5 PFU/ml in the case of animal viruses and 10^7 PFU/ml for bacteriophages) of purified virus was added to each tube. Tubes were immediately vortexed to mix the contents and then agitated at 300 to 400 rpm on a shaker table. At desired times, ranging from 2 to 60 min, tubes were removed from the shaker apparatus and spun at 12,000 rpm in an Eppendorf microcentrifuge (Brinkmann Instruments, Inc., Westbury, N.Y.) for 1 min. Pellets containing silica plus adsorbed virus were discarded, and supernatants were assayed for unadsorbed virus. Plaque assays for polioviruses were done on confluent BGM cell monolayers grown in 1-ounce (30-ml) glass prescription bottles, as previously described (3). Bacteriophages were assayed by the PFU method (10). At each time point, experimental tubes were matched with control tubes containing only virus and buffer, thus providing a means of monitoring any virus inactivation due to buffer pH.

RESULTS

EM of charge-modified silica. The EM of untreated Syloid 266 silica indicated that the particles bear a negative surface charge over the pH range 4.0 to 8.5 (Fig. 2). Indications that refluxing silica particles with selected organosilanes produced the desired surface modifications were deviations from the silica electrophoretic mobility curve by samples of reacted silica (Fig. 2). The most positive surface charge existed on the silicas bearing quaternary and primary amine groups. These retained their positive charge throughout the pH range tested. Carboxyl groups, contributed to the silica surface by succinic anhydride treatment, gave rise to particles of extreme negative surface charge. The carboxylated silica remained negatively charged over the entire pH range of interest.

Virus pI. Poliovirus 1 (strain LSc) was isoelectric at pH 6.6. A second strain of poliovirus 1 (strain Brunhilde) focused at pH 7.1. Phage MS-2 was isoelectric at pH 3.9. Unsuccessful attempts were made to focus reovirus types 1 (strain Lang) and 3 (strain Dearing). However, reovirus type 3 has been reported to be isoelectric at pH 3.9 by column focusing techniques (1).

Adsorption of low-pI viruses: MS-2, T2, and reovirus. The adsorption of MS-2 to the variously charged silica particles is shown in Fig. 3. At pH 4.0, i.e., near the virus pI, 0.5 to 1.5 logs of virus were removed from the liquid phase onto each of the silica types. At pH 6.4, almost no adsorption occurred to the carboxylated silica, although adsorption to the primary and quaternary amine-modified silicas was substantial. A similar pattern was noted at pH 8.5. Virus removal by each of the silicas was pH dependent, since

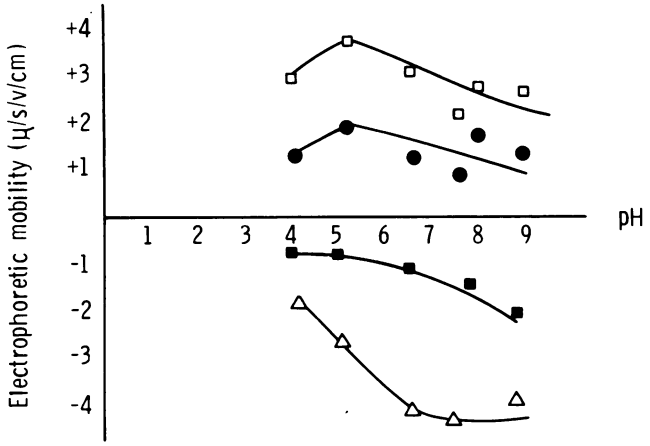

FIG. 2. EM curves for untreated and silane-reacted silica. Symbols: □, silica plus quaternary amine; ●, silica plus primary amine; △, silica plus carboxyl group; ■, untreated silica.

adsorption to the negatively charged carboxylated silica decreased with increasing pH, and adsorption to the positively charged amines was enhanced as pH was raised above the virus pI, i.e., when virions had a net negative charge on their surfaces.

Adsorptive behavior was very similar for reovirus (Fig. 4). Some adsorption to all types of silica occurred at pH 4.0 for both types 1 and 3 of the virus. At pH 6.4, reovirus type 1 adsorbed well to the amine-bearing silicas, but not to the carboxylated particles. Results were similar at pH 8.7. Reovirus type 3 adsorbed best to the amine-bearing silicas also at high pH and best to the carboxylated silica at low pH.

Coliphage T2 adsorption paralleled MS-2 and reovirus results (Fig. 5). Some adsorption occurred to all types of silica at the lower pH. An increase in pH enhanced adsorption to the positively charged particles. In the range of 2 pH units above the virus pI, adsorption was negligible to the carboxylated silica.

Adsorption of near-neutral-pI viruses: poliovirus 1 strains LSc and Brunhilde. The adsorption patterns of poliovirus 1 (strains LSc and Brunhilde) at pH 4.0, 6.4, and 8.5 are

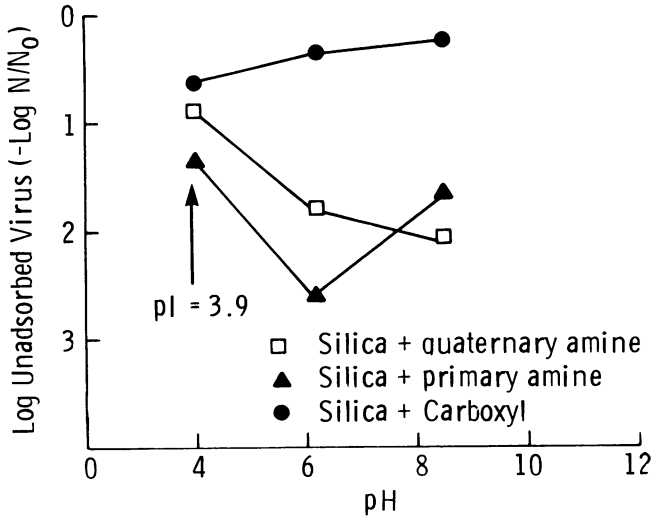

FIG. 3. MS-2 adsorption to modified silica.

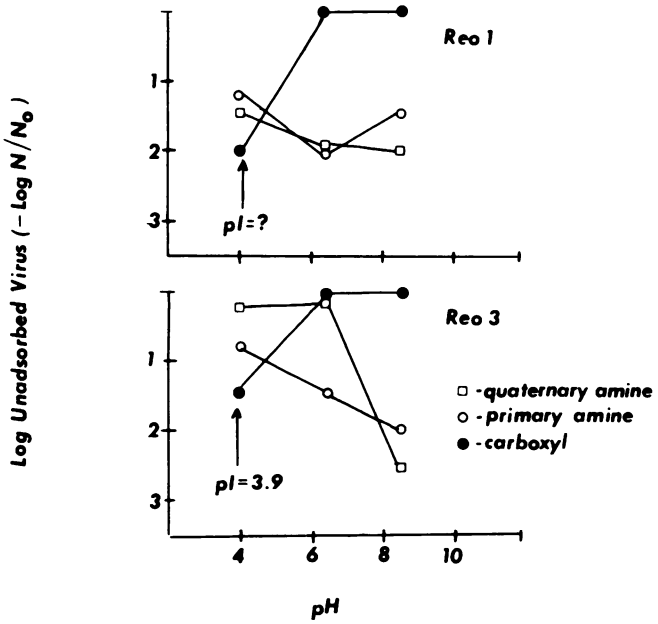

FIG. 4. Reovirus type 1 and 3 adsorption to modified silica.

shown in Fig. 6 and 7. The results for the two strains are nearly identical. In contrast to results for the other viruses, the greatest degree of selective adsorption was noted at pH 4.0. The virus adsorbed well to the carboxylated silica at this pH, but not to the positively charged silicas. As pH was increased toward the virus pI, some adsorption, though minimal, occurred onto all types of silica. Very little adsorption of virus to the carboxylated silica occurred at pH 8.5, whereas adsorption to the positively charged silica was enhanced.

To investigate virus adsorption at pH conditions above the virus pI, experiments were conducted with strain LSc at pH 9.5 and 10.0. At high pH, adsorption was greatest to the positively charged silicas and negligible to negatively charged particles (Fig. 7).

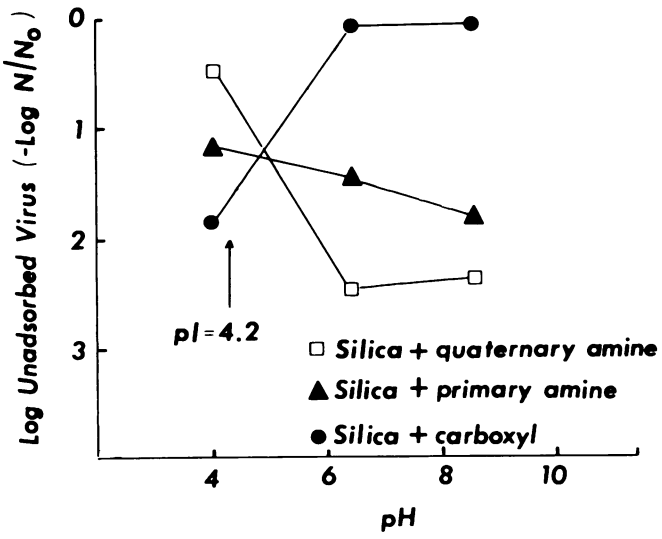

FIG. 5. T2 adsorption to modified silica.

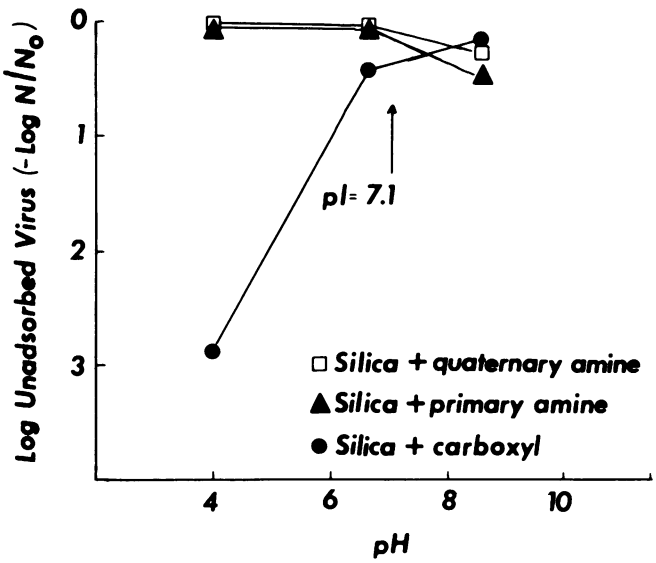

FIG. 6. Poliovirus 1 (strain Brunhilde) adsorption to silica.

DISCUSSION

Results of the adsorption experiments showed that variation of pH greatly influenced the degree of virus adsorption to silica particles. The pH of the dispersion medium determines the relative surface charge states of the virus and adsorbent by controlling the ionization on their surface groups. Figure 1 shows the three types of organic groups added to silica to give the desired surface charge characteristics. Whereas untreated silica carries a net negative charge over the pH range 4.0 to 8.5 (Fig. 2), explained by the fact that the surface hydroxyl groups were ionized to $-O^-$ above the reported pI of 2.0 (8), the amine-modified silicas carry a net positive surface charge over the same pH range. Such a change results from ionization of the amino groups to $-NH_3^+$. A carboxylated silica was likewise prepared, the chemistry of which is shown in Fig. 1. A strongly negative charge results over the pH range of interest, due to ionization of carboxyl groups to $-COO^-$.

Viruses used for adsorption studies had pI values of 3.9 to 7.1. Preliminary studies with MS-2 (pI 3.9) and poliovirus 1 (strain Brunhilde; pI 7.1) were done. The results indicated a pattern of adsorption to the three types of silica, apparently governed by the charge state of the virion relative to that of the adsorbent. At pH values near the virus pI, some adsorption occurred on all three silica types (Fig. 3 and 6). Such a result is not unexpected, since viral proteins in an isoelectric state would be likely to neither strongly attract nor strongly repel surfaces in a highly specific fashion. Increasing pH above the virus pI, as shown in Fig. 3 for MS-2, provided a net negative virion surface charge and greatly enhanced adsorption onto the positively charged silicas. Conversely, adsorption onto the negatively charged carboxylated silica became negligible as pH increased. Results of similar experiments with poliovirus 1 are shown in Fig. 6. At pH 4.0, well below the virus pI, adsorption to amine-bearing silica was negligible compared with the very significant adsorption to the carboxylated adsorbent. As pH increased toward the virus pI, the adsorption to the negatively charged silica decreased sharply, which we interpret to be a reflection of the change of the surface charge of poliovirus 1 from strongly positive at pH 4.0 to much less positive at pH 6.4. At pH 8.5, poliovirus adsorbed slightly more to the positively charged silicas, suggesting that, as with MS-2, virus adsorption to the primary and quaternary amines was enhanced when virus charge was negative. To investigate whether further increases of pH would result in even more enhancement of poliovirus adsorption to the positively charged silicas as well as a decrease in adsorption to carboxylated particles, a follow-up study was undertaken. The LSc strain of poliovirus 1 was used and, as before, adsorption was tested at pH 4.0, 6.4, and 8.5. Additional experiments were done at pH 9.5 and 10. Results confirmed that pH conditions which caused the virus to carry a highly negative surface charge promoted good adsorption of virus onto the positively charged silicas (Fig. 7). As expected, no significant adsorption onto the negatively charged silica occurred at high pH.

The proposed relationship among the degree of adsorption, virus pI, adsorbent surface charge state, and system pH conditions was further supported by studies of coliphage T2 and reovirus types 1 and 3. T2 is reportedly isoelectric at pH 4.2 (12), a value consistent with adsorption results shown in Fig. 5. At pH 4.0, slightly below the virus pI, some slightly preferential adsorption onto carboxylated silica was noted. However, some adsorption did occur onto the amine-bearing silicas, suggesting that pH 4.0 is below but near the virus pI.

Adsorption data for reovirus appear in Fig. 4. The pI for reovirus type 1, although unobtainable from published literature, can be predicted. At pH 4.0 more virus adsorbed to the carboxylated silica than to the amine-modified silica, suggesting a positive virion charge state (if only slightly so) and a pI slightly greater than 4.0.

Reovirus type 3 has a reported pI of 3.9 and therefore would be expected to adsorb in a way quite similar to MS-2 virus under the conditions of our study. Instead it behaved in a manner that would be expected of a virus with a pI closer to pH 5. This apparent discrepancy suggests that under the conditions of this study the charge densities of the capsid proteins were altered sufficiently to change the pI of the virus without causing virion inactivation. Since pI is a measure of net surface change, it can be imagined that any disruption of the integrity of the surface proteins which

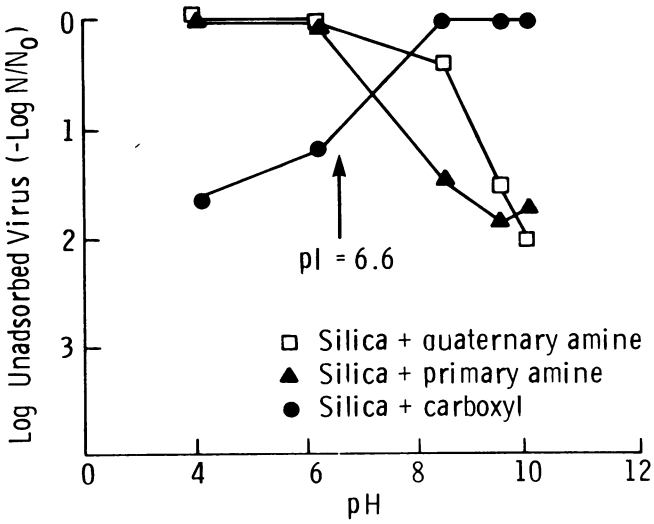

FIG. 7. Poliovirus 1 (strain LSc) adsorption to modified silica.

results in changes in surface-exposed areas of local charge (as might be induced by buffer components) may alter the pI. The effect of buffer salts on the Dearing strain of reovirus type 3 has been reported by Taylor and Bosmann (14). They found that NaCl concentrations of 1 to 100 mM had no apparent effect on the virus. It is likely, then, that NaCl (0.08 M in our buffer) was not responsible for the elevated pI of reovirus type 3 indicated by adsorption results at pH 4.0, despite findings by Salo and Cliver (11) that the presence of NaCl enhanced poliovirus inactivation at low pH in their studies. It is also unlikely that the reovirus was altered by the pH conditions alone, as the infectivity of reovirus is stable in buffers of pH 2.2 to 8.0 (4). Taylor and Bosmann did report on other buffer salts of interest, however. They found that whereas phosphate and sulfate from buffers did not interact with the surface of virions, acetate apparently did block some negative charges on the reovirus surface. In their study, the ultimate effect of the acetate-virus interaction was reflected in an elevation of the pI to 4.8 when reovirus type 3 was suspended in buffer containing 1 and 10 mM sodium acetate. The buffer used in the present study contained 0.03 M sodium acetate. It seems likely that the increase in apparent pI indicated by the adsorption data is a real phenomenon brought about inadvertently by exposure of the reovirus to the chosen buffer.

The results of this study indicate the very significant role of electrostatic forces in governing virus-surface interactions. The data likewise suggest that a knowledge of the virus pI makes it possible to predict its adsorptive interactions with a surface of known charge, as long as the suspending medium conditions are well defined and controlled. Conversely interpretation of adsorption behavior of a virus of unknown pI may lead to accurate appraisals of the surface charge conditions of the virus.

ACKNOWLEDGMENT

This work was funded in part by the U.S. Environmental Protection Agency under assistance agreement CR-811147-01-0 to the University of Arizona.

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