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Evaluation of Microspheres as Surrogates for *Cryptosporidium parvum* Oocysts in Filtration Experiments

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The size and surface characteristics of a surrogate particle and *Cryptosporidium parvum* oocysts are important in determining the ability of the particle to mimic the behavior of *C. parvum* oocysts in filtration and particle transport experiments. The ζ potential, hydrophobicity, and filterability of a surrogate particle, 5 μm carboxylated latex microspheres, and oocysts were compared for a variety of solution conditions. *C. parvum* oocysts had a slightly negative ζ potential (−1.5 to −12.5 mV) at pH 6.7 over a wide range of calcium concentration (10^{-6}−10^{-1} M), while the fluorescent microspheres were more negatively charged under the same conditions (−7.4 to −50.2 mV). After exposure to 5 mg of C/L of Suwanee River natural organic matter (NOM), the ζ potentials of both particles became significantly more negative, with the microspheres consistently maintaining a more negative ζ potential than the oocysts. Alum was able to neutralize the negative ζ potentials of both particles when in the presence of NOM, but nearly twice the dosage was required for the microspheres. NOM also affected the hydrophobicity of the particles by increasing the hydrophobicity of the relatively hydrophilic oocysts and decreasing the hydrophobicity of the relatively hydrophobic microspheres. A bench-scale filtration system removed less microspheres (40.3 ± 1.5%) than oocysts (49.7 ± 2.9%) when 0.01 M $CaCl_2$ was supplied as coagulant. After preexposure to 5 mg of C/L of NOM, the removals of both particles declined significantly, and the removals of microspheres (13.7 ± 1.5%) and oocysts (16.3 ± 1.5%) were similar. Finally, the removal efficiencies of microspheres and oocysts in the presence of NOM increased to 69.3 ± 3.5% and 67.7 ± 6.4%, respectively, when alum was supplied as coagulant at the optimum dosage needed to destabilize the oocysts. These experimental results suggest that microspheres can be used to provide a conservative estimate of oocyst removal in filters containing hydrophilic negatively charged filter media.

Introduction

Cryptosporidium parvum is a protozoan pathogen frequently found in surface waters in the United States (*1*−*3*), and it has even been detected in groundwater at low concentrations (*2*). The oocyst is the disinfection-resistant stage of *C. parvum* that is released into the environment via the feces of infected host organisms. This waterborne pathogen causes cryptosporidiosis, a severe gastrointestinal illness than can prove fatal in immunocompromised persons (*4*, *5*).

Recently, a great deal of research has been devoted to developing approaches for limiting human exposure to this organism via drinking water supplies. For example, watershed management programs have been developed to reduce oocyst loading to surface waters. Because this organism is known to occur in many drinking water sources (*1*−*3*), research has also focused on oocyst removal or inactivation during water treatment. Unfortunately, the most commonly used drinking water disinfectants in the United States, free chlorine and monochloramine, are not effective at inactivating *C. parvum* oocysts at typical dosages and contact times used in practice (*45*). While increasing the chlorine dosage may improve oocyst inactivation efficiency, high chlorine dosages impart unpalatable taste and odor to the water and will likely result in the formation of unacceptable levels of halogenated disinfection byproducts. Ozone is much more effective than free chlorine or monochloramine for inactivating oocysts (*6*, *45*), but ozonation is more costly than chlorination, and bromate formation is a concern for bromide-containing waters (*7*, *8*). Ultraviolet light irradiation (UV) is another promising disinfection alternative (*9*, *10*), but UV is currently not in widespread use for disinfection of potable water.

Because of the resistance of oocysts to inactivation by common disinfectants, emphasis has been placed on oocyst removal by traditional particle removal processes including sedimentation, dissolved air flotation, and filtration (*11*−*13*). Filtration is considered the most important treatment barrier for oocyst removal in water treatment plants. In addition, filtration in natural porous media can inhibit the transport of oocysts through aquifers (*14*). A few studies in the peer-reviewed literature report results for viable oocyst removal by filtration systems including granular media filters (e.g., ref *11*) and bag filters (*15*); however, a significant concern in performing filtration experiments with viable *C. parvum* oocyts, especially pilot-scale or full-scale studies, is the potential health risk for research personnel. On the other hand, it is unclear how well the results using inactivated oocysts or synthetic particles represent the behavior of viable oocysts. Therefore, finding a reliable surrogate is very important for reducing human health risk while not compromising the value or usefulness of the results.

Oocysts range in size from 4 to 7 μm, with a mean of approximately 5 μm (*16*). Several different particles have been used as surrogates for viable oocysts in experiments designed to test the performance of particle removal technologies (e.g., rapid filters) including formalin-inactivated oocysts (*17*, *18*), heat-inactivated oocysts (*19*), and fluorescent microspheres (*15*, *19*−*21*).

Li et al. (*15*) reported good agreement between log removals of 4−6-μm polystyrene microspheres and *C. parvum* oocysts by bag filtration systems with a nominal pore size of 1 μm. The particle removal mechanism was physical straining, and the oocysts and microspheres were similar in size. Therefore, it is not surprising that the removals of oocysts and microspheres were in good agreement. In a rapid filter, however, the major particle removal mechanism is not straining but deposition onto the surfaces of the filter media grains (*22*). Amburgey et al. (*19*) compared removals of heat-inactivated oocysts (55 °C for 30 min) and 4.5-μm carboxylated polystyrene microspheres in conventional and biologically active deep-bed (152 cm) filters containing granular activated carbon (GAC) or anthracite coal. The researchers

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reported similar removals across all filters, but the removal of microspheres (2.0 log) was consistently greater than that of heat-inactivated oocysts (1.5 log). Emelko et al. (*20*) observed ~5 log removal of both formalin-inactivated oocysts and microspheres in pilot-scale dual media filters under optimal operating conditions. A linear relationship was reported between the log removals of the two particles over a range of operating conditions (log microsphere removal = 0.85 log oocyst removal + 0.35; $R^2 = 0.96$). The aforementioned studies provide useful information on the use of surrogate particles in filtration experiments, but there appears to be a lack of research comparing the surface properties and removals of surrogate particles and viable oocysts.

Although particle size also affects particle removal in rapid filters, the surface characteristics of the particles and the media determine the collision efficiency, which is also very important for effective particle removal (*23*). Collision efficiency is defined as the fraction of particles that attach to a collector (i.e., filter media grain) after colliding with it, and its value ranges from 0 (no attachment) to 1 (100% attachment) (*23*). The surface properties of suspended particles and hence collision efficiency are significantly affected by solution conditions, including pH, ionic strength, and the presence of natural or synthetic organic macromolecules (*24*). Natural organic matter (NOM) is the term used to refer to the naturally occurring, typically anionic, organic molecules and macromolecules that are ubiquitous in water supplies. NOM can sorb onto solid surfaces immersed in water including those of suspended particles and alter their surface properties (*25*). Therefore, NOM can affect the coagulation and flocculation of particles and their deposition in filters (*24*, *25*).

Several research groups have investigated the surface properties of *C. parvum* oocysts including measurement of electrophoretic mobility (*12*, *26*−*29*) and hydrophobicity as adhesion to octane (*27*) or as adhesion to polystyrene (*29*). Many investigators report ζ potentials rather than mobilities. ζ potentials are computed from the mobility values typically using the Helmholtz−Schmoluchowski equation. The reported surface properties vary because of differences in one or more of the following factors: source of the oocysts and purification method used (*29*), addition of chemicals or antibiotics to preserve the oocysts and limit aggregation (*26*), age of the oocysts (*27*, *29*), and solution used to prepare the oocysts for the experiments (*26*, *27*). Furthermore, when oocysts enter a natural water body, their surface properties are likely to be affected by the chemical composition of the water (*26*), and these changes could affect oocyst removal in filters (*30*). Thus, to identify a reliable surrogate, the surface properties of the surrogate and its removal in filters must be tested over a wide range of solution conditions. Therefore, the main objective of this research was to compare the surface characteristics of microspheres and oocysts under different solution conditions in order to determine the ability of microspheres to serve as surrogates for oocysts in filtration experiments.

Experimental Section

Materials. *Particles. C. parvum* oocysts were purchased from the Sterling Parasitology Laboratory, Department of Veterinary Science and Microbiology, University of Arizona. The oocysts were collected from infected Holstein calves, screened through sieves, and concentrated by centrifugation. The oocysts were then isolated from the feces by discontinuous sucrose gradients followed by microcentrifuge-scale cesium chloride gradients (*31*, *32*). The purified oocysts were placed in a 0.01% Tween 20 solution containing 100 units of penicillin, 100 μg of streptomycin, and 100 μg of gentamicin/mL to retard bacterial growth. The oocyst suspensions (~10^8 oocysts/mL) were stored at 4 °C and used within 1 year of acquisition. Fluorescent latex microspheres were purchased from Polysciences Inc. (catalog no. 16592) as a 2.5 wt % aqueous suspension (~5×10^8 microspheres/mL) and were stored in the dark at 4 °C. The fluorescent microspheres are comprised of polystyrene with surface carboxyl groups.

The size of the oocysts was determined by measuring 30 randomly selected oocysts on a hemacytometer using light microscopy under 400× magnification and an image analysis system. The image analysis system was calibrated with a micrometer. The mean size of the oocysts showed minor variation for two different shipments (1, 4.92 ± 0.43 μm; 2, 4.47 ± 0.37 μm). The mean diameter of the microspheres (4.69 ± 0.26 μm) was determined in a similar manner. Mean diameter values (oocysts, shipment 2, 4.31 ± 0.24; microspheres, 4.56 ± 0.11) determined using a particle counter (Multisizer3 Coulter Counter) calibrated with nearly monodisperse suspensions of microspheres were in good agreement with those determined by microscopy. The specific gravities of the oocysts and microspheres were 1.025−1.07 (*16*) and 1.05 (provided by manufacturer), respectively.

Chemicals. All chemicals used in this research including $NaHCO_3$, $CaCl_2$, NaOH, and concentrated HNO_3 were reagent grade. Stock solutions were prepared in distilled and deionized (DI) water obtained from a Milli-Q water purification system. Suwannee River whole NOM was obtained from the International Humic Substances Society as a freeze-dried powder (48.8% carbon, 7.0% ash). The NOM was dissolved in buffered water (5×10^{-5} M $NaHCO_3$, pH 6.7−7.0) to 5 mg/L as C for the experiments.

Filtration Experiments. A bench-scale filtration system was used to compare the clean-bed removals of microspheres and oocysts. The filter consisted of a 2.54 cm (1 in.) i.d. × 30.5 cm (12 in.) polycarbonate plastic column. A schematic diagram of the filtration system used in this research is provided elsewhere (*33*). The column was packed with 0.55-mm spherical glass beads (MO-SCI Corporation) to a depth of 25 cm (10 in.) and a porosity of 40%. Relatively uniform spherical glass beads were used to provide a filter media with a well-characterized shape that was needed for modeling and analysis of particle collision efficiencies. Prior to being installed in the column before each filter run, the glass beads were thoroughly cleaned by a multistep procedure similar to that used by Franchi (*34*): (i) wash with distilled water, (ii) ultrasonicate in 0.01 M NaOH for 15 min, (iii) ultrasonicate in distilled water and repeat until the UV light absorbance at 254 nm of the rinse water is close to zero (below 0.010 cm^{-1}), (iv) ultrasonicate in 1 M HNO_3 for 20 min, and (v) final rinse with distilled and deionized water.

A filter loading rate of 5 m/h (2 gpm/ft^2) was used for all experiments. A bromide tracer experiment demonstrated that the system approximated plug-flow conditions with a mean hydraulic residence time of 6.6 min and a dispersion number of 0.02. All experiments with this system were performed at room temperature (20−25 °C). Calcium chloride (10^{-2} M) served as the coagulant for two sets of filtration experiments, and alum (75 μM Al^{3+}) was used for the other. Although calcium is not typically used as a coagulant in water treatment practice, it has been used to destabilize particles in other filtration studies (e.g., ref *35*) and also plays an important role in the coagulation of turbidity and organic matter in the environment (*36*). The solution pH values for the filtration experiments were 6.7−7.0 for experiments with Ca^{2+} alone, 6.2 with NOM and Ca^{2+}, and 4.4 with NOM and alum. Clean glass beads have negative ζ potentials at pH 7 for Ca^{2+} concentrations of 10^{-2} M and lower (*35*). Soda lime glass is largely comprised of silica (~74%), which is typically negatively charged at pH values of 2 or higher because of deprotonation of surface silanol groups (*24*).

A filtration experiment was initiated by first pumping the filtration solution through the filter for 30 min prior to the

introduction of particles. Then, a step input of a combined suspension of oocysts and microspheres ($\sim 3 \times 10^3$/mL of each) was introduced into the filter followed by effluent monitoring. Three composite effluent samples were collected for 2 min each during the pseudo-steady-state period of the breakthrough curve (time ≥10 min) (*33*). The three effluent samples, along with three samples from the particle feed reservoir, were enumerated using microscopy as described below.

Analytical Procedures. *Enumeration of Particles. C. parvum* oocysts and microspheres were enumerated by direct counting using microscopy. Water samples containing both oocysts and microspheres were vacuum-filtered onto a 0.22-μm pore size (25 mm diameter) black polycarbonate membrane filter (Millipore). The volume filtered was 50 mL for samples of the influent water from the bench-scale filtration experiments and 80−84 mL for effluent samples. After filtration, 330 μL of immunofluoresent antibody solution (Waterborne Inc.) was applied to the filter and allowed to react for 40 min. The antibody solution was removed from the filter by applying vacuum, and the filter was then mounted on a glass microscope slide. The fluorescently labeled oocysts and fluorescent microspheres were counted by epifluorescence microscopy at 400× magnification with a Nikon microscope (model Eclipse E600) equipped with mercury vapor lamp, color video camera, computer, and image analysis software. The microspheres were significantly brighter than the oocysts. Thus, the microspheres could be counted separately by the installation of two neutral density filters (ND4 and ND16) that effectively blocked the fluorescence of the oocysts. Then, the neutral density filters were removed, and the total number of particles in the field was determined with the oocyst count obtained by the difference. At least 20 randomly selected fields (area = 0.00052 cm^2) were counted per membrane filter (filtration area = 2.5 cm^2). The average count per field was computed and multiplied by the number of fields per filter (4800) to obtain the total number of particles in the sample. Recoveries of oocysts and microspheres using this method were 83% and 102%, respectively.

Particle Characterization. The oocysts and microspheres were characterized by determining the electrophoretic mobility and hydrophobicity for a variety of solution conditions.

The electrophoretic mobilities of the particles were determined using a Lazer Zee Meter (model 501) at an applied voltage of 100 V. A small aliquot ($\sim$100 μL) of the stock particle suspension (oocysts or microspheres) was diluted with 100 mL of a prepared solution in a 250-mL beaker and mixed for 30 min on a stir plate. The diluted suspension was then placed into the sample cell for measurement of electrophoretic mobility. The measurements were made by manually adjusting the speed of a rotating prism until the entire field of laser-illuminated particles appeared to be stationary. The instrument computed the electrophoretic mobilities from the rotational speed of the prism and then converted the mobility values to ζ potentials using the Helmholtz−Smoluchowski equation. By changing the composition of the diluent solution, the effects of NOM, calcium concentration, and alum concentration on ζ potential were evaluated. The pH was in the range of 6.7−7.0 for the mobility measurements except for those with NOM, alum, or both. The pH of the buffered solution after NOM addition was 6.2, and it decreased with increasing alum dosage (see Figure 2).

Hydrophobicity was evaluated by measuring the partitioning of particles between hexadecane ($C_{16}H_{34}$) and water (*37*, *38*). The effect of NOM on particle hydrophobicity was evaluated by performing partitioning experiments both in the presence of NOM (5 mg C/L) and in the absence of NOM. For the partitioning experiment, 500 μL of an oocysts or microsphere suspension (10^6−10^7/mL) in buffered water (5×10^{-5} M $NaHCO_3$, pH 6.7−7.0) was added to a 1.5-mL microcentrifuge tube. Then, 500 μL of hexadecane was added, and the tube was vigorously mixed by hand-shaking for approximately 30 s. After being mixed, the hexadecane and water phases were allowed to separate under quiescent conditions for at least 5 min. The aqueous phase was gently mixed to resuspend any settled particles by withdrawing and reinjecting 100 μL by micropipet at least 3 times prior to removing 100 μL for measurement of the particle concentration using a particle counter (Multisizer3 Coulter Counter). The oocyst concentration in the hexadecane phase was calculated by mass balance from the particle counts in the aqueous phase of the hexadecane-water vials and the control vials (no hexadecane). Finally, the K_{hw} value was obtained by dividing the particle concentration in hexadecane by that in water. Each partitioning experiment was repeated 4−6 times, and all experiments were performed at room temperature (20−25 °C).

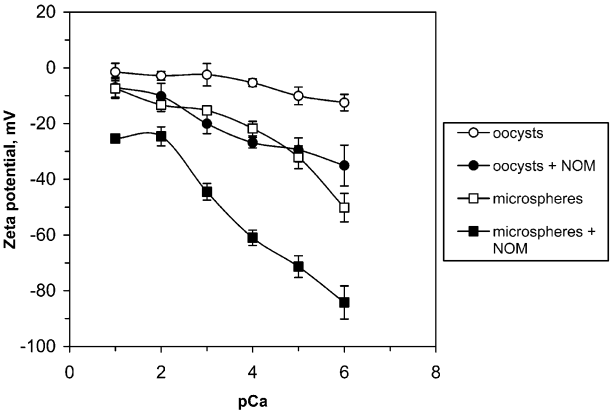

FIGURE 1. Comparison of the ζ potentials of *C. parvum* oocysts (circles) and microspheres (squares) in the presence of 5 mg of C/L of NOM (solid symbols) and the absence of NOM (open symbols) as a function of calcium concentration at pH 6.7. Error bars represent one standard deviation from the mean of three measurements (oocysts) or five measurements (microspheres). (pCa = −log[Ca^{2+}] where [Ca^{2+}] is the calcium concentration in moles per liter).

Results and Discussion

Particle Surface Characterization. *ζ Potential.* The ζ potentials of both oocysts and microspheres in the presence and absence of NOM over a wide range of Ca^{2+} concentration are shown in Figure 1. At Ca^{2+} concentrations ranging from 10^{-6} to 10^{-1} M in the absence of NOM, the oocysts had a slightly negative ζ potential (−1.5 to −12.5 mV). These results agree well with some ζ potential values reported in the literature (*28*, *29*) but were significantly less negative than values reported in other studies (*26*, *27*). The differences in reported values are likely due to differences in oocyst purification. Brush et al. (*29*) reported significant differences in electrophoretic mobility depending on the oocyst purification procedure used and little effect of aging on mobility. The negative ζ potential of the oocysts is likely due to the presence of deprotonated carboxyl groups, as the oocyst surface is known to be covered by proteins (*39*).

The fluorescent microspheres consistently had a much more negative ζ potential than the oocysts over a similar range of Ca^{2+} concentrations. At a Ca^{2+} concentration of 10^{-6} M, the ζ potential of the microspheres was -50.2 ± 5.1 mV, but as the calcium concentration was increased to 0.1 M, the ζ potential became less negative (-7.4 ± 3.4 mV). The negative ζ potential of the microspheres results from deprotonated carboxyl groups on the surface, as is suspected for oocysts. Furthermore, the effects of Ca^{2+} on the ζ potential of the

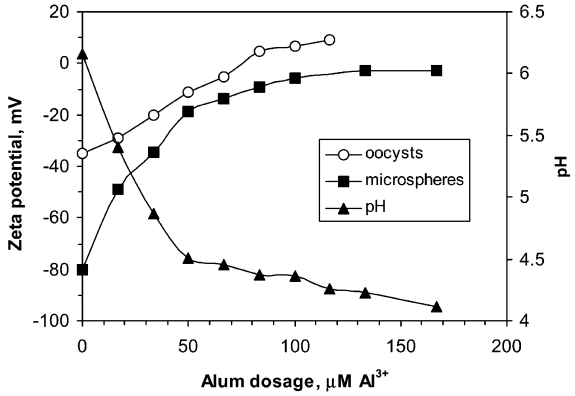

FIGURE 2. Effect of alum dosage on solution pH and on the ζ potential of microspheres and oocysts in the presence of 5 mg of C/L of NOM. The data points represent single measurements.

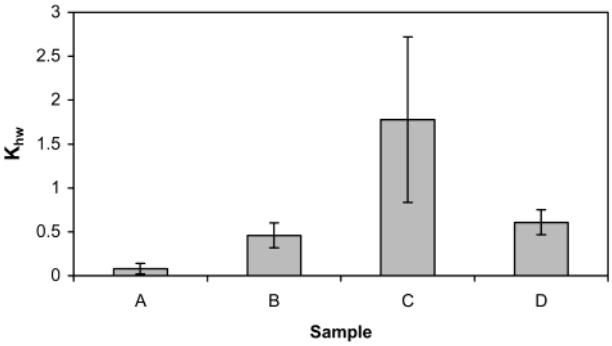

FIGURE 3. Comparison of K_{hw} values for oocysts in the absence of NOM (A), oocysts in the presence of 5 mg of C/L of NOM (B), microspheres in the absence of NOM (C), and microspheres in the presence of 5 mg of C/L of NOM (D). Error bars represent one standard deviation from the mean of four (oocysts) or six (microspheres) measurements.

particles can be attributed to a combination of electrical double-layer compression and specific interaction of Ca^{2+} with the particle surface resulting in charge neutralization (*35*).

After exposing the oocysts to 5 mg/L of NOM as C, the ζ potential decreased significantly as compared to "clean" oocysts over the entire range of Ca^{2+} concentrations (Figure 1). At 10^{-6} M Ca^{2+}, the ζ potential of the oocysts was -35 ± 7.3 mV, which is nearly 20 mV more negative than the value for clean oocysts. Ongerth and Pecoraro (*26*) also observed a decrease in ζ potential when oocysts were exposed to a 2.4 mg/L fulvic acid solution. As the Ca^{2+} concentration was increased, the ζ potential became less negative until it was approximately zero at a Ca^{2+} concentration of 0.1 M (Figure 1).

Similar to the oocysts, the ζ potential of the microspheres became more negative relative to clean microspheres after exposure to 5 mg of C/L of NOM. At a Ca^{2+} concentration of 10^{-6} M, the ζ potential of the microspheres was -84 ± 5.9 mV in the presence of NOM, which is nearly 34 mV more negative than the value for clean microspheres of -50.2 ± 5.1 mV. When the Ca^{2+} concentration was increased, the ζ potential of microspheres in the presence of NOM became less negative. However, even at 0.1 M Ca^{2+}, the microspheres had a ζ potential of -24.6 ± 3.4 mV, suggesting that electrostatic repulsion would still impede flocculation of the particles and their deposition onto negatively charged surfaces.

It appears that the NOM adsorbed onto the surfaces of the negatively charged oocysts and microspheres despite the expected electrostatic repulsion at circumneutral pH values. The adsorption of humic acid onto negatively charged bacteria was attributed to hydrophobic interactions by Fein et al. (*40*), who observed increasing extent of adsorption as pH decreased. NOM adsorption onto negatively charged surfaces can also be mediated by polyvalent cations that form complexes with negatively charged groups on both the NOM and the surface (*36*, *41*). Although the particles were equilibrated with NOM in the absence of added polyvalent cations such as Ca^{2+}, the contribution of this mechanism cannot be dismissed as there are likely polyvalent cations present in the NOM sample (7 wt % ash).

The effect of alum addition on the ζ potential was also evaluated because alum is a commonly used coagulant in drinking water treatment. The ζ potential of both microspheres and oocysts in the presence of NOM (5 mg of C/L) became less negative with increasing alum dosage (Figure 2). At alum dosages greater than ~75 μM as Al^{3+}, the ζ potential of the oocysts became positive. Bustamante et al. (*12*) also observed a reversal in ζ potential to positive values for oocysts at sufficiently high dosages of the coagulants alum and ferric chloride. The ζ potential appeared to plateau at slightly negative values for the microspheres at alum dosages of 130 μM as Al^{3+} (Figure 2). Thus, the dosage required to nearly neutralize the surface charge on the microspheres (~130 μM as Al^{3+}) was much greater than that for the oocysts (~75 μM as Al^{3+}). It is important to note that the addition of alum to the weakly buffered solution resulted in a significant decrease in pH. This pH decrease likely contributed to the ζ potentials becoming less negative by increasing the protonation of surface carboxylic acid groups.

The microspheres consistently exhibited a more negative ζ potential than the oocysts over a range of solution conditions. These results suggest that when microspheres are filtered, they will have lower collision efficiencies and, thus, lower removal efficiencies than oocysts.

Hydrophobicity. Particle surface hydrophobicity was quantified by measuring the hexadecane−water partitioning coefficient (K_{hw}). K_{hw} serves as a relative measure of hydrophobicity with increasing values corresponding to increasing hydrophobicity. The K_{hw} value obtained for *C. parvum* oocysts was 0.08 ± 0.06 (Figure 3), which indicated that the oocysts preferentially partitioned into the water phase and were relatively hydrophilic. However, the K_{hw} value obtained for microspheres was 1.78 ± 0.94, which indicated that the microspheres preferentially partitioned into the hexadecane phase and were more hydrophobic than the oocysts. Values of percent adhesion to hexadecane for 20 different strains of bacteria reported by van Loosdrecht et al. (*38*) ranged from 0 to 83%, which corresponds to K_{hw} values of 0−5, respectively. The K_{hw} value for both particles studied in this research fell within this range.

Preequilibration of the particles with NOM affected the hydrophobicities of the two types of particles. The K_{hw} value for oocysts increased from 0.08 ± 0.06 to 0.46 ± 0.14, while the K_{hw} value for microspheres decreased from 1.78 ± 0.94 to 0.61 ± 0.14 (Figure 3). Thus, NOM increased the hydrophobicity of the hydrophilic oocysts and decreased the hydrophobicity of the relatively hydrophobic microspheres. A likely reason for the seemingly contradictory effects on particle hydrophobicity is that the NOM has both hydrophobic (e.g., aromatic) and hydrophilic (e.g., carboxyl) functional groups. Thus, it is possible that the NOM could alter the hydrophobicity of different particles in opposite directions, depending on the initial surface chemistry of the particles.

The surface hydrophobicities of both particle and collector are important in particle adhesion. For example, adhesion of bacteria to a hydrophobic polystyrene surface increased with increasing bacterial hydrophobicity; adhesion to a hydrophilic glass surface was not significantly affected by changes in bacterial hydrophobicity unless electrostatic

TABLE 1. Comparison of Experimentally Determined Removal Efficiencies and Calculated Collision Efficiencies for Oocysts and Microspheres in Bench-Scale Filtration Experiments[a]

conditions				removal efficiency (%)		collision efficiency	
NOM (mg of C/L)	Ca^{2+} (M)	alum (μm of Al^{3+})	run	oocysts	microspheres	oocysts	microspheres
0	0.01	0	1	48	39	0.80	0.65
			2	53	40	0.92	0.68
			3	48	42	0.80	0.72
			mean[b]	49.7 ± 2.9	40.3 ± 1.5	0.84 ± 0.07	0.68 ± 0.03
5	0.01	0	1	15	12	0.20	0.17
			2	16	15	0.21	0.22
			3	18	14	0.24	0.20
			mean[b]	16.3 ± 1.5	13.7 ± 1.5	0.22 ± 0.02	0.19 ± 0.02
5	0	75	1	65	69	1.8	1.9
			2	75	73	2.4	2.1
			3	63	66	1.7	1.7
			mean[b]	67.7 ± 6.4	69.3 ± 3.5	2.0 ± 0.4	1.9 ± 0.2

[a] The solution pH values for the filtration experiments were 6.7−7.0 for experiments with Ca^{2+} alone, 6.2 with NOM and Ca^{2+}, and 4.4 with NOM and alum. [b] Mean ± standard deviation.

repulsion was negligible (*38*, *42*). The results from the K_{hw} measurements in this research suggest that the use of microspheres as a surrogate may result in overestimation of oocyst removal when the filter media surfaces are hydrophobic. This statement is supported by the results of Amburgey et al. (*19*) discussed above, who observed greater removals of microspheres than (inactivated) oocysts in GAC and anthracite filters in the presence of a low concentration (2 mg of C/L) of preozonated NOM. Nevertheless, the results of this research suggest that the difference between the particle hydrophobicities and removals in filters containing hydrophobic media will be diminished when the water is rich in natural organic matter.

Bench-Scale Filtration Experiments. The bench-scale filtration system removed less microspheres (40.3 ± 1.5%) than oocysts (49.7 ± 2.9%) when 0.01 M $CaCl_2$ was supplied as coagulant (Table 1). After preexposure to 5 mg of C/L of NOM, the removals of both particles declined significantly, and the removals of microspheres (14 ± 1.7%) and oocysts (16.3 ± 1.5%) were similar. This decrease in particle removal in the presence of NOM was due to increased electrostatic repulsion between the particles and filter media. The removals of both particles in the presence of NOM (oocysts, 67.7 ± 6.4%; microspheres, 69.3 ± 3.5%) increased significantly when alum was supplied as coagulant at the optimal dosage for oocyst destabilization in the presence of NOM (75 μM Al^{3+}).

In pilot-scale filters under optimal operating conditions with alum as coagulant, Huck et al. (*18*) observed oocyst removals of ~3 log units or 99.9% for one facility and ~5 log units or 99.999% for another. Furthermore, Edzwald et al. (*17*) reported >5 log removal of oocysts for pilot plant systems (>3 log removal attributed to filtration) employing alum with the dosage optimized for *Cryptosporidium* and *Giardia* removal. Clearly, the removals in these pilot-scale systems are more than 2 log units greater than the highest removals observed in this study (63−75%). These large differences are likely due to one or more of the following factors: (i) the flocculation of the particles prior to filtration was employed in the pilot studies; (ii) the presence of other particles in the water (in addition to the oocysts) enhanced flocculation; (iii) the filter beds in the pilot studies were comprised of sand and anthracite and were of greater depth (0.7−0.9 m); and (iv) the pilot filter beds were ripened prior to measuring the optimum oocyst removals. Thus, the experiments discussed in this paper are more reflective of direct filtration systems, but the low bed depth of the bench-scale system and the use of clean-bed or nonripened filters resulted in much lower removals than would be expected in full-scale systems.

Collision efficiency values were computed from the breakthrough concentrations and a particle mass balance equation using the corrected Rajagopalan and Tien (*43*) expression for the collector efficiency as described in Logan et al. (*44*). The mean collision efficiency value for microspheres was lower than that for oocysts at 0.01 M Ca^{2+} in the absence of NOM, but the values were similar in the presence of 5 mg C/L of NOM (Table 1). Furthermore, the values for both particles decreased significantly in the presence of NOM. Thus, NOM strongly influences the surface properties of both particles and appears to minimize the differences between them.

The highest observed collision efficiency values (1.9 ± 0.2 for microspheres, 2.0 ± 0.4 for oocysts) were obtained with alum as coagulant in the presence of NOM. Values in excess of 1.0 are theoretically impossible when discrete particles are filtered through a clean bed of collectors. The high collision efficiency values suggest that particle flocculation may have occurred prior to the particles reaching the filter bed, that filter ripening occurred, or both. In addition, Yao et al. (*23*) suggested that flocculation within the pores of the filter bed could increase the removal efficiency of particles with size greater than 1 μm. A value close to 1.0 was expected for the oocysts as the alum dosage (75 μM Al^{3+}) was selected such that they would be completely destabilized (Figure 2). Despite the suboptimal alum dosage for the microspheres (ζ potential of approximately −15 mV), the collision efficiency was still very high. The high collision efficiency value for the microspheres at this alum dosage was likely because of a combination of factors including: (i) a lowering of the solution pH with protonation of some of the acidic functional groups on the NOM and elsewhere; (ii) the ability of the free aluminum and aluminum hydroxide species to form complexes with and to neutralize the negative charges on the particles and the filter media; and (iii) flocculation of the microspheres and oocysts.

Environmental Significance. The results from this research suggest that the differences in surface characteristics between *C. parvum* oocysts and latex microspheres, and the potential effects on removal efficiency should be considered when making predictions of oocyst removals from filtration experiments where microspheres served as surrogates. In experiments with hydrophilic negatively charged filter media, microspheres had a similar or lower removal efficiency than oocysts. Thus, microspheres should provide a similar or conservative estimate of oocyst removal removals by such filter media (e.g. glass beads or sand). Based on results from the particle surface characterization experiments, however, microspheres likely will not provide a conservative estimate

of oocyst removal when relatively hydrophobic filter media (e.g., GAC) is used. In addition, the presence of NOM had two important effects: (i) the ζ potential of both oocysts and microspheres became significantly more negative, which decreased their probability of removal in the filters; and (ii) it improved the agreement between the surface properties and removal efficiencies for the two types of particles under the conditions tested. Despite the detrimental effects of NOM on particle removal, effective removal of oocysts in the presence of NOM was achieved by applying alum at the optimum dosage for particle destabilization.

Although these experiments were designed to compare the behavior of oocysts and carboxylated latex microspheres in water treatment filters, the experimental results also have some potential implications for oocyst transport in natural environments. The results suggest that oocysts will effectively move through sandy groundwater aquifers containing soft or low ionic strength water (i.e., $\leq 10^{-3}$ M Ca^{2+}) and significant organic matter concentrations. Furthermore, the transport of the microspheres is likely to be much greater that that of oocysts under low ionic strength conditions. On the other hand, oocyst transport should be very limited in relatively hard waters with low organic matter concentrations. When the groundwater is hard or of high ionic strength (i.e., $> 10^{-3}$ M Ca^{2+}) and both particles are coated with natural organic matter, the microspheres should effectively mimic the transport of oocysts. Finally, because this work only evaluated latex microspheres, more work is needed to investigate other particles such as inactivated oocysts for use as surrogates for viable oocysts in filtration and groundwater transport studies.

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Literature Cited

(1) Clancy, J. L. *J. Am. Water Works Assoc.* **2000**, *92*, 55−66.
(2) Hancock, C. M.; Rose, J. B.; Vasconcelos, G. J.; Harris, S. I.; Klonicki, P. T.; Sturbaum, G. D. *J. Am. Water Works Assoc.* **2000**, *92*, 117−123.
(3) LeChevallier, M. W.; Norton, W. D.; Lee, R. G. *Appl. Environ. Microbiol.* **1991**, *57*, 2610−2616.
(4) Fayer, R. *Cryptosporidium and Cryptosporidiosis*; CRC Press: Boca Raton, FL, 1997.
(5) Tate, C. H.; Arnold, K. F. In *Water Quality and Treatment*, 4th ed.; Pontius, F. W., Ed.; McGraw-Hill: New York, 1990; pp 63−156.
(6) Rennecker, J. L.; Kim, J. H.; Corona-Vasquez, B.; Mariñas, B. J. *Environ. Sci. Technol.* **2001**, *35*, 2752−2757.
(7) Galey, C.; Mary-Dile, V.; Gatel, D. *J. Am. Water Works Assoc.* **2001**, *93*, 105−115.
(8) von Gunten, U.; Driedger, A.; Gallard, H. *Water Res.* **2001**, *35*, 2095−2099.
(9) Clancy, J. L.; Bukhari, Z.; Hargy, T. M.; Bolton, J. R.; Dussert, B. W.; Marshall, M. M. *J. Am. Water Works Assoc.* **2000**, *92*, 97−104.
(10) Cotton, C. A.; Owen, D. M.; Cline, G. C.; Brodeur, T. P. *J. Am. Water Works Assoc.* **2001**, *93*, 82−94.
(11) Shaw, K.; Walker, S.; Koopman, B. *J. Am. Water Works Assoc.* **2000**, *92*, 103−111.
(12) Bustamante, H. A.; Shanker, S. R.; Pashley, R. M.; Karaman, M. E. *Water Res.* **2001**, *35*, 3179−3189.
(13) Plummer, J. D.; Edzwald, J. K.; Kelley, M. B. *J. Am. Water Works Assoc.* **1995**, *87*, 85−95.
(14) Harter, T., Wagner, S.; Atwill, E. R. *Environ. Sci. Technol.* **2000**, *34*, 62−70.
(15) Li, S. Y.; Goodrich, J. A.; Owens, J. H.; Willeke, G. E.; Schaefer, F. W.; Clark, R. M. *J. Am. Water Works Assoc.* **1997**, *89*, 90−99.
(16) Medema, G. J.; Schets, F. M.; Teunis, P. F. M.; Havelaar, A. H. *Appl. Environ. Microbiol.* **1998**, *64*, 4460−4466.
(17) Edzwald, J. K.; Tobiason, J. E.; Parento, L. M.; Kelley, M. B.; Kaminski, G. S.; Dunn, H. J.; Galant, P. B. *J. Am. Water Works Assoc.* **2000**, *92*, 70−84.
(18) Huck, P. M.; Coffey, B. M.; Emelko, M. B.; Maurizio, D. D.; Slawson, R. M.; Anderson, W. B.; van den Oever, J.; Douglas, I. P.; O'Melia, C. R. *J. Am. Water Works Assoc.* **2002**, *94*, 97−111.
(19) Amburgey, J. E.; Amirtharajah, A.; Arrowood, M. J.; Spivey, N. C. *Proceedings of the American Water Works Association Water Quality Technology Conference*; 2001.
(20) Emelko, M. B.; Huck, P. M.; Douglas, I. P.; van den Oever, J. *Proceedings of the American Water Works Association Water Quality Technology Conference*; 2000.
(21) Emelko, M. B.; Douglas, I. P.; van den Oever, J. *Proceedings of the American Water Works Association Water Quality Technology Conference*; 2001.
(22) Cleasby, J. L. In *Water Quality and Treatment*, 4th ed.; Pontius, F. W., Ed.; McGraw-Hill: New York. 1990; pp 455−560.
(23) Yao, K.; Habibian, M. T.; O'Melia, C. R. *Environ. Sci. Technol.* **1971**, *5*, 1105−1112.
(24) Amirtharajah, A.; O'Melia, C. R. In *Water Quality and Treatment*, 4th ed.; Pontius, F. W., Ed.; McGraw-Hill: New York, 1990; pp 269−365.
(25) Jekel, M. R. *Water Res.* **1986**, *20*, 1543−1554.
(26) Ongerth, J. E.; Pecoraro J. P. *J. Environ. Eng.* **1996**, *122*, 228−231.
(27) Drozd, C.; Schwartzbrod, J. *Appl. Environ. Microbiol.* **1996**, *62*, 1227−1232.
(28) Rice, E. W.; Fox, K. R.; Miltner, R. J.; Lytle D. A.; Johnson C. H. *J. Am. Water Works Assoc.* **1996**, *88*, 122−130.
(29) Brush, C. F.; Walter, M. F.; Anguish, L. J.; Ghiorse, W. C. *Appl. Environ. Microbiol.* **1998**, *64*, 4439−4445.
(30) Hsu, B. M.; Huang, C.; Pan, J. R. *Water Res.* **2001**, *35*, 3777−3782.
(31) Arrowood, M. J.; Sterling, C. R. *J. Parasitol.* **1987**, *73*, 314−319.
(32) Arrowood, M. J.; Donaldson, K. *J. Eukaryotic Microbiol.* **1996**, *43*, 5, 89S.
(33) Dai, X.; Hozalski, R. M. *Water Res.* **2002**, *36*, 3523−3532.
(34) Franchi, A. Deposition and Reentrainment of Colloids in Porous Media: Effects of Natural Organic Matter and Solution Chemistry. Ph.D. Dissertation, Johns Hopkins University, Baltimore, MD, 2000.
(35) Tobiason, J. E.; O'Melia, C. R. *J. Am. Water Works Assoc.* **1988**, *80*, 54−64.
(36) Stumm, W.; Morgan, J. J. *Aquatic Chemistry*, 2nd ed.; John Wiley and Sons: New York. 1981; p 671.
(37) Rosenberg, M. *FEMS Microbiol. Lett.* **1984**, *22*, 289−295.
(38) van Loosdrecht, M. C. M.; Lyklema, J.; Norde, W.; Schraa, G.; Zehnder, A. J. B. *Appl. Environ. Microbiol.* **1987**, *53*, 1893−1897.
(39) Ranucci, L.; Muller, H. M.; LaRosa, G.; Reckmann, I.; Morales, M. A. G.; Spano, F.; Pozio, E.; Crisanti, A. *Infect. Immun.* **1993**, *61*, 2347−2356.
(40) Fein, J. B.; Bioly, J. F.; Guclu, K.; Kaulbach, E. *Chem. Geol.* **1999**, *162*, 33−45.
(41) Busch, P. L.; Stumm, W. *Environ. Sci. Technol.* **1968**, *2*, 49−53.
(42) van Loosdrecht, M. C. M.; Lyklema, J.; Norde, W.; Zehnder A. J. B. *Microbiol. Rev.* **1990**, *54*, 75−87.
(43) Rajagopalan, R.; Tien, C. *AIChE J.* **1976**, *22*, 523−533.
(44) Logan, B. E.; Jewett, D. G.; Arnold, R. G.; Bouwer, E. J.; O'Melia, C. R. *J. Environ. Eng.−ASCE* **1995**, *121*, 869−873.
(45) Korich, D. G.; Mead, J. R.; Madore, M. S.; Sinclair, N. A.; Sterling, C. R. *Appl. Environ. Microbiol.* **1990**, *56*, 1423−1428.

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