

Cryptosporidium Oocyst Surface Macromolecules Significantly Hinder Oocyst Attachment

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The role *Cryptosporidium parvum* oocyst surface macromolecules play in controlling oocyst adhesion (deposition) kinetics to quartz surfaces has been investigated utilizing a radial stagnation point flow system. Deposition kinetics and corresponding attachment efficiencies of viable oocysts were compared with those after treatment with a digestive enzyme (proteinase K) to cleave these surface macromolecules. Low deposition rates were observed with viable oocysts over the entire range of ionic strengths (KCl) investigated, even at ionic strengths as high as 100 mM where the Derjaguin–Landau–Verwey–Overbeek (DLVO) theory of colloidal stability predicts the absence of an electrostatic energy barrier. “Electrosteric” repulsion between the oocyst surface macromolecules and the quartz surface is surmised to cause these low deposition rates and attachment efficiencies. However, after removal of these surface macromolecules by the digestive enzyme, increased attachment efficiencies were observed over the entire range of ionic strengths. This significant increase in the deposition kinetics was seen despite the oocysts having a more negative zeta potential following the removal of the surface macromolecules. After treatment with proteinase K, the oocysts no longer experienced electrosteric repulsive forces, and their deposition kinetics followed the general behavior predicted by DLVO theory.

Introduction

Cryptosporidium parvum is a protozoan parasite which is ubiquitous in natural waters. The parasite infects the gastrointestinal tract of both humans and animals causing the disease cryptosporidiosis (1, 2). *C. parvum* exists as an oocyst in the environment and is resistant to a number of environmental stresses, including chlorination during drinking water treatment (3).

Harris and Petry (4) have viewed *Cryptosporidium* oocysts by thin sectioning with a transmission electron microscope, suggesting the existence of three distinct layers. An acidic glycoprotein was hypothesized to make up the outermost layer of the oocyst wall. The inner layer of the oocyst wall also appeared to be a filamentous glycoprotein, while the central layer of the wall was thought to be a rigid, complex lipid. Studies on the electrokinetic characteristics of the oocyst surface indicate it to be negatively charged at ambient pH,

with an isoelectric point falling between pH values of 2 and 3 (5–9). Recent studies (8–11) suggest that the acidic macromolecules on the oocyst surface impart steric repulsion with solid surfaces, which may significantly hinder oocyst attachment.

Considine et al. (10, 11) examined the interaction forces between *C. parvum* oocysts and both soda-lime glass and silica nitride surfaces using an atomic force microscope (AFM). Both the magnitude and decay lengths of the measured interaction forces in the presence of a monovalent salt (KCl) were much greater than predictions based on the Derjaguin–Landau–Verwey–Overbeek (DLVO) theory of colloidal stability (12, 13). This finding implies that an additional long-range repulsive force exists in this system, besides electrostatics, resulting in the large experimental decay lengths. The additional repulsive force has been attributed to the presence of macromolecules on the oocyst surface, which extend into solution as a brush like structure. These polymers have been postulated to impart a steric repulsion with the silica surfaces (8–11). Variation in solution pH or electrolyte concentration (KCl) imposed little change to the magnitude of this steric interaction; however, divalent salt (CaCl₂) was able to compress the brush layer to varying extents (10, 11). In a related study, Butkus et al. (14) witnessed a lack of aggregation of *Cryptosporidium* oocysts in 500 mM NaCl, which also suggests the presence of a non-DLVO force. The authors attributed this behavior to Lewis acid–base forces or to steric interactions. More recently, Byrd and Walz (15) demonstrated that the decay lengths of repulsive force profiles between *Cryptosporidium parvum* oocysts and an AFM silica particle probe are essentially independent of the ionic strength and always much larger than the theoretical Debye lengths. In addition, the magnitude of the force was found to be nearly the same for NaCl and CaCl₂ electrolytes, suggesting that the long-range repulsive forces are primarily steric in nature.

We have recently studied the adhesion kinetics of viable oocysts to ultrapure quartz surfaces in a radial stagnation point flow (RSPF) system (8, 9). In the presence of a monovalent salt (KCl) at ambient pH (5.5–5.7), oocyst deposition rates and corresponding attachment efficiencies were low, even at ionic strengths as high as 100 mM, where DLVO theory predicts the absence of an electrostatic energy barrier. This unusually low deposition rate in a monovalent salt solution was attributed to “electrosteric” repulsion between the oocyst surface macromolecules and the quartz surface. However, in the presence of a divalent salt (CaCl₂), oocyst deposition rates increased over the entire range of ionic strengths investigated, but the attachment efficiencies remained well below unity (8). An increase in the overall deposition kinetics was also seen after treatment with either heat or formalin, which most likely denatures the surface glycoproteins (9).

In this study, we unravel the role of oocyst surface macromolecules in controlling the deposition kinetics of *Cryptosporidium* oocysts onto a quartz surface. Deposition rates were determined for viable oocysts as well as for oocysts which had their surface polymers cleaved with a digestive enzyme—proteinase K. By treating the oocysts with proteinase K, the surface glycoproteins are removed, thus reducing or even eliminating the steric interactions occurring between the oocyst and the quartz surface. Comparison of the oocyst deposition behavior and surface properties before and after treatment with proteinase K reveals the role these surface macromolecules have in controlling the adhesion of *C. parvum* oocysts to solid surfaces in aquatic environments.

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Materials and Methods

Cryptosporidium Oocyst Source and Preparation. Viable *C. parvum* oocysts were purchased from the University of Arizona Sterling Parasitology Laboratory (SPL). The oocysts were shed from a calf infected with the Iowa isolate from the National Animal Disease Center in Ames, IA. The oocysts were purified at SPL by discontinuous sucrose and cesium chloride centrifugation gradients as described elsewhere (8, 16). Before the experiments, the oocysts were spun down twice at 12 000 rpm for 1.5 min (Eppendorf centrifuge 5415D), and the supernatant was removed by a pipet and replaced with 1 mL of deionized water (Barnstead). Purified oocysts were diluted to the desired concentration and suspended in the solution chemistry of interest prior to the deposition experiments. All oocysts used in the following study were from the same batch, meaning that they were shed from the same calf, during the same shed event. Fluorescence microscopy was used to determine the number concentration of *Cryptosporidium* oocysts before the deposition experiments, as described in our recent publications (8, 9).

Cryptosporidium Oocyst Characterization. The electrophoretic mobility of the *Cryptosporidium* oocysts was determined immediately before a deposition experiment under solution chemistries similar to those used in the corresponding deposition run (1, 3.16, 10, 31.6, and 100 mM KCl). The measurements were performed at 25 °C (± 1 °C) using a ZetaPALS analyzer (Brookhaven Instruments Corp., Holtsville, NY). Electrophoretic mobilities were converted to zeta potentials using the Smoluchowski equation. This equation is adequate because of the relatively large size of the oocysts and the ionic strengths used (12).

To determine the oocyst average size, images of the oocysts (ca. 10^4 oocysts/mL) were taken with an inverted microscope (Axiovert 200m, Zeiss, Thornwood, NY) operating in phase contrast mode. Image analysis software (ImageJ, NIH) was used for determining the average major and minor axes of the oocysts. The resulting equivalent spherical diameter of the *Cryptosporidium* oocyst was calculated to be 4.6 μm .

Cryptosporidium oocyst relative hydrophobicity was measured by the microbial adhesion to hydrocarbon (MATH) test (17, 18). A 4 mL oocyst suspension in 10 mM KCl (approximately 10^5 oocysts/mL) was transferred to test tubes, each containing 1 mL of dodecane. The test tubes were then vortexed at full speed for 2 min using a touch mixer (model 231, Fisher Scientific) and then allowed to stand for 15 min to allow phase separation. After 15 min, 1 mL of the aqueous phase was removed using a pipet and the number of oocysts were enumerated using the fluorescence microscopy technique described previously. This concentration was compared to the initial bulk oocyst concentration to determine the fraction of oocysts partitioned in the hydrocarbon phase.

Digestion of Oocyst Surface Macromolecules. *Cryptosporidium* oocyst samples were adjusted to a concentration of approximately 5×10^8 per mL to perform the digestion of the oocyst surface macromolecules with proteinase K (Sigma-Aldrich). All proteolytic digests were performed at a proteinase K concentration of 0.1 mg/mL (19), with background concentrations of 5 mM EDTA, 0.01 g/L SDS, and 10 mM Tris-HCl (pH 8.0) (4). Digests were then incubated at 37 °C and 260 rpm for 2.5 h (4). Next, the oocysts were separated from the digested surface polymers and washed with deionized water (Barnstead) by spinning them down twice at 12 000 rpm for 1.5 min (Eppendorf centrifuge 5415D). To verify that the overall integrity of the *Cryptosporidium* was not compromised by exposure to proteinase K, the oocysts were stained with DAPI by the technique described previously, and viewed under a fluorescent microscope. The oocysts appeared to be intact, still containing the sporozoites. It is

important to point out that all oocysts used in this study were from the same batch, namely they were shed from the same calf during the same shed event.

High Performance Liquid Chromatography. After digestion of the oocyst surface macromolecules with proteinase K, the sample was run through a high performance liquid chromatograph (HPLC) to verify their effective removal. An HP 1090 liquid chromatograph (Hewlett-Packard) was used in conjunction with a Supelco Biowide pore C18-5 column and an HP 1046A fluorescence detector. Each sample was first filtered through a 0.45 μm filter (Millex-HV) before injection into the HPLC. After injection of the sample (15 μL), a 10 min linear gradient from 100% HPLC grade water to 50% acetonitrile (ACN) was injected through the column. Next, a one minute linear gradient from 50% ACN to 95% ACN occurred, remaining at this concentration for two minutes. The run concluded with the 95% ACN solution decreasing linearly to 100% HPLC grade water over a two minute time period and remaining at this concentration for three minutes. This procedure separates the macromolecules present in the sample based on their hydrophobic nature. All runs were performed at a flow rate of 0.5 mL/min and a temperature of 50 °C.

Quartz Surface Preparation and Characterization. The high purity (99.9% SiO_2) quartz cover slides (25 mm in diameter and 0.1 mm thick) were acquired from Electron Microscopy Sciences (Ft. Washington, PA). All cover slides were thoroughly cleaned before performing deposition experiments with the oocysts as described elsewhere (8, 9). To create electrostatic conditions favorable for deposition, the quartz slides were chemically modified using a 0.2% (v/v) mixture of (amino-ethylaminomethyl)phenethyltrimethoxysilane (Gelest, Inc., Tullytown, PA) in ethanol. After the aminosilane solution was spread onto the quartz surface ($\sim 15 \mu\text{L}$), the slide was cured at 130 °C for 90 min, followed by a deionized water rinse. Details on this chemical modification of the quartz surfaces with aminosilane are given in our recent publications (20, 21).

A streaming potential analyzer (EKA, Brookhaven Instruments Corp.) with an asymmetric clamping cell was used to determine the electrokinetic properties of the quartz cover slides. Measurements were obtained in monovalent salt (KCl) solutions in ionic strengths ranging from 1 to 100 mM, corresponding to the solution chemistries of the deposition experiments. The instrument was first rinsed with 1 L of deionized water followed by 0.5 L of the electrolyte solution used in the measurement. Prior to taking the streaming potential measurements, the quartz slide was equilibrated with the corresponding fresh electrolyte solution for 10 min. The zeta potential was calculated from the measured streaming potential as described elsewhere (20).

Radial Stagnation Point Flow System. A radial stagnation point flow system (RSPF) was utilized to determine the oocyst deposition kinetics. Details on the stagnation point flow cell and the image analysis software utilized during these experiments are described in our previous publications (8, 9). The images were analyzed to determine the deposition flux (number of oocysts per area per time), which was used to calculate the oocyst transfer rate in the radial stagnation point flow system.

Cryptosporidium oocyst deposition kinetics in the radial stagnation point flow system were quantified as the transfer rate coefficient, k_D (8, 9). Deposition kinetics were also presented in terms of the attachment efficiency, α . The attachment efficiency is calculated by normalizing the actual oocyst transfer rate coefficient for each experiment by the transfer rate under completely favorable (nonrepulsive) electrostatic conditions, $k_{D,\text{fav}}$:

$$\alpha = \frac{k_D}{k_{D,\text{fav}}} \quad (1)$$

Deposition kinetics under favorable electrostatic conditions were determined for both viable oocysts and the oocysts treated with proteinase K. To achieve favorable electrostatic conditions, the quartz surface was chemically modified with aminosilane by the procedure outlined earlier to create a positive charge on the surface. Deposition runs were then performed in the RSPF system with the chemically modified cover slip at 88.5 mM KCl and ambient pH (5.5–5.7) for both oocyst types. The average favorable transfer rate coefficient ($k_{D,\text{fav}}$) for the viable oocysts was determined to be 5.35×10^{-7} m/s; similarly, the average favorable transfer rate for the oocysts treated with proteinase K was determined to be 5.20×10^{-7} m/s. These results were then verified by carrying out a deposition run at pH 2.5, where both the oocyst and the pure quartz surface zeta potentials are near their isoelectric points (8). Results from this experiment at pH 2.5 with oocysts treated with proteinase K yielded a transfer rate coefficient of 4.70×10^{-7} m/s. Because of the similarity between all the favorable transfer rates, the attachment efficiency, α , was calculated using the average $k_{D,\text{fav}}$ of these three runs, 5.08×10^{-7} m/s ($\pm 0.82 \times 10^{-7}$ m/s).

Deposition Experiments in the Radial Stagnation Point Flow System. The oocyst deposition rate onto the quartz surface was determined in the presence of monovalent (KCl) salt solutions. Transfer rates were determined for 1, 3.16, 10, 31.6, 100, and 177 mM solutions with a target oocyst concentration of ca. 2.5×10^6 oocysts per mL. For each experiment, the influent oocyst concentration was verified by directly visualizing and counting the oocysts using the fluorescence microscopy technique described previously. The oocyst suspension was collected during each run in the RSPF system, and by adding the necessary amount of stock KCl solution the ionic strength was raised to achieve the desired ionic strength for the next deposition experiment. Deposition experiments were performed at a flow rate of 5 mL/min (0.027 m/s), corresponding to a capillary Reynolds number of 26.53 and a particle Peclet number of 65.21. All experiments were carried out at an ambient pH (5.5–5.7) and a temperature of 25 °C (± 1 °C).

Results and Discussion

Electrokinetic Properties of *Cryptosporidium* Oocysts and Quartz Surfaces. The zeta potentials of the *Cryptosporidium* oocysts along with the quartz surface are presented in Figure 1. Both surfaces are negatively charged and become less negative as the ionic strength of the solution is increased. The overall decrease in the zeta potential as the KCl concentration is increased can be attributed to double-layer compression (12). These zeta potentials were used to calculate the DLVO interaction energy profiles between the oocysts and the quartz surface as presented later in this paper.

A wide variability in zeta potentials has been previously reported for *Cryptosporidium* oocysts. Our reported values are consistent with those published in the literature. Previously reported values include: ζ between -8.1 and -8.7 mV in 1 mM KCl at neutral pH for viable oocysts (9); ζ between -17.2 and -30.6 mV in 1 mM KCl and between -6.5 and -17.5 mV in 10 mM KCl at ambient pH (5.5–5.7) for viable oocysts (8); $\zeta = -30$ mV at ambient pH in 1 mM KCl (22); $\zeta = -25 \pm 2.8$ mV in DI water at pH 6 for viable oocysts (7); ζ between -19 and -36 mV in 1 mM NaCl at pH 6 for viable oocysts (5); and ζ between -37 and -42 mV at neutral pH for viable oocysts (23).

Knowledge of the exact composition of the oocyst surface which gives rise to its pH-dependent charge behavior is limited. Researchers have determined the oocyst surface

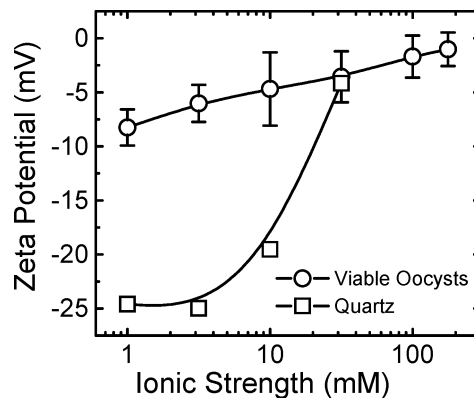


FIGURE 1. Zeta potentials of viable *Cryptosporidium* oocysts and quartz cover slip as a function of ionic strength (KCl) at pH 5.5–5.7 and a temperature of 25 °C (± 1 °C).

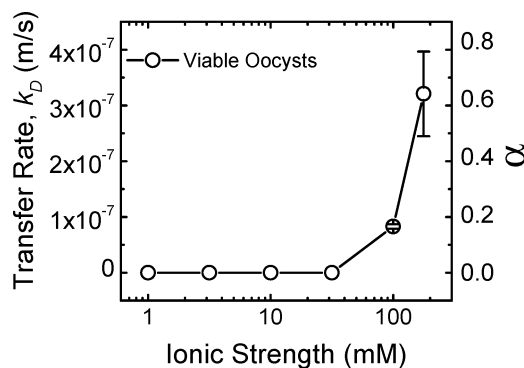


FIGURE 2. *Cryptosporidium* oocyst deposition kinetics onto a quartz surface as a function of ionic strength (KCl). The deposition kinetics are expressed as the oocyst transfer rate, k_D , and attachment efficiency, α . The capillary flow rate in the RSPF was 5.0 mL/min, resulting in a capillary Reynolds number of 26.5 and a particle Peclet number of 65.2. Other experimental conditions employed were an ambient pH (5.5–5.7) and a temperature of 25 °C (± 1 °C).

contains the amino acids cysteine, proline, and histidine (24). Karaman et al. (5) suggested the presence of carboxylate and/or phosphate groups based on a fitted pK_a value of 2.5. The presence of the carboxylate groups could be associated with cysteine rich proteins and glycoproteins on the oocyst surface (5). Nanduri et al. (25) identified glucose, galactose, mannose, xylose, and ribose on the surface of the *Cryptosporidium* oocyst.

Deposition Kinetics of Viable *Cryptosporidium* Oocysts. Viable oocyst transfer rates (k_D) and corresponding attachment efficiencies (α) are presented in Figure 2 as a function of monovalent salt (KCl) concentration. At ionic strengths up to 100 mM KCl, the transfer rate and corresponding attachment efficiency of the oocysts onto the quartz substrate remain negligible. The attachment efficiency remains at zero through an ionic strength of 31.6 mM, before rising to 0.16 at 100 mM and to 0.63 at 177 mM. An attachment efficiency of zero means that no deposition could be observed over the twenty minute run at the given oocyst concentration and hydrodynamic conditions. To explain the low attachment efficiency, even at high ionic strengths, we turn to the DLVO theory of colloidal stability.

To calculate the DLVO interaction energy profile, the repulsive electrostatic interaction energy was determined by using the constant surface potential interaction expression of Hogg et al. (26), and the expression of Gregory (27) was used to determine the retarded van der Waals contribution. A sphere-plate geometry was assumed when calculating the interaction energies. In absence of literature values for the Hamaker constant of the oocyst–water–quartz media, a

TABLE 1. Energy Barrier Height as a Function of Ionic Strength (KCl) for Viable and Proteinase K Treated Oocysts as Calculated by DLVO Theory^a

ionic strength (mM)	viable (kJ)	proteinase K (kJ)
1	260.3	461.2
3.16	105.9	∞
10	NB ^b	45.4
31.6	NB	NB
100	NB	NB

^a Interaction energies were calculated using experimentally determined zeta potentials (Figures 1 and 4), a Hamaker constant of 6.5×10^{-21} J, and an oocyst diameter of $4.6 \mu\text{m}$. ^b No Energy Barrier.

value of 6.5×10^{-21} J was chosen, similar to that reported for other microbes particles interacting with quartz in an aqueous medium (28). The calculated energy barriers from DLVO theory for all ionic strengths investigated are presented in Table 1.

The calculated energy barriers indicate that, for viable oocysts, sizable energy barriers exist at both 1 mM (260.3 kJ) and 3.16 mM (105.9 kJ), which should inhibit the deposition of the oocysts onto the quartz surface. However, at ionic strengths of 10 mM or greater, electrostatic energy barriers cease to exist. Even in the absence of an energy barrier at the higher ionic strengths, the attachment efficiency of the oocysts is far from reaching unity. We attribute this behavior to an electrosteric repulsive force due to the oocyst surface macromolecules as delineated below.

Role of Surface Macromolecules in Controlling Oocyst Deposition Kinetics. An additional repulsive force must be present to explain the low attachment efficiency of the *Cryptosporidium* oocysts to the quartz substrate in the presence of a monovalent salt (KCl). This is especially the case at high ionic strengths where DLVO theory predicts the absence of an electrostatic energy barrier and deposition remains very low. We surmise that this additional repulsive force is ascribed to an electrosteric repulsion as we reported in our previous publications (8, 9).

Surfaces comprising polyelectrolytes are subjected to both electrostatic and steric repulsive forces when interacting with solid surfaces (12, 29, 30). Previous studies have suggested that the oocyst surface contains anchored glycoproteins (4, 8–11, 15) stretching into solution due to electrostatic repulsion between surface ionizable groups distributed along the polypeptide backbone. It has been suggested that the formation of this brush like structure imparts a steric repulsion with the quartz substrate.

Divalent cations have shown the ability to compress the polyelectrolyte layer to varying extents, and subsequently reduce the electrosteric repulsion with solid surfaces. By utilizing an AFM, Considine et al. (10, 11) and Byrd and Walz (15) witnessed decay lengths much greater than those expected based on classical DLVO interactions in the presence of a monovalent salt. However, the brush layer did compress to varying extents in the presence of divalent calcium ions. In a related study (8, 9), we have witnessed significantly higher deposition rates onto an ultrapure quartz surface in the presence of divalent calcium ions, when compared to oocysts in a monovalent (KCl) salt solution. Nonetheless, the attachment efficiency was still much smaller than predictions based on DLVO theory. Therefore, the removal of the oocyst surface polymers will allow us to investigate their role in controlling the overall deposition rate of *Cryptosporidium* oocysts onto quartz surfaces.

Effect of Surface Polymer Removal on Oocyst Surface Properties. In the previous sections, we attributed the low attachment efficiency of the oocysts to an electrosteric repulsion caused by the surface macromolecules interacting

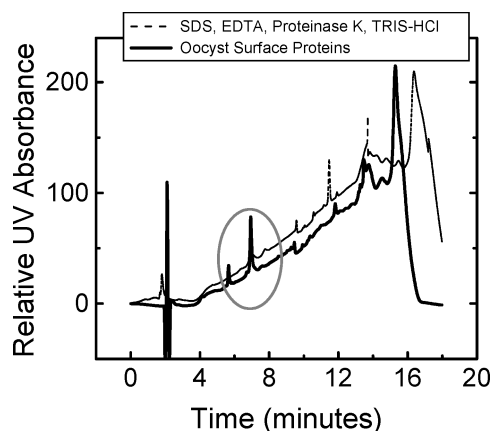


FIGURE 3. HPLC chromatograph (—) showing a sample containing oocyst surface macromolecules with background SDS, EDTA, proteinase K and TRIS–HCl, and (---) a sample containing SDS, EDTA, proteinase K, and TRIS–HCl. The latter serves as a control. Both runs were done at a flowrate of 0.5 mL/min and at a temperature of 50 °C.

with the solid quartz surface. After the removal of the outer glycoprotein layer of the *Cryptosporidium* oocysts with proteinase K, the sample was spun down by the previously described procedure, and the supernatant was removed. This supernatant, containing the oocyst surface macromolecules along with SDS, EDTA, TRIS–HCl, and proteinase K still present from the digestion, was injected into a high performance liquid chromatograph (HPLC). A second sample, containing SDS, EDTA, proteinase K, and TRIS–HCl, was also run through the HPLC to serve as a blank. A comparison of the two chromatographs (Figure 3) will allow us to determine if the surface polymers were effectively removed from the oocyst surface.

From Figure 3, we can see two distinct peaks (circled in the chromatograph) exist in the solution containing the surface macromolecules at approximately 5 min after the injection. These peaks are not present in the sample containing only the background solution for the digestion. This experiment was repeated with consistent results. We, therefore, attribute these peaks to the macromolecules which were removed from the oocyst surface. The effect of the surface polymer removal on the oocyst electrokinetic and hydrophobic properties will now be discussed.

(a) Oocyst Electrokinetic Properties. The zeta potentials as a function of ionic strength of viable *Cryptosporidium* oocysts and oocysts after treatment with proteinase K in the presence of KCl at ambient pH (5.5–5.7) are presented in Figure 4a. Both sets of oocysts are negatively charged, and become less negative as the ionic strength of the solution is increased due to double-layer compression (12). It is also clear that the oocyst zeta potential becomes more negative after the surface polymers were removed by treatment with proteinase K.

The zeta potentials as a function of pH (from pH 2 to 9) of the viable oocysts before and after treatment with proteinase K are presented in Figure 4b. As shown, the isoelectric point of both fall between pH 2 and 3, consistent with values previously reported in the literature (5–7, 12). Generally, the zeta potential becomes more negative with increasing solution pH as expected for surfaces with acidic functional groups. The zeta potential of the oocysts after the removal of the surface polymers becomes more negative over all pH values above 3. To rule out that this deviation was not due to SDS or EDTA (present in the Protease K solution) adsorbing to the oocyst surface, samples of viable *Cryptosporidium* were mixed with both SDS and EDTA, and washed with the technique described previously. Zeta potentials were

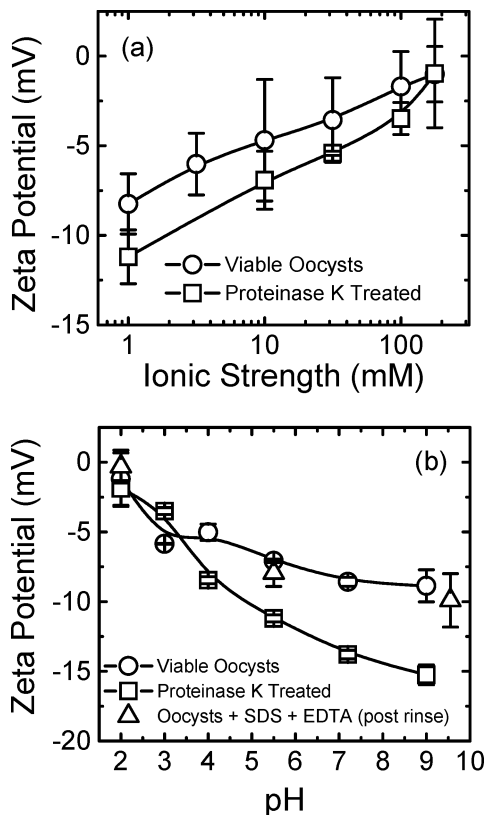


FIGURE 4. Zeta Potentials of viable *Cryptosporidium* oocysts and *Cryptosporidium* oocysts after treatment with proteinase K: (a) Dependence on ionic strength (KCl) at pH 5.5–5.7. (b) Dependence on solution pH. Zeta potentials of oocysts after removal of SDS and EDTA with rinsing procedure are also presented in (b).

then taken at three different pH values (shown as open triangles in Figure 4b). As can be seen from the figure, the overall zeta potential of the viable *Cryptosporidium* after washing off the SDS and EDTA remains unchanged indicating that the SDS and EDTA were effectively removed by our washing technique.

As previously described, the surface of the oocyst contains anchored glycoproteins which extend into solution forming a brush like structure (4, 10, 11, 15). Glycosolated proteins are ubiquitous components of extra-cellular matrixes and cellular surfaces (31). They are composed of proteins covalently bonded to carbohydrates. Nanduri et al. (25) identified glucose, galactose, mannose, xylose, and ribose on the surface of *Cryptosporidium* oocysts, thus verifying the presence of these uncharged sugar groups. Therefore, removing the oocyst surface proteins will, in effect, remove the covalently bound carbohydrates extending into solution. Proteinase K was chosen because it is a highly aggressive protease, which cleaves peptide bonds at the carboxylic sides of aliphatic, aromatic, or hydrophobic amino acids (19). However, Harris et al. (4) verified, by transmission electron microscopy, that exposure of the oocysts to proteinase K for 2.5 h did not disrupt the central, rigid layer of the wall. Thus, the overall integrity of the oocyst would remain intact after treatment with this protease. The presence of this “brush layer” consisting of uncharged sugar groups shifts the electrokinetic plane of shear outward (32), resulting in a less-negative oocyst zeta potential. This can explain the relatively low zeta potentials reported in the literature by previous researchers (listed above). After the removal of the glycosolated proteins, the plane of shear is closer to the surface, which explains the more negative zeta potentials of the oocyst surface after treatment with proteinase K (Figure 4).

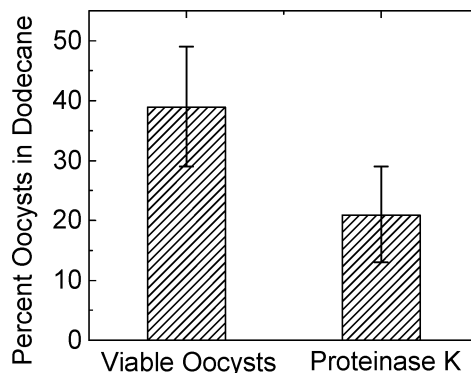


FIGURE 5. Fraction of *Cryptosporidium* oocysts partitioned in the hydrocarbon (dodecane) phase as determined by the MATH test at an ambient pH of 5.5–5.7 and a temperature of 25 °C (± 1 °C).

(b) *Oocyst Hydrophobicity.* The relative hydrophobicity of viable oocysts and oocysts after treatment with proteinase K was determined using the MATH test. By evaluating the concentration of oocysts partitioned into dodecane using the previously described procedure, the fraction of hydrophobic oocysts was determined (Figure 5). The average of three experiments was used to determine the relative hydrophobicity for both viable and proteinase K treated oocysts. Viable oocysts partitioned at a higher percentage (39%) than those after the removal of the oocyst surface macromolecules (21%).

The *Cryptosporidium* oocysts exhibit hydrophilic properties, consistent with the findings of previous researchers (7, 9, 33). However, the fact that there exists a slight decrease in the overall hydrophobic nature of the oocysts after the removal of the surface macromolecules is not surprising. As stated previously, the oocyst surface contains glycosolated proteins, namely proteins with carbohydrates covalently bonded to their surface (31). Therefore, if we have successfully cleaved the surface glycoproteins, the carbohydrates will be removed to a certain extent. Removal of these uncharged moieties should result in an increase in the hydrophilic nature of the oocyst surface. We also can attribute this difference in the surface hydrophobicity to an overall change in the exposed functional groups on the oocyst surface due to the removal of these macromolecules.

Effect of Oocyst Surface Macromolecule Removal on Deposition Rate. The transfer rates (k_t) and corresponding attachment efficiencies (α) of oocysts before and after treatment with proteinase K are presented in Figure 6 as a function of monovalent salt (KCl) concentration. Attachment efficiencies of viable oocysts remain extremely low, even at high ionic strengths where the DLVO theory of colloidal stability predicts the absence of an energy barrier. As we discussed earlier, this additional repulsive force was attributed to an electrosteric repulsion. However, after treatment with proteinase K and the removal of the oocyst surface polymers, a much higher attachment efficiency is seen over the range of ionic strengths investigated. To better explain the deposition kinetics of the proteinase K treated oocysts, we once again turn to DLVO theory.

The calculated height of the DLVO energy barriers (Table 1) indicates a sizable repulsive barrier at an ionic strength of 1 mM (461.2 kT) which inhibits the deposition of the *Cryptosporidium* oocysts at this salt concentration, and explains the negligible deposition seen at 1 mM. However, at an ionic strength of 10 mM, a much smaller energy barrier exists (45 kT), which results in an oocyst attachment efficiency of 0.63. At ionic strengths of 31.6, 100, and 177 mM, repulsive energy barriers cease to exist, and attachment efficiencies of 0.91, 0.85, and 0.90 result at these respective salt concentrations.

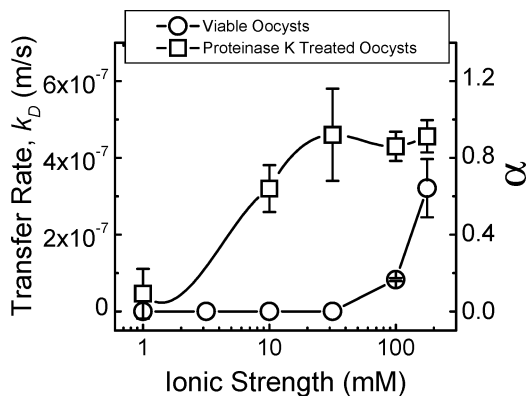


FIGURE 6. Viable and proteinase K treated oocyst deposition kinetics onto a quartz surface as a function of ionic strength (KCl). The deposition kinetics are expressed as the oocyst transfer rate, k_D , and attachment efficiency, α . The capillary flow rate in the RSPF was 5.0 mL/min, resulting in a capillary Reynolds number of 26.53 and a particle Peclet number of 65.21. Other experimental conditions employed were an ambient pH (5.5–5.7), and a temperature of 25 °C (± 1 °C).

From the preceding experimental data, it is obvious that classic DLVO theory fails to explain the unusual low transfer rates and corresponding attachment efficiencies of viable (untreated) *Cryptosporidium* in a monovalent salt solution, signifying the presence of an additional electrosteric repulsion between oocyst surface macromolecules and the quartz surface. However, after the surface polymers are removed with a protease, the oocysts behave like “model colloidal” particles, and follow the DLVO theory of colloidal stability much more closely. For instance, the attachment efficiency and the transfer rate increase with increasing ionic strength. At an ionic strength of 31.6 mM where the electrostatic energy barrier vanishes, the attachment efficiency rises to near unity (0.91). The attachment efficiencies continue to remain near unity at ionic strengths of 100 and 177 mM, signifying that oocyst deposition reached a point of transport-limited rate. After the surface macromolecules are removed, the oocysts no longer experience an additional electrosteric repulsive force with the quartz surface. Therefore, DLVO theory can be used to explain the oocysts deposition kinetics much more closely.

Acknowledgments

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