

# Transport of *Cryptosporidium* Oocysts in Porous Media: Role of Straining and Physicochemical Filtration<sup>†</sup>

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The transport and filtration behavior of *Cryptosporidium parvum* oocysts in columns packed with quartz sand was systematically examined under repulsive electrostatic conditions. An increase in solution ionic strength resulted in greater oocyst deposition rates despite theoretical predictions of a significant electrostatic energy barrier to deposition. Relatively high deposition rates obtained with both oocysts and polystyrene latex particles of comparable size at low ionic strength (1 mM) suggest that a physical mechanism may play a key role in oocyst removal. Supporting experiments conducted with latex particles of varying sizes, under very low ionic strength conditions where physicochemical filtration is negligible, clearly indicated that physical straining is an important capture mechanism. The results of this study indicate that irregularity of sand grain shape (verified by SEM imaging) contributes considerably to the straining potential of the porous medium. Hence, both straining and physicochemical filtration are expected to control the removal of *C. parvum* oocysts in settings typical of riverbank filtration, soil infiltration, and slow sand filtration. Because classic colloid filtration theory does not account for removal by straining, these observations have important implications with respect to predictions of oocyst transport.

## Introduction

*Cryptosporidium parvum* has been identified as one of the most important contaminants found in drinking water and is associated with a high risk of waterborne illness (1, 2). This protozoan parasite is transmitted via the fecal–oral route in its environmentally resistant stage—the oocyst (1, 3, 4). Ingestion of a small number of viable oocysts can lead to cryptosporidiosis, a diarrheal disease that can prove fatal for immunocompromised individuals (1, 2, 5, 6). Over the past

two decades, several outbreaks of cryptosporidiosis related to both groundwater and surface water contamination have been reported in Europe and North America (3, 7, 8).

Resistance of the *C. parvum* oocyst to conventional disinfection processes poses a significant challenge to the protection of drinking water supplies from contamination (2, 9–11). As a result, water utilities are showing increased interest in oocyst removal in porous media such as riverbank filtration, deep-bed (granular) filtration, and slow sand filtration to control drinking water quality (12–14). However, the mechanisms governing the transport and filtration behavior of *C. parvum* oocysts in these settings are not well understood to allow the development of predictive models for oocyst removal.

Compared to other microbial pathogens, such as bacteria and viruses, studies on the removal mechanisms of *C. parvum* oocysts in flow through saturated porous media are relatively scarce. Previous studies involved the use of slow sand filters (12, 15), deep-bed filters (14, 16–21), and columns packed with natural sediments (22, 23) as well as glass and polystyrene beads (24–26). Although significant removal of oocysts has been observed, these studies were not designed to improve understanding of the key mechanisms controlling the filtration behavior of *C. parvum*.

In most natural and engineered aquatic systems, *C. parvum* oocysts and sediment (collector) surfaces are negatively charged, giving rise to repulsive electrostatic interactions. Yet, the role of physicochemical filtration (i.e., surface interactions between oocysts and the porous medium) has not been examined in previous studies. In particular, the influence of solution chemistry (e.g., changes in ionic strength) on the deposition rate of oocysts in columns packed with sand has not been previously investigated. While Hsu et al. (25) reported the effects of physicochemical filtration (i.e., ionic strength and pH) on the removal of *C. parvum* oocysts and *Giardia* cysts, their experiments were conducted in columns packed with glass or polystyrene beads, neither of which is representative of the variety of mineral surfaces, grain sizes, and grain shapes of granular media encountered in riverbank filtration or deep-bed and slow sand filtration.

In this paper, we systematically investigate the key factors governing the filtration behavior of *C. parvum* oocysts in saturated porous media. A laboratory-scale column packed with a high-purity quartz sand is used to study the retention of oocysts under repulsive electrostatic conditions. Experiments conducted with polystyrene latex particles of comparable size provide insight into the mechanisms controlling the removal of oocysts. In addition, the retention of both *C. parvum* oocysts and polystyrene latex particles in columns packed with glass beads is studied in order to determine the relative importance of grain shape.

## Materials and Methods

***Cryptosporidium parvum* Oocysts.** Heat-inactivated oocysts (60 min at 80 °C) were obtained from the Sterling Parasitology Laboratory (SPL) at the University of Arizona. The oocysts were shed from a calf infected with the Iowa isolate from Dr. Harley Moon (National Animal Disease Center, Ames, IA). Oocysts were purified (at SPL) using discontinuous sucrose and cesium chloride centrifugation gradients and stored (in the dark at 4 °C) in an antibiotic solution containing 0.01% Tween 20, 100 U of penicillin, and 100 µg/mL of gentamicin. Before use, oocysts were spun down twice at 12 000 rpm for 1 min, and the supernatant was replaced with 1 mL of deionized water (DI) (Nanopure Infinity, Barnstead Ther-

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molyne Corporation, Dubuque, IA). Prior to transport experiments, oocysts were diluted to the desired concentration ( $10^5$  oocysts/mL) in the solution chemistry of interest.

**Porous Media.** The first set of experiments was conducted using a column packed with ultrapure (99.99%  $\text{SiO}_2$ ) quartz sand (Unimin Corporation, Spruce Pine, NC). The sand was sorted by nylon sieves (U.S. standard mesh size 60 (0.250 mm) and 100 (0.150 mm)) and thoroughly cleaned to remove metal and organic impurities using the procedure of Litton and Olson (27). Cleaning the sand involved soaking in 12 N HCl for at least 24 h, washing with DI water until the measured pH reached a value of about 5.6, and finally heating for 8 h at 800 °C. Cleaned sand was stored under vacuum and rehydrated by boiling in DI water prior to packing the column for each experiment. Sand grains were imaged with an environmental scanning electron microscope (ESEM, XL-30, FEI Company, Peabody, MA) to determine the shape of the collector grains.

A second set of experiments was conducted with the *C. parvum* oocysts in a column packed with uniform soda-lime glass beads (Class V, MO-SCI Corporation, Rolla, MO). The manufacturer reported the average diameter of the glass beads as 0.230 mm. The beads were selected to be similar in size to the average grain diameter of the quartz sand. ESEM images of the glass beads were taken for comparison with those of the quartz grains. The glass beads were thoroughly cleaned to remove grease and other impurities. The beads were soaked in a surfactant solution (2% Extran MA02, EM Science, Gibbstown, NJ) for 1 h, rinsed with DI water, sonicated (Aquasonic 150T, VWR Scientific Products, West Chester, PA) for 15 min in a detergent solution (2% RBS 35, Pierce Biotechnology Inc., Rockford, IL), rinsed with DI water, soaked for 24 h in a solution containing sulfuric acid and NOCHROMIX (Godax Laboratories, Inc., Takoma Park, MD), and finally rinsed with DI water until the measured pH reached a value of about 5.6.

**Model Particles.** Surfactant-free polystyrene latex colloids (Interfacial Dynamics Corporation, Portland, OR) with carboxyl functional groups were used as model particles. A wide range of particle sizes was selected (0.32, 1.0, 1.9, and 4.1  $\mu\text{m}$ ) to further examine and verify proposed mechanisms governing the filtration of *C. parvum* oocysts. In addition to the experiments conducted with *C. parvum* oocysts, column experiments were carried out using the latex particles in columns packed with clean quartz sand and soda-lime glass beads.

**Characterization of *C. parvum* Oocysts.** Microelectrophoresis (Zeta-PALS, Brookhaven Instruments Corporation, Holtsville, NY) was used to characterize the electrokinetic properties of the *C. parvum* oocysts over the range of ionic strengths (1, 3.16, and 10 mM KCl) used in the column experiments. Electrophoretic mobility was measured at 25 °C ( $\pm 1$  °C) using oocyst suspensions ( $10^5$  oocysts/mL) prepared in the background electrolyte of interest (1, 3.16, or 10 mM KCl) at an unadjusted pH (5.6–5.8).  $\zeta$  potentials were calculated from the measured electrophoretic mobilities using the Smoluchowski equation (28).

The nominal size of the *C. parvum* oocysts was determined by analyzing images taken in an inverted fluorescent microscope operating in phase contrast mode (Axiovert 200m, Zeiss, Thornwood, NY). A drop of an oocyst suspension ( $10^6$  oocysts/mL in DI water) was placed on a microscope slide, and several images were recorded. Using an image processing program (ImageJ, NIH), the average lengths of the major and minor axes of the oocysts were determined to be  $3.9 \pm 0.7$  and  $3.4 \pm 0.6 \mu\text{m}$ , respectively. The corresponding equivalent spherical diameter of the oocysts was calculated as 3.6  $\mu\text{m}$ .

**Characterization of Porous Media.** Streaming potentials of the granular (quartz sand) porous medium were measured by a streaming potential analyzer (EKA, Brookhaven Instru-

ments Corporation, Holtsville, NY). The quartz sand was wet-packed into a cylindrical cell to a bed depth of 3 cm, rinsed extensively with DI water, and equilibrated with electrolyte solution. Measurements were taken at a fixed pH (5.7) over a range of ionic strengths (1, 3.16, and 10 mM KCl) corresponding to the solution conditions used in the column experiments. Streaming potentials were converted to  $\zeta$  potentials using the Helmholtz–Smoluchowski equation (29).

The grain size distribution of the quartz sand was determined by standard sieve analysis. About 460 g of dry sand was loaded into a series of brass sieves and vibrated for 20 min on a sieve shaker (RX-29 RO-TAP, Tyler, Mentor, OH). The sieve analysis yielded an average grain diameter ( $d_{50}$ ) of 0.21 mm and a coefficient of uniformity ( $d_{60}/d_{10}$ ) of 1.26.

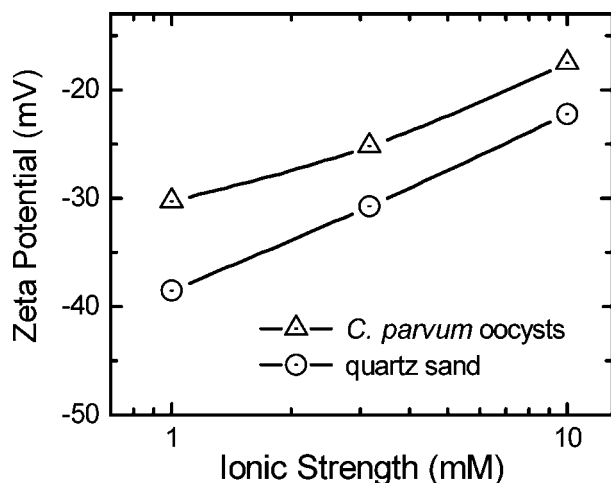
**Column Experiments.** Transport experiments were conducted by pumping a suspension of *C. parvum* oocysts or latex particles through a glass chromatography column packed with clean quartz grains or soda-lime glass beads. An adjustable-height column (Omnifit USA, Toms River, NJ) with an inner diameter of 1 cm was used. The porous medium (quartz sand or soda-lime glass beads) was wet-packed to a height of 7.1 cm with vibration to minimize any layering or air entrapment. Standard gravimetric methods were used to determine a column packing porosity of 0.43 for the quartz sand and 0.36 for the glass beads.

Column experiments were conducted over a range of ionic strengths (1, 3.16, and 10 mM KCl) with no pH adjustment (pH 5.6–5.8). Prior to each experiment, the packed column was equilibrated by pumping (model 200 syringe pump, KD Scientific Inc., New Hope, PA) 10 pore volumes of the background electrolyte solution through the column at a constant approach (superficial) velocity of 0.042 cm/s. A suspension of *C. parvum* oocysts ( $10^5$  oocysts/mL) or model latex colloids ( $10^7$ – $10^8$  particles/mL) of the same background electrolyte composition was pumped for at least 8 pore volumes followed by a particle-free background electrolyte solution (at least 10 pore volumes). A constant influent particle concentration was maintained by including a miniature magnetic stir bar in the particle solution syringe. For the experiments conducted with the *C. parvum* oocysts, effluent samples were collected at 1-min intervals using a fraction collector (FRAC-100, Amersham Biosciences, Piscataway, NJ), and oocyst concentrations were determined as described below. In all experiments conducted with model latex colloids, the particle concentration at the column outlet was monitored on-line using optical density measurements with a UV/visible spectrophotometer (Hewlett-Packard model 8453) and a 1-cm flow-through cell.

**Enumeration of *C. parvum* Oocysts.** Influent and effluent samples were filtered with vacuum assistance onto 25 mm diameter polycarbonate membranes (0.22  $\mu\text{m}$  average pore diameter) (Osmonics Inc., Minnetonka, MN). One milliliter of DAPI (4',6-diamidino-2-phenylindole) stain was applied to each membrane in the dark and filtered after 15 min. The oocysts were counted using an epifluorescent microscope (excitation 372 nm, emission 456 nm; BX41, Olympus America, NY). At least 20 fields of view were counted on each membrane. The validity of the oocyst enumeration technique was verified by repeating the counts of several sample membranes to demonstrate reproducibility.

## Results and Discussion

**Electrokinetic Properties of *C. parvum* Oocysts and Quartz Grains.** The  $\zeta$  potentials of the *C. parvum* oocysts and the clean quartz grains as a function of ionic strength are presented in Figure 1. Both the oocysts and the quartz grains are negatively charged at the pH investigated (5.6–5.8), and their  $\zeta$  potentials become less negative with increasing ionic strength. Oocyst  $\zeta$  potentials determined here ( $-30 < \zeta < -18$  mV) are comparable to values previously reported in



**FIGURE 1.**  $\zeta$  potentials of *Cryptosporidium parvum* oocysts and clean quartz grains as a function of ionic strength at unadjusted pH of 5.6–5.8.

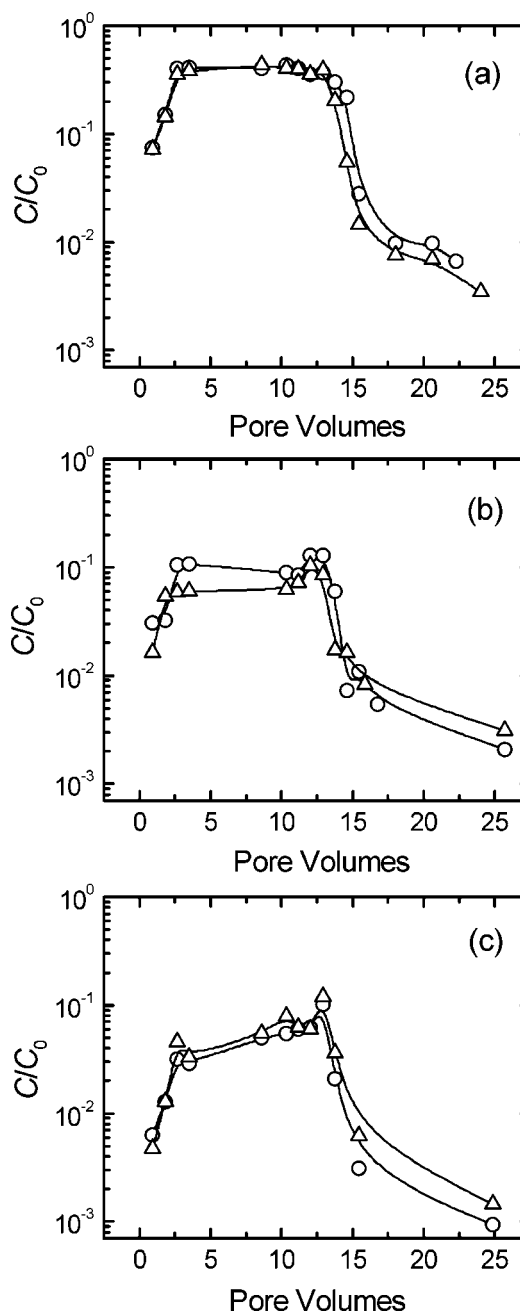
the literature:  $-30 < \zeta < -20$  mV at pH 6 for live oocysts (30),  $\zeta \approx -27$  mV in 1 mM NaCl at pH 6 for Co<sup>60</sup>-irradiated oocysts (31),  $\zeta \approx -25$  mV in DI at pH 6 for live oocysts (32), and  $\zeta \approx -22$  mV in DI at pH 6 for heat-inactivated oocysts (33).

Knowledge of the structure and chemical surface properties of the *C. parvum* oocyst wall is limited. Previous studies (31–33) have shown that the oocyst surface is negatively charged at pH values greater than 3.5. Although the nature of the surface groups has not been identified, it is known that the oocyst wall contains cysteine, proline, and histidine (34, 35). Karaman et al. (31) further suggested the presence of carboxylic and/or phosphate groups based on a fitted  $pK_a$  value of 2.5. The carboxylic groups could be associated with cysteine-rich proteins and glycoproteins on the oocyst surface (31).

**Transport of *C. parvum* Oocysts in Quartz Sand.** Oocyst breakthrough curves obtained at 1, 3.16, and 10 mM ionic strength are presented in replicate in Figure 2 panels a–c, respectively. Here, the normalized effluent oocyst concentration ( $C/C_0$ ) is plotted as a function of the number of pore volumes passed through the column. The effect of ionic strength on oocyst deposition is in qualitative agreement with the Derjaguin–Landau–Verwey–Overbeek (DLVO) theory of colloidal stability (36, 37). That is, the deposition of *C. parvum* oocysts on quartz sand increases ( $C/C_0$  decreases) with increasing ionic strength (Figure 2).

Both the *C. parvum* oocysts and the quartz grains have considerable negative  $\zeta$  potential at 1 mM ionic strength (Figure 1). Under these solution conditions, available expressions for electrostatic double-layer interaction—either interaction at constant surface potential (38), constant surface charge (39), or linear superposition approximation (37)—and retarded van der Waals interaction (40) for a sphere-plate geometry predict significant electrostatic energy barrier to deposition ( $>2400$  kT). Hence, based on DLVO interaction energy calculations, *C. parvum* oocysts are not expected to deposit onto the quartz grain surfaces under these solution conditions. Despite these DLVO predictions, however, significant oocyst deposition ( $C/C_0 \approx 0.41$ ) is observed at the lowest ionic strength examined (Figure 2a). This unusually high degree of deposition in the presence of such significant energy barriers will be discussed in detail later in this paper.

**Deposition Rate of *C. parvum* Oocysts.** The experimental results presented in Figure 2 can be compared quantitatively by calculating the oocyst deposition rate coefficient,  $k_d$  (41–43):



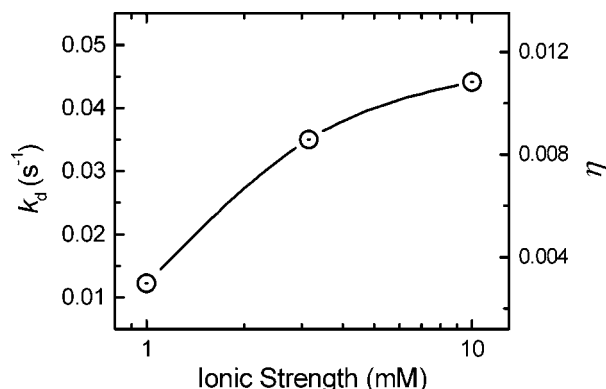
**FIGURE 2.** Breakthrough curves for two replicate experiments conducted with *C. parvum* oocysts in columns packed with clean quartz grains at three different solution ionic strengths: (a) 1 mM KCl; (b) 3.16 mM KCl; and (c) 10 mM KCl. Other experimental conditions were as follows: approach velocity = 0.042 cm/s, porosity = 0.43, mean grain diameter = 0.21 mm, pH 5.6–5.8, and temperature = 22–23 °C.

$$k_d = -\frac{U}{\epsilon L} \ln\left(\frac{C}{C_0}\right) \quad (1)$$

where  $U$  is the approach (superficial) fluid velocity,  $\epsilon$  is the porosity of the porous medium, and  $L$  is the packed column length. In determining  $k_d$ , the initial “clean bed”  $C/C_0$  at 2 pore volumes was used. The deposition rate coefficient is also directly related to the single-collector removal efficiency ( $\eta$ ) via (41, 43, 44):

$$k_d = \frac{3(1-\epsilon)}{2\epsilon d_c} U \eta \quad (2)$$



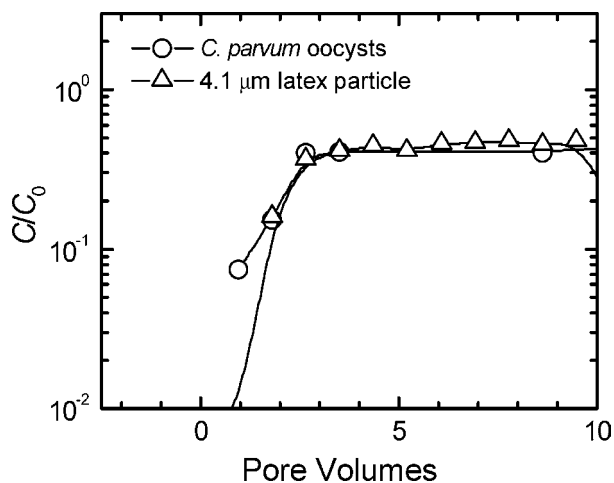


**FIGURE 3.** Influence of ionic strength on the *C. parvum* oocyst deposition rate coefficient ( $k_d$ ) and the corresponding single-collector removal efficiency ( $\eta$ ) in a column packed with quartz sand. Each data point represents the average value determined from all experiments conducted at a given ionic strength. Oocyst deposition rate coefficients were calculated using breakthrough concentrations ( $C/C_0$ ) in Figure 2 and eq 1.

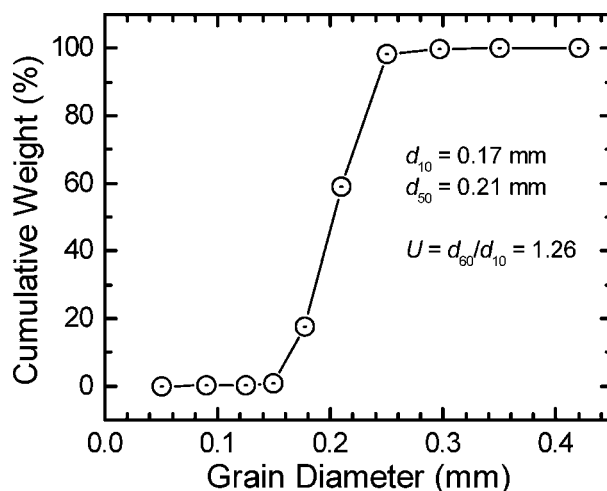
where  $d_c$  is the collector diameter. In Figure 3, the oocyst deposition rate coefficient ( $k_d$ ) and the corresponding single-collector removal efficiency ( $\eta$ ) are plotted as a function of ionic strength. As mentioned earlier, the results are in qualitative agreement with DLVO theory, that is, at higher ionic strength, the diffuse double layer is compressed causing a reduction in the repulsive electrostatic double-layer forces and an increase in the rate of oocyst deposition. The change in solution composition from 1 to 10 mM KCl yielded an increase in  $k_d$  from  $1.2 \times 10^{-2}$  to  $4.4 \times 10^{-2} s^{-1}$  and a corresponding increase in  $\eta$  from  $3.0 \times 10^{-3}$  to  $1.1 \times 10^{-2}$ .

In the previous section, we discussed the presence of a high electrostatic energy barrier to oocyst deposition at the lowest ionic strength examined (1 mM KCl). Despite this, we note here significant oocyst removal ( $C/C_0 \approx 0.41$ ), which is reflected by the relatively high value for the oocyst deposition rate coefficient ( $k_d$ ) in comparison to typical microbial or colloidal deposition rates observed in physicochemical filtration under similar conditions (41, 42, 45, 46). This high degree of oocyst removal at the lowest ionic strength examined suggests that there may be another mechanism contributing to the attenuation of oocysts in the fluid phase, which has not been considered.

**Comparison to Model Particle Deposition.** In Figure 4, the breakthrough curve obtained with *C. parvum* oocysts at 1 mM ionic strength (from Figure 2a) is compared with the experimental results obtained with a polystyrene latex particle of similar size (4.1  $\mu m$ ) under identical solution conditions. This comparison clearly indicates that *C. parvum* oocysts behave like colloidal particles of comparable size in filtration in saturated porous media. However, it is surprising that the filtration behavior of the latex particles is similar to that of the *C. parvum* oocysts given the significant differences in their electrokinetic properties. As indicated in Figure 1, the  $\zeta$  potential of the *C. parvum* oocyst at 1 mM ionic strength was  $-30$  mV. The  $\zeta$  potential of the 4.1  $\mu m$  latex particle was determined in the same manner (in 1 mM KCl, pH 5.6–5.8), and a value of  $-60$  mV was obtained. In DLVO theory, the height of the electrostatic energy barrier to deposition is extremely sensitive to the surface potential (or, in this case,  $\zeta$  potential). Such a large difference in  $\zeta$  potential should result in a significant change in the degree of particle removal, with the latex particle exhibiting a considerably lower deposition rate (47). Thus, the remarkably similar filtration behavior of *C. parvum* oocysts and 4.1  $\mu m$  latex particles at 1 mM ionic strength strongly suggests that a physical mechanism, independent of solution chemistry, may play a key role in the removal of oocysts.



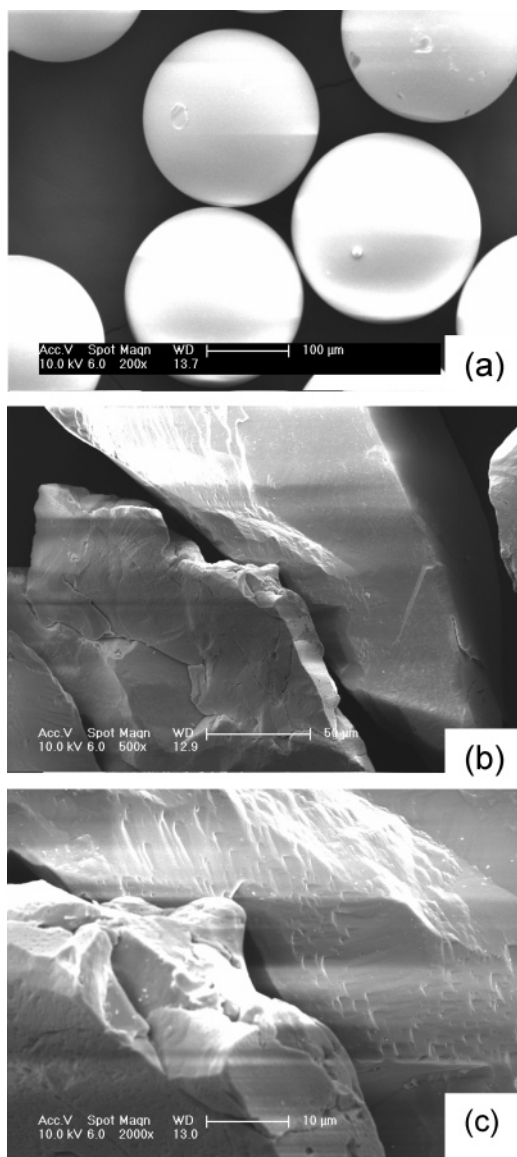
**FIGURE 4.** Breakthrough curves for experiments conducted with *C. parvum* oocysts and 4.1  $\mu m$  model latex particles in columns packed with clean quartz sand at 1 mM ionic strength. Other experimental conditions include approach velocity = 0.042 cm/s, porosity = 0.43, mean grain diameter = 0.21 mm, pH 5.6–5.8, and temperature = 22–23 °C.



**FIGURE 5.** Grain size distribution for quartz sand obtained by sieve analysis.

**Is Straining an Important Capture Mechanism?** The experimental results obtained with *C. parvum* oocysts in columns packed with a high-purity quartz indicate that a mechanism other than physicochemical filtration may be contributing to the removal of oocysts from the pore fluid. In particular, we observed significant removal of oocysts at the lowest ionic strength examined (1 mM), despite DLVO predictions to the contrary. We also showed that the filtration behavior of *C. parvum* oocysts in columns packed with clean quartz sand is nearly identical to that of model latex particles of similar size even though the latex particles possess significantly more negative  $\zeta$  potentials. These observations all point to the presence of a physical removal mechanism, such as straining, which is not influenced by electrostatic interactions. In this section, we further investigate the potential role of physical straining in the transport behavior of *C. parvum* oocysts.

The grain size distribution of the quartz sand obtained by sieve analysis is shown in Figure 5. This analysis indicates that the size distribution of the quartz grains is quite uniform (i.e., coefficient of uniformity,  $d_{60}/d_{10}$ , close to unity). Sakthivadivel (48, 49) developed a model to predict the potential for straining based on the system geometry which indicates that straining could have a significant influence



**FIGURE 6. ESEM images of (a) spherical glass bead collectors (200 $\times$  magnification, 100  $\mu$ m bar) and irregularly shaped quartz grains at (b) 500 $\times$  magnification (50  $\mu$ m bar) and (c) 2000 $\times$  magnification (10  $\mu$ m bar).**

when the ratio of the particle diameter to the median grain diameter ( $d_p/d_{50}$ ) is greater than 0.05. Similarly, Herzig et al. (50) proposed a limiting ratio of 0.154 for predicting straining of particles in constrictions. In the present study, the ratio of the oocyst to median quartz grain diameter has a value of 0.018, which, considering these geometric models, suggests that straining should not be important.

#### **(a) Grain Shape Plays an Important Role in Straining.**

A number of studies (48, 49, 51–53) indicate that straining could be important when the ratio of the particle to median grain diameter ( $d_p/d_{50}$ ) is less than 0.05, and even when it is as low as 0.002. Geometric models developed for predicting straining potential (48–50) consider a porous medium consisting of uniform spherical collectors, such as the glass beads used in this study. ESEM images of the glass beads (200 $\times$  magnification), shown in Figure 6a, demonstrate the uniform sphericity of the collectors. Although sieve analysis of the quartz sand suggested a fairly uniform grain size distribution, as indicated by the relatively low coefficient of uniformity ( $d_{60}/d_{10} = 1.26$ ), microscopic examination did reveal the grains to be quite irregular in shape. ESEM images

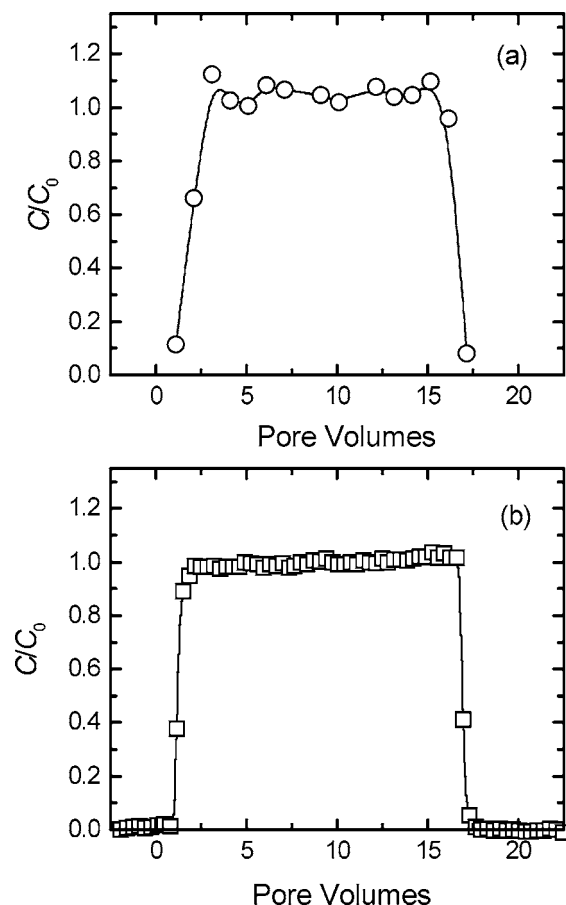
of the quartz grains taken at 500 $\times$  and 2000 $\times$  magnifications are shown in Figure 6, panels b and c, respectively. These images clearly demonstrate how the angular shape of the quartz grains can result in more irregular packing in comparison to uniform spherical collectors. This variation in the shape of the individual sand grains can contribute significantly to the porous medium straining potential as it may give rise to a very wide pore size distribution. Irregular packing can result in much smaller pore sizes than those expected based on the mean grain diameter. Colloidal particles, such as *C. parvum* oocysts, may become trapped in these interstices that are too small to allow passage.

To further verify our hypothesis that straining was an important capture mechanism in our experimental system, we conducted additional experiments with both *C. parvum* oocysts and a model latex particle of similar size (4.1  $\mu$ m) in columns packed with uniform, spherical soda-lime glass beads similar in size to the quartz grains. Furthermore, these experiments were conducted in DI water to minimize the influence of physicochemical filtration on the transport behavior of the *C. parvum* oocysts and the model colloidal particles. At the very low ionic strength of the DI water, the electrostatic double-layer repulsion between particles and collector grains is substantial so that particle deposition (or physicochemical filtration) should be negligible. Thus, in this type of experiment, any removal in the packed column can be attributed to the influence of a physical mechanism such as straining. The results of these experiments are plotted in Figure 7a,b for the *C. parvum* oocysts and the 4.1  $\mu$ m latex particles, respectively. Both the *C. parvum* oocysts and the model particles demonstrated complete breakthrough ( $C/C_0 \approx 1$ ). These data indicate that straining does not play a role in the removal of these particles in a porous medium consisting of uniform and spherical collector grains of a similar mean diameter as the quartz sand.

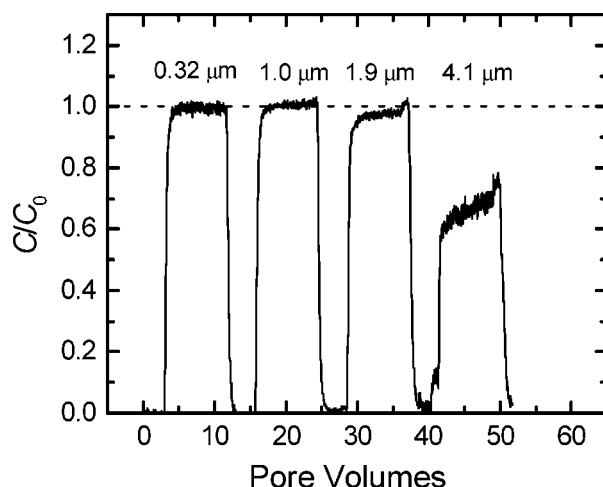
#### **(b) Further Evidence for Straining—Breakthrough with Increasing Particle Size.**

To demonstrate the effect of the irregularly shaped quartz grains on particle straining, similar experiments were conducted using DI water (pH 5.6–5.8) in a column packed with clean quartz grains. The breakthrough curves obtained with model latex particles of increasing size (0.32, 1.0, 1.9, and 4.1  $\mu$ m) suspended in DI water are plotted in Figure 8. As discussed previously, experiments conducted in DI water allow for the exclusion of the influence of physicochemical filtration on the removal of colloidal particles in the packed porous medium. In effect, both the 0.32 and 1.0  $\mu$ m latex particles exhibit no removal ( $C/C_0 \approx 1$ ) after passage in the column packed with quartz grains. The degree of removal for the 1.9  $\mu$ m colloid is also minimal ( $C/C_0 \approx 0.97$ ). However, the 4.1  $\mu$ m latex particle, which is of comparable size to *C. parvum* oocysts, exhibits significant removal under identical experimental conditions ( $C/C_0 \approx 0.63$ ). The latter removal rate corresponds to deposition rate coefficient  $k_d = 6.4 \times 10^{-3} \text{ s}^{-1}$ —several orders of magnitude greater than particle deposition rates commonly observed for similar physicochemical conditions (41, 42, 45, 46). These results clearly underline the significant role of straining as a capture mechanism for *C. parvum* oocysts in this type of porous medium. Furthermore, when compared to the data obtained with the 4.1  $\mu$ m latex particles in the column packed with glass beads (Figure 7) where  $C/C_0 \approx 1$ , the result obtained here ( $C/C_0 \approx 0.63$ ) highlights the influence of the irregular shape of the quartz grains.

**Role of Physicochemical Filtration.** In the previous section, we demonstrated the importance of straining with respect to the removal of *C. parvum* oocysts in saturated porous media. Furthermore, careful inspection of Figure 3 previously revealed a high degree of removal (i.e., large  $k_d$  or  $\eta$  value) for the experiment conducted at 1 mM KCl with respect to typical filtration rates expected for a particle of



**FIGURE 7.** Breakthrough curves for experiments conducted in columns packed with soda-lime glass beads with (a) *C. parvum* oocysts and (b) 4.1  $\mu\text{m}$  model latex particles suspended in DI water. Other experimental conditions were as follows: approach velocity = 0.042 cm/s, porosity = 0.36, mean bead diameter = 0.23 mm, unadjusted pH (varied between 5.6 and 7.1), and temperature = 22–23 °C.



**FIGURE 8.** Breakthrough curves for experiments conducted with model latex particles of increasing diameter (0.32, 1.0, 1.9, and 4.1  $\mu\text{m}$ ) suspended in DI water in a column packed with clean quartz sand. Other experimental conditions include approach velocity = 0.042 cm/s, porosity = 0.43, mean grain diameter = 0.21 mm, pH 5.6–5.8, and temperature = 22–23 °C.

comparable size at low ionic strength. As discussed earlier, this high removal rate is mostly attributed to straining. However, we can also note in Figure 3 that the degree of

oocyst removal increases considerably with increasing ionic strength. This would not be the case if physical straining were the sole mechanism controlling the transport behavior of *C. parvum* oocysts in porous media. Hence, the results presented in Figure 3 confirm that physicochemical filtration (or oocyst deposition) also contributes significantly to the removal of oocysts in saturated porous media.

**Implications.** Straining was found to be an important removal mechanism for *C. parvum* oocysts in a porous medium having a relatively uniform grain size distribution and a moderate particle to average grain diameter ratio. The results presented in this study suggest that the ratio of microbial particle to mean grain size cannot be used as the sole predictor of straining potential. Instead, the shape irregularity, or angularity, of the sediment grains should also be considered. Because natural subsurface environments are highly heterogeneous with respect to grain size and shape, straining is expected to be a dominant removal mechanism for *C. parvum* oocysts in such settings. Furthermore, the observed straining of *C. parvum* oocysts suggests that other pathogenic protozoa that are larger in size, such as cysts of *Giardia* spp. and oocysts of *Cyclospora* sp., would be removed much more effectively by straining. Therefore, the classic colloid filtration theory, which does not account for the influence of straining, cannot be used to model and estimate (oo)cyst removal and travel distances in granular porous media settings such as bank filtration, groundwater aquifers, and slow sand filters.

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