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Comparison of transport and attachment behaviors of *Cryptosporidium parvum* oocysts and oocyst-sized microspheres being advected through three minerologically different granular porous media

Arvind Mohanram [a], Chittaranjan Ray [b], Ronald W. Harvey [c,d,*], David W. Metge [c], Joseph N. Ryan [d], Jon Chorover [e], D.D. Eberl [c]

[a] Department of Molecular Bioscience and Bioengineering, 1955 East-West Road, Agricultural Science 218, University of Hawaii at Manoa, Honolulu, HI 96822, USA
[b] Department of Civil & Environmental Engineering and Water Resources Research Center, University of Hawaii at Manoa, 2540 Dole Street, Honolulu, HI 96822, USA
[c] U. S. Geological Survey, 3215 Marine Street, Boulder, CO 80303, USA
[d] Department of Civil, Environmental and Architectural Engineering, 428 UCB, Boulder, CO 80309, USA
[e] Department of Soil, Water and Environmental Science, Shantz 429, University of Arizona, Tucson, AZ 85721-0038, USA

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ABSTRACT

In order to gain more information about the fate of *Cryptosporidium parvum* oocysts in tropical volcanic soils, the transport and attachment behaviors of oocysts and oocyst-sized polystyrene microspheres were studied in the presence of two soils. These soils were chosen because of their differing chemical and physical properties, i.e., an organic-rich (43–46% by mass) volcanic ash-derived soil from the island of Hawaii, and a red, iron (22–29% by mass), aluminum (29–45% by mass), and clay-rich (68–76% by mass) volcanic soil from the island of Oahu. A third agricultural soil, an organic- (13% by mass) and quartz-rich (40% by mass) soil from Illinois, was included for reference. In 10-cm long flow-through columns, oocysts and microspheres advecting through the red volcanic soil were almost completely (98% and 99%) immobilized. The modest breakthrough resulted from preferential flow-path structure inadvertently created by soil-particle aggregation during the re-wetting process. Although a high (99%) removal of oocysts and microsphere within the volcanic ash soil occurred initially, further examination revealed that transport was merely retarded because of highly reversible interactions with grain surfaces. Judging from the slope of the substantive and protracted tail of the breakthrough curve for the 1.8-μm microspheres, almost all (>99%) predictably would be recovered within ~4000 pore volumes. This suggests that once contaminated, the volcanic ash soil could serve as a reservoir for subsequent contamination of groundwater, at least for pathogens of similar size or smaller. Because of the highly reversible nature of organic colloid immobilization in this soil type, *C. parvum* could contaminate surface water should overland flow during heavy precipitation events pick up near-surface grains to which they are attached.

* *Corresponding author.* U. S. Geological Survey, 3215 Marine Street, Boulder, Colorado 80303, United States. Tel.: +1 303 541 3034; fax: +1 303 447 2505.
E-mail addresses: arvind@hawaii.edu (A. Mohanram), cray@hawaii.edu (C. Ray), rwharvey@usgs.gov (R.W. Harvey), dwmetge@usgs.gov (D.W. Metge), joseph.ryan@colorado.edu (J.N. Ryan), chorover@cals.arizona.edu (J. Chorover), ddeberl@usgs.gov (D.D. Eberl).

Surprisingly, oocyst and microsphere attachment to the reference soil from Illinois appeared to be at least as sensitive to changes in pH as was observed for the red, metal-oxide rich soil from Oahu. In contrast, colloidal attachment in the organic-rich, volcanic ash soil was relatively insensitive to changes in pH in spite of the high iron content. Given the fundamental differences in transport behavior of oocyst-sized colloids within the two volcanic soils of similar origin, agricultural practices modified to lessen *C. parvum* contamination of ground or surface water would necessitate taking the individual soil properties into account.

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1. Introduction

Groundwater contamination by the pathogenic protozoa *Cryptosporidium parvum* is a cause of serious public health concern. *C. parvum* is an opportunistic protozoan pathogen that infects the small intestine of mammals including humans and cattle, resulting in cryptosporidiosis (LeChevallier et al., 1991). Outside the host, the thick-walled, environmental form of this parasite (oocysts), can remain viable and potentially infective for up to 6 months (Fayer et al., 1997). Oocysts often contaminate drinking water supplies, following their transport from sources such as dairy cattle operations and wastewater effluents (LeChevallier et al., 1991). They are highly resistant to common methods of chemical disinfection employed in water treatment, particularly chlorination (Fayer et al., 1997). Consequently, treatment plants can be faced with a considerable challenge in order to achieve the 4-log removal from source water under the Long Term 2 Enhanced Surface Water Treatment Rule (USEPA, 2006). Cost-effective removal commonly involves immobilization within granular-media, often accomplished by sand or riverbank filtration (Ray et al., 2002; Tufenkji et al., 2002).

Despite the potent health impacts and difficulty of inactivation of *C. parvum*, incomplete data exist regarding the fate and transport of these pathogens in soils and aquifers. A number of controlled laboratory studies on oocysts transport have focused on model granular media such as quartz sand (Tufenkji et al., 2004) or glass beads (Dai and Hozalski, 2002). These studies are helpful for a more mechanistic understanding of oocysts deposition processes. However, more information is needed on how these results might relate to transport and deposition in the multi-component, terrestrial subsurface. The few studies that have addressed oocyst attachment in natural media (Hijnen et al., 2005; Harter et al., 2000) have involved so-called "permanently charged" soils in which charge derives dominantly from isomorphically-substituted phyllosilicate clay minerals, such as smectite and vermiculite, that impart a net negative structural charge that is relatively independent of pH (Sposito, 2008). However, several cases of oocyst contamination of surface and groundwater have been reported in regions with "variably charged" soils such as in the southeast US (LeChevallier et al., 2000) and Brazil (Bushen et al., 2007), where the clay mineralogy is dominated by kaolinite and (oxy) hydroxides of iron and aluminum, including hematite and gibbsite. These secondary minerals exhibit variable charge whose magnitude and sign depend on the pH, ionic strength (I), and ionic composition of the soil water (Uehara and Gillman, 1981). Although it has been reported that metal oxides provide favorable interfacial regions for attachment of oocysts in model sediments (Abudalo et al., 2005), no study to date has reported on the transport behavior of oocysts in tropical soils of volcanic origin, where these mineral phases can predominate. Although these soils may be rich in iron and aluminum oxides, high concentrations of organic carbon (e.g., Trumbore, 1993) could mask the electrostatic attraction of the metal oxide surfaces (Pieper et al., 1997), thereby enhancing microbial transport. Consequently, there is a need to assess the efficacy of different types of tropical, volcanic soils to immobilize oocysts under relevant conditions.

The overall objective of this paper is to compare the transport and attachment behaviors of oocysts and oocyst-sized microspheres in two volcanic soils from Hawaii having fundamentally different physicochemical properties. Because considerably more research has been previously conducted on permanent-charged soils (Bradford and Bettahar, 2005; Tufenkji et al., 2004; Darnault et al., 2004), an agricultural soil from Illinois was also included as a reference. The pH dependency was assessed using static batch experiments, and the magnitude and reversibility of attachment were assessed using flow-through column experiments in order to compare the manner in which oocyst-sized colloids would be transported in the aforementioned soils.

2. Methods

2.1. Cryptosporidium parvum oocysts

Formalin-inactivated oocysts were obtained from Sterling Parasitology Laboratory (SPL) at the University of Arizona and prepared according to the methods outlined by Abudalo et al. (2005).. The final oocyst concentration used for the column experiments was between $2 \times 10^6 - 5 \times 10^6$ mL^{-1}. The oocysts were enumerated by epifluorescence microscopy. Samples containing the oocysts were stained with 4,6-dia-midino-2-phynylindole (DAPI, 0.1 mg mL^{-1} solutions, 15 min contact time), filtered with vacuum assistance (0.34 bar) onto black polycarbonate membranes (1.0-μm pore diameter, Osmonics), prepared with a cover slide and immersion oil, and counted manually using an epifluorescence microscope (Nikon Optiphot-2, 788× magnification, 350 nm excitation, 470 nm emission). Oocysts were enumerated in at least 100 optical fields for every sample. The average diameter of the oocysts was measured by flow cytometry analysis using DAPI-stained oocysts. The flow cytometer (Biorad, HY Bryte) was calibrated using fluorescent microspheres of 3 different sizes

(1.8-μm, 2.9-μm, and 4.9-μm). The electrophoretic mobility of the oocysts was measured at a temperature of 22 °C by laser Doppler microelectrophoresis (Zeta PALS Potential Analyzer-Brookhaven Instruments) for pH values of 3, 6, and 9 in 10^{-3}M NaCl at a concentration of about 4×10^5 oocyst mL^{-1}. Electrophoretic mobilities were converted to zeta potential (ζ) using the Smoluchowski equation (Masliyah and Bhattacharjee, 2006).

2.2. Microspheres

We used a mixture consisting of fluorescent carboxylated, polystyrene microspheres of two diameters: 1.8-μm and 4.9-μm (Type "BB", brilliant blue) and 2.9-μm (Type, fluorescent green) (Polysciences, Warrington, Pennsylvania). These were used to create the polydispersed microsphere suspension that collectively bracketed the 2—5 μm variation in oocyst size commonly found in the environment (Harvey et al., 2008). The concentration of the microspheres used in the column studies were 0.5—1 × 10^7 mL^{-1} (1.8 μm) and 4 × 10^6 mL^{-1} (2.9 μm and 4.9 μm). The electrophoretic mobility of microspheres was measured by laser Doppler micro electrophoresis at pH values 3, 6 and 9 in 10^{-3} M NaCl at a concentration of about 4 × 10^6 microspheres mL^{-1}.

2.3. Soil characteristics

Two compositionally different tropical volcanic soils were chosen because they differed substantively in terms of organic and mineral contents and degrees of weathering. The weathered red volcanic clayey (Poamoho) soil was collected on Oahu from the Poamoho Agricultural Experiment Station (College of Tropical Agriculture and Human Resources (CTAHR), University of Hawaii), which is 200 m above sea level and receives ~1.0 m of annual rainfall. The red soil (derived from a lava flow of about 2.5 million years in age) is used for pineapple production and is intensely weathered silty clay Oxisol of the Wahiawa series, and is classified as Rhodic Eutrustox (Wan and El-Swaify, 1997). In contrast, the organic rich-volcanic ash (Lalamilo) soil, collected from a field near the town of Waimea, Hawaii, at an elevation of 350 m, is derived from a lava flow that is about 150,000 years old, and is subjected to about 0.3 m of annual rainfall. As a result, this soil is considerably less weathered, and contains a lower mass concentration of iron oxides. The latter soil is used to grow cabbage, lettuce and soybean (Deenik et al., 2006) and is a silty loam Inceptisol of the Waimea series, typic Eutrandepts, medial and amorphic family (Soil Survey Staff: http://websoilsurvey.nrcs.usda.gov).

The temperate agricultural soil used for comparative purposes is a 10,000 year old, black loamy Midwest agricultural (Drummer) soil that was collected on the campus of University of Illinois at an elevation of 200 m in a region that receives 0.9 m of annual precipitation. It is used for growing corn and soybeans and is classified as silty clay loam Mollisol of the Drummer series in the Endoaquolls family (David et al., 1997).

The three soils were autoclaved at 121 °C for 20 min, oven-dried at 105 °C overnight, and sieved to obtain a common grain size corresponding to their d$_{50}$ of the aggregates (red, clayey volcanic soil) and corresponding soil particles. In the rest of the text, the red, clayey volcanic soil; the high-organic volcanic ash; and the black loamy Midwestern agricultural soil will be referred to, respectively, as "Poamoho", "Lalamilo", and "Drummer" soils. Physical, chemical and mineralogical data on these three soils are given in Tables 1 and 2.

2.4. X-ray diffraction

The mineralogy of the soils were examined by X-ray diffraction (XRD; Siemens model D 500) using Cu Kα radiation from 5° to 65° 2Θ, with 0.02° 2Θ steps and scan time of 2s step^{-1}. A graphite monochromator eliminated high background Fe fluorescence. The XRD intensity was converted to mineral weight (%) data using an option in the RockJock computer program and according to the method given in Eberl and Smith (2009).

2.5. Solutions

All solutions were prepared using filter-sterilized deionized water (18 MΩ cm^{-1} resistivity; Milli-Q, Millipore Corp., Bedford, MA). For the flow-through column studies, influent pH was maintained at 7.2 by adding 10^{-2} M NaOH as necessary. For the experiments in static columns the solution pH was adjusted as necessary with 10^{-2} M NaOH and HCl. Nitrate (10^{-3} M, added as NaNO$_3$) was used as a conservative tracer and measured by ultraviolet absorption (220 nm wavelength) using a spectrophotometer (Spectronic/Unicam, Genesys 10).

2.6. Static columns

The attachment studies with oocysts and microspheres were conducted with 20 mL static columns (25 mm diameter, 120 mm height) modified from those described by Scholl and Harvey (1992). Columns were prepared using polypropylene mesh (105 μm pore size) as an underlayment, on top of which was placed about 5 g of soil. Another polypropylene mesh was laid on top of the added soil and 4 g of glass beads (1 mm, diameter) were carefully placed on top of the mesh. This ensured that the soil bed did not move when washing with

Table 1 — Characteristics of granular porous media used for the experiments.			
Property	Poamoho, Oahu	Lalamilo, Hawaii	Drummer, Illinois
Order	Oxisol	Inceptisol	Mollisol
Type	Clay loam	Silt loam	Silty clay loam
Sampled soil interval (A-horizon, mm)	0—300	0—300	0—300
Avg. grain density (g/cm^3)	2.66	2.37	2.65
Bulk density (g/cm^3)	0.92	0.70	1.20
Porosity[a]	0.66	0.70	0.55
pH[b] (1:1, KCl)	5.30	5.80	5.60
d$_{50}$ (mm)	0.65	0.21	1.00
Avg. grain fraction used (mm)	0.35	0.20	1.00
Pore volume (mL) in flow-through column	39	39	33

a Calculated from the ratio of the bulk to particle density.
b This is the pH of the soil; the experimental pH condition was 7.2.

Table 2 – Mineralogy of granular porous media used for the experiments. The range represents the maximum and minimum values of six samples for each soil type.

Property (w/w %)	Poamoho soil	Lalamilo soil	Drummer soil
Non-clay materials			
Quartz	0	0–1	40
Plagioclase feldspar	0	12–15	0
Potassium feldspar	0	0	8
Magnetite	0–1	2–3	0
Hematite	5–8	0	0
Apatite	0–1	0–1	0
Goethite	0–1	0	0
Maghemite	17–19	14–16	2
Organic matter	0	43–46	13
Total non-clay	24–32	82–84	63
Clay materials			
Smectite	0–5	7–13	14
Amorphous kaolinite	29–45	0	1
Illite	8–19	6–9	0
Halloysite	15–25	0	2
Phlogopite	0	0	2
Muscovite	0	0	8
Total clay	68–76	16–19	27

background solution and prevented the low bulk density (0.7 g cm^{-3}) Lalamilo soils from floating. The columns were saturated and water level maintained at about 35 mm above the soil bed. The static columns were equilibrated by rinsing with at least 200 pore volumes of background solution. The pore volume of Poamoho and Lalamilo soil columns was ~5 mL, whereas that of Drummer soils was ~3.5 mL. One mL of oocysts and microsphere suspensions ($2 \times 10^6 - 5 \times 10^6$ colloidal particles/mL) were added to the top of the column. From the bottom of the static columns, 1.0 mL of pore fluid was withdrawn in order to draw the suspension into the granular media. Previous kinetic studies revealed that the >70% of microspheres and oocysts attached within the first 2 h and equilibrium was reached in less than 4 h. After 4 h, 12 pore volumes of background solution (10^{-3}M NaCl) were passed through the column to remove unattached particles. The eluent was collected in a 30 mL capacity, acid-washed, baked (400 °C) glass vial. Fractional attachment of oocysts and microspheres was calculated based upon the number of oocysts or microspheres recovered compared to the number that were added at pH 3, 6, and 9. All experiments were run in triplicates at 22 °C.

2.7. Flow-through columns

Transport studies were conducted using flow-through glass chromatography columns (25 mm diameter, 100 mm length). A 5 mL injection loop provided a "pulse input" of colloids to the column. Pore volumes (calculated by the time taken for tracer to breakthrough) for the Poamoho and Lalamilo was 39 mL, while that of Drummer soil was 33 mL. Polypropylene mesh (105-μm openings) was installed inside the polytetra-fluoroethene end caps on both ends of the column.

Two different sets of flow-through column studies were employed. The first set was conducted using the Poamoho and the Drummer soils only. The soil fractions were dry-packed

with vibration to minimize any layering. Carbon dioxide (2%) was passed through the column for 1 h to remove residual air from the pore spaces. The second set of experiments was conducted with Lalamilo soil using only the 1.8 μm micro-spheres in the absence of oocysts or 4.9-μm microspheres. Two types of studies were conducted, i.e., one employed unaltered soil and the other used soil in which the soil organic matter (SOM) had been removed by chemical oxidation using sodium hypochlorite (NaOCl) as modified from Siregar et al. (2005). For both experiments, the influent pH was 8.5.

Standard gravimetric methods were used to determine the porosity, bulk density and particle density of the soils. The background solution was fed to the column through poly-propylene tubing using a computer-controlled piston pump (stainless steel, 500 mL volume, ISCO model 500D). The tracer solution, oocysts, and microspheres suspensions were added to the background solution using a high performance liquid chromatography injector (stainless steel; Supelco Rheodyne) and injection loop (stainless steel, 5.0 mL volume). For all experiments, the pump was filled with the background elec-trolyte solution (10^{-3} M NaCl) and the injection loop was filled with the conservative tracer nitrate (added as 10^{-3} M NaNO$_3$; pH 5.6), oocysts (2×10^6 mL^{-1}), and microspheres ($0.5-1 \times 10^7$ mL^{-1} (1.8-μm) and 4×10^6 mL^{-1} (2.9-μm and 4.9-μm)). The sodium nitrate and oocysts were co-injected as a single pulse. The I of the carrier and injection fluids were the same. A fraction collector and glass test tubes were used to collect the column effluent.

Before each experiment, several hundred pore volumes of background solution were passed though the column until the pH and specific conductance levels of inlet and effluent solu-tions were the same. Equilibration time for each soil column took approximately 1 week. The pumping rates were set to produce pore velocities of 1.6 ± 0.1 m d^{-1} for all the experi-ments. At least three pore volumes of breakthrough were monitored for all experiments except those involving the Lalamilo soils, where 10 pore volumes were collected. Numerical integration of the breakthrough curve was carried out only for the Lalamilo soils. Two replicates flow-through tests were performed using the Poamoho, Drummer and unaltered Lalamilo soil, whereas only one flow-through column test was performed using the chemically altered Lalamilo soil.

2.8. Transport modeling of colloid transport

The one dimensional advective dispersion equation for colloid transport and removal of colloids by physiochemical filtration for homogenous granular porous media under saturated flow conditions is given as:

$$\frac{\partial C}{\partial t} + \frac{\rho_b}{\varepsilon}\frac{\partial S}{\partial t} = D\frac{\partial^2 C}{\partial x^2} - v\frac{\partial C}{\partial x} \qquad (1)$$

where C is the colloid concentration in the aqueous phase at a distance x and time t, D is the hydrodynamic dispersion coefficient, v is the interstitial colloid velocity, S is the concentration of attached colloid, ρ_b is the bulk density of the granular porous media, and ε is the porosity.

The change in microbial concentration due to attachment and detachment processes is given as:

$$\frac{\partial(\rho_b S_{att})}{\partial t} = k_a \varepsilon C - k_r \rho_b S_{att} \qquad (2)$$

where S_{att} is the attached concentration of the colloid, k_a and k_r are the first order attachment and first order detachment rate coefficients respectively.

Colloid filtration theory can be incorporated in this model by using k_a term (Ryan and Elimelech, 1996):

$$k_a = \frac{3}{2}\frac{(1-\varepsilon)}{d_g}\eta\alpha v \qquad (3)$$

where d_g is the grain size diameter, η is the single-collector removal efficiency, α is the attachment efficiency and v is the pore water velocity. The value of collector efficiency accounts for the colloid removal due to diffusion, sedimentation and interception and calculated according to Tufenkji and Elimelech (2004).

The attachment and detachment rate coefficients, collector efficiency, collision efficiency were calculated using the "Colloid Filtration Model". The breakthrough curve data, obtained were used to fit parameters k_a, k_r, dispersivity and collision efficiency using HYDRUS-1D computer code (Simunek et al., 2005) as described by equations (1) to (3). Finally, the extent of colloid removal within the soil columns and the retardation of peaks and center of mass were calculated for all the colloids.

3. Results

3.1. Physical/mineralogical composition

The data from the XRD analysis are presented in Table 2. The range represents the maximum and minimum values of six samples for each soil type. For the Poamoho soils, the iron oxide composition varied between 17%–19% w/w. Clay minerals comprised between 68%–76% of the total mass, a majority of which were identified as kaolinite. For the Lalamilo soil, the organic matter made up 43%–46% of the total mass, whereas iron oxide (maghemite) made up 14%–16%. In contrast, the Drummer soils were rich in both quartz (40%) and organic matter (13%) with a considerably smaller content (2%) of iron oxide.

3.2. Colloid size and charge

The average diameter of oocysts was measured as $3.6 \pm 0.3\ \mu m$ (± 1 standard deviation) by flow cytometry and confirmed by epifluoresence microscopy. The buoyant density of oocysts was reported by Abudalo et al., 2005 as $1.075 \pm 0.005\ g\ cm^{-3}$ using Percoll density gradient centrifugation.

The zeta potentials for the microspheres and oocysts were negative (Fig. 1) for the pH used in the static and flow-through column experiments. The microspheres became more negatively charged with increasing pH from 3 to 9. The 4.9-μm microspheres were more negatively charged (-0.12 mV at pH 3 and -79 mV at pH 9) compared with the 1.8-μm microspheres (-21 mV at pH 3 and -53 mV at pH 9). However, the oocysts exhibited a substantive negative charge (ζ of -17 mV) at pH 6, but were nearly uncharged at pH 3 and at pH 9 (ζ of
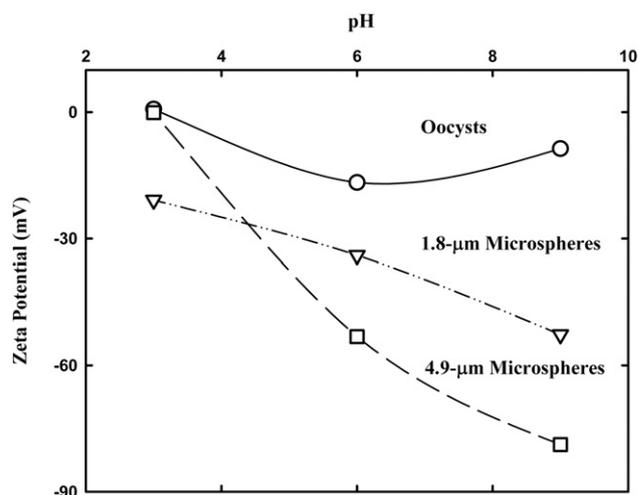

Fig. 1 — ζ of formalin inactivated *C. parvum* oocysts (4×10^5 mL^{-1}) and oocysts sized microspheres (4×10^6 mL^{-1}) suspended in 10^{-3}M NaCl as measured by Doppler micro electrophoresis. 10^{-2} M-Sodium hydroxide and Hydrochloric acid were used to adjust the pH.

only $+0.7$ mV and -9 mV, respectively). As expected, the absolute value of the zeta potential decreased with increase in I with the addition of sodium hydroxide, as reported earlier (Abudalo et al., 2005; Hsu and Huang, 2002). The maximum standard deviation for replicate ζ values determinations was 2.6 mV.

3.3. Static columns

A comparison of the pH dependencies of oocyst and microsphere attachment for the three soil types is shown in Fig. 2. For the microspheres, attachment was strongly pH dependent in the Drummer and Poamoho soils, but relatively independent of pH in the presence of Lalamilo soils. For the 1.8-μm microspheres, fractional attachment in the presence of Drummer soil decreased from 90% at pH 3 to 50% at pH 9 and for Poamoho soil from 99% at pH 3 to 74% at pH 9. However, fractional attachment in the presence of Lalamilo soil remained nearly constant at 67% from pH 3 to 9. Similarly, in response to a pH increase from 3 to 9, fractional attachment of 4.9-μm microspheres dropped from 99% to 79% for Drummer soils and from 99% to 82% for Poamoho soils. For the oocysts, fractional attachment was strongly pH-dependent in the Drummer soils (decreasing from 92% at pH 3 to 29% at pH 9), insensitive to pH (ranging from 98 to 99%) for the Lalamilo soils and slightly pH dependent for Poamoho soil (decreasing from 99% at pH 3 to 86% at pH 9).

3.4. Flow-through columns

3.4.1. Poamoho soil
Fractional breakthrough (C/C$_o$) was plotted as a function of the number of pore volumes passing through the 100-mm long column (Figs. 3–5). For all experiments, peak breakthrough
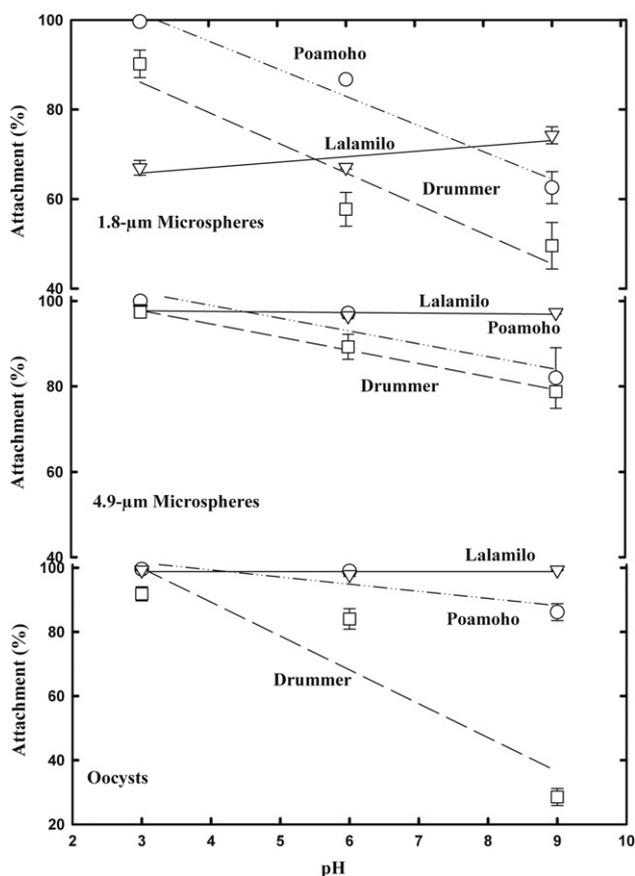
Fig. 2 — Attachment (%) at 22 °C ± 1 °C of *C. parvum* oocysts and microspheres ($2 \times 10^6 - 5 \times 10^6$ mL^{-1}) to 5 g soil packed in 120 mm static columns. Fractional attachment determined by difference between concentration of colloid added and recovered subsequent to flushing. The trend lines indicate best-fit linear regression for each soil. The correlation coefficients varied between 0.78 and 0.98 at $p \leq 0.001$. The error bars represent standard errors of measurement between triplicate measurements.

Fig. 3 — Dimensionless concentration histories at 22 ± 1 °C for conservative tracer, *C. parvum* oocysts, 1.8-μm and 4.9-μm microspheres being advected in 10^{-3}M NaCl through 100 mm Poamoho soil column ($d_{50} = 0.65$ mm) at 1.6 m d^{-1} and pH 7.2. The measurements of colloids were done for three pore volumes.

Fig. 4 — Dimensionless concentration histories at 22 ± 1 °C for 1.8-μm microspheres being advected in 10^{-3}M NaCl through 100 mm Lalamilo soil column ($d_{50} = 0.21$ mm) at 1.6 m d^{-1} and pH 8.5. Arrow indicates the point of nitrate breakthrough.

occurred earlier for oocysts and microspheres than for the conservative tracer (nitrate). For the Poamoho soils (Fig. 3), there was instantaneous breakthrough of the oocysts at 0.46 pore volume, PV, versus 1.0 PV for nitrate and 0.62 PV and 0.72 PV, respectively, for the 4.9-μm and 1.8-μm microspheres. Replicate values of the retardation of microspheres and oocysts based upon time of arrival for the peak (RF_p) and center of masses (RF_{cm}) relative to those of the conservative tracer (nitrate) for the three soils are given in Tables 3 and 4. For the three soil types, there were no significant differences in retardation among replicate tests. Only about 2% and 1%, respectively, of the 3.6-μm oocysts and 4.9-μm microspheres were transported through the entire length of the column as compared with 18% of the smaller (1.8-μm) microspheres. The retardation factor of the microsphere peak (0.72) through the Poamoho soil was slightly lower than retardation factor estimated for the center of mass (0.79). Calculated attachment and detachment rate coefficients for the 1.8-μm microspheres were 39.6 d^{-1} and 1.1 d^{-1}, respectively.
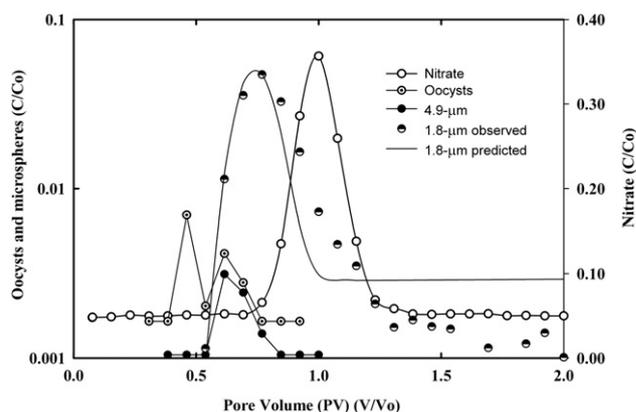
Fig. 5 – Dimensionless concentration histories at 22 ± 1 °C for conservative tracer, *C. parvum* oocysts, 1.8-μm and 4.9-μm microspheres being advected in 10^{-3}M NaCl through 100 mm Drummer soil column (d_{50} = 1 mm) at 1.6 md^{-1} and pH 7.2. The measurements of colloids were done for three pore volumes.
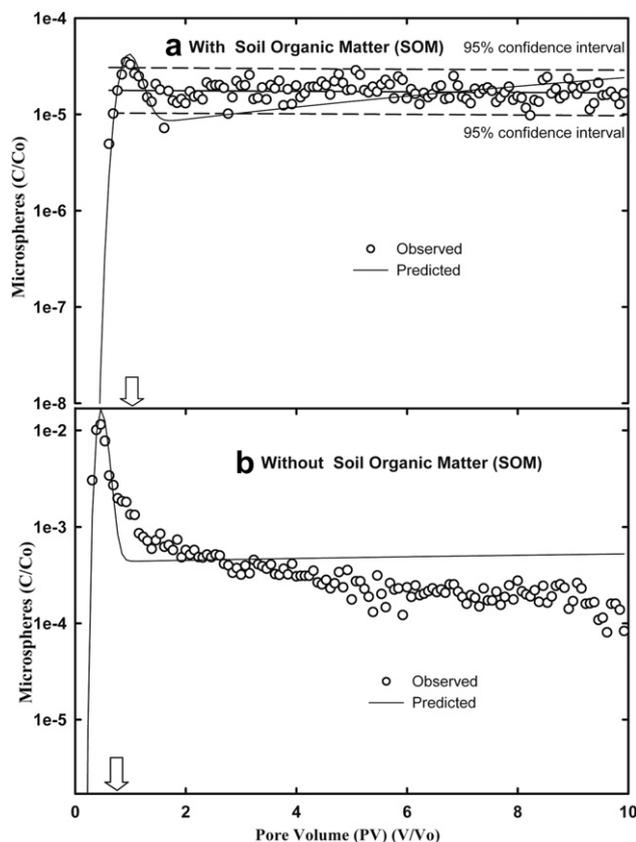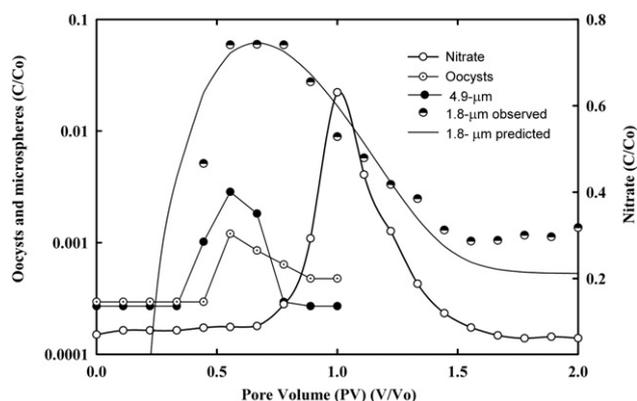
3.4.2. Lalamilo soil

The effect of removal of soil organic matter (SOM) on the transport of 1.8-μm microspheres in the Lalamilo soils is depicted in Fig. 4. Dimensionless concentrations of microspheres in the effluent of flow-through columns packed with the unaltered organic-rich Lalamilo soil peaked at ∼3.5×10^{-5} during the first collected pore volume, but exhibited substantive breakthrough (tailing behavior) for the remainder of the experiment. For the subsequent 9 pore volumes following the initial peak, fractional breakthrough of microspheres averaged ∼1.8×10^{-5}. Extrapolation based upon the slope of the tailing portion of the breakthrough curves in the two replicate tests, suggested that substantive breakthrough could have continued for several thousand pore volumes had the test not been terminated.

A subsequent column study employing Lalamilo soil where much of the SOM had been removed by chemical oxidation exhibited a peak in fractional breakthrough of 1.8-μm microspheres (1.2×10^{-2}) that was unexpectedly more than 300 times higher than that observed for the unaltered soil. Also, the onset of detectable microsphere breakthrough (at 0.84 PV) occurred significantly earlier (at 0.54 PV) in the absence of the SOM. Although most (95%) of the 1.8-μm microspheres were also immobilized in the latter column study, attachment was

considerably less reversible. The retardation factors for 1.8-μm microspheres also differed substantively between unaltered and chemically oxidized Lalamilo soil. For the unaltered soil, the retardation factor based upon transport of peak breakthrough (0.84) differed from that based upon the center of mass (1800—2100) by more than 2000 fold. On the other hand, retardation factors for the chemically oxidized soil were more similar (0.54 based upon peak breakthrough versus 0.91 based upon the center of mass). Moreover, the attachment rate coefficients (k_a) for the unaltered Lalamilo soil was faster (94.3 d^{-1}) than that observed for the chemically oxidized soil (k_a = 8.6 d^{-1}), whereas the detachment rates were almost equal (0.36 d^{-1}). However, it is anticipated that any range of parameter values in the model (Table 3) would do a similarly good job.

3.4.3. Drummer soil

Transport of oocysts and microspheres through the quartz-rich Drummer soils is shown in Fig. 5. Peak abundance of oocysts and microspheres broke through much earlier (at 0.67 PV) relative to that of nitrate (at 1.0 PV). For the 1.8-μm microspheres, retardation based upon peak breakthrough and upon centers of mass were similar (0.67 and 0.64, respectively). As observed for the Poamoho soil, the vast majority of oocysts and 4.9-μm microspheres were immobilized within the column. Only 0.4% of oocysts and 0.7% of 4.9-μm microspheres were recovered in the eluent, although 25% of the smaller (1.8-μm) microspheres were transported through the entire length of the column. The calculated attachment rate coefficient for the latter microspheres (36 d^{-1}) was considerably faster than that for detachment (0.09 d^{-1}).

4. Discussion

4.1. pH dependency of colloidal attachment

Although the zeta potentials for oocysts and oocyst-sized microspheres at 1 mM I were clearly pH-dependent (Fig. 1), pH had little effect on the propensity of either colloid for attachment within the Lalamilo soil. The high-organic content (43%—46%) in this tropical volcanic ash soil may preclude the microspheres from interacting directly with the otherwise prevalent maghemite surfaces (Table 2). The specific manner in which SOM affects the pH-dependency of microbial

Table 3 – Removal efficiencies of 1.8-μm microspheres, retardation coefficients and fitted parameters (k_a, k_r) obtained from the data of the breakthrough curve of different granular porous media.				
Granular media	% Removed[a]/decimeter travel distance	k_a (day)$^{-1}$	k_r (day)$^{-1}$	R^2
Poamoho soil (pH-7.2)	82.0	39.60	1.11	0.92[b]
Lalamilo soil (pH-8.5) unaltered soil	99.9	94.30	0.36	0.34
Lalamilo soil (pH-8.5) treated (SOM extracted)	99.6	8.60	0.35	0.89
Drummer soil (pH-7.2)	98.6	36.00	0.09	0.94[c]

a Oocysts: 97.8% (Poamoho soil), 98.6% (Drummer soil).
b The corresponding values for oocysts and 4.9-μm colloids were 0.70 and 0.89 respectively.
c The corresponding values for oocysts and 4.9-μm colloids were 0.99.

Table 4 – Summary of the retardation coefficients, and cumulative recovery of all colloids for replicate experiments.

Soil	Colloid	Run	Retardation[a] factor for peak breakthrough (RF_p)	Retardation factor for center of mass (RF_{cm})	$\Sigma(C/Co)$	p value
Poamoho	1.8-μm	1	0.72	0.79	0.180	0.101
		2	0.85	0.78	0.012	0.144
	4.9-μm	1	0.62	0.65	0.010	0.177
		2	0.77	0.74	0.001	0.183
	Oocysts	1	0.46	0.54	0.018	0.172
		2	0.54	0.51	0.004	0.181
Drummer	1.8-μm	1	0.67	0.64	0.250	0.062
		2	0.62	0.70	0.088	0.134
	4.9-μm	1	0.55	0.54	0.007	0.178
		2	0.55	0.64	0.009	0.180
	Oocysts	1	0.67	0.59	0.004	0.183
		2	0.27	0.24	0.005	0.177
Lalamilo soil (pH-8.5) (with SOM)	1.8-μm	1	0.67	1800–2100[b]	0.003	NA
		2	0.69	1200–1300[b]	0.001	NA
Lalamilo soil (pH-8.5) treated (SOM extracted)	1.8-μm	1	0.54	0.91	0.004	NA

a Calculated as ratios of time required to reach peak abundance for microspheres to time to peak concentration for the conservative tracer.
b Simulated data, extrapolated for 99% recovery of mass.

attachment in soils is difficult to predict, in part, because of its structural complexity. However, findings from other studies suggest that even modest quantities of SOM can greatly diminish the pH-dependency of microbial attachment onto iron-rich soils and sediments (Harvey et al., 2010). Consequently, perturbations in pore water pH following precipitation events would be expected to have a lesser effect upon re-entrainment of oocysts in the organic-rich Lalamilo soils than in the other two soils.

In contrast, attachment of oocysts and microspheres in the low-organic, weathered volcanic (Poamoho) soil from Hawaii was strongly pH-dependent (Fig. 2). The pH-dependency of colloidal attachment, particularly for the well-defined, carboxylated microspheres, was surprisingly similar to that observed for our reference Midwestern agricultural (Drummer) soil, despite the disparity in mineral composition (Table 2) of the two soils. The steady decrease in attachment (increase in transport potential) with increasing pH was consistent with concomitant increases in the magnitude of negative charge of the colloid, judging from the zeta potential (Fig. 1). This would occur as the metal oxide-rich grain surfaces become less electropositive. Consequently, at higher pH, the microspheres and grain surfaces should become more electrostatically repulsive.

It was less clear why oocyst attachment within the Drummer soil was more sensitive to changes in pH than was attachment in the Poamoho soil. Our initial hypothesis was that attachment would be much more pH sensitive in the presence of Poamoho soil than in Drummer soil, because the net charges of the grain surfaces in the former are dominated by abundant iron and aluminum oxides. We suspected that under alkaline conditions (pH 9) less attachment (more transport) of oocysts occurred within the Drummer soil because of the presence of quartz, which constituted up to 40% of the bulk material and presumably promoted stronger negative charges on the grain surfaces relative to the iron/ aluminum oxide dominated Poamoho soil. Harvey et al. (2002) found a 70% decrease in fractional attachment of native

bacteria in the presence of iron-coated quartz sand collected from the Cape Cod aquifer when pH was increased from 5.8 to 7.9.

Although the larger (4.9-μm) carboxylated microspheres closely replicated the pH-dependency of oocyst attachment behavior within the two volcanic soils, the pH dependencies of microsphere- and oocyst attachments in the reference Drummer soil were clearly different (Fig. 1). In contrast to the carboxylated latex microspheres, oocyst surfaces are highly complex with multiple layers of glycoprotein and glycolipids that collectively exhibit a different pattern in which zeta potential varies with pH. Glycoproteins on the oocyst surface would be expected to exhibit a different pH dependent charge behavior due to the presence of functional groups associated with the cysteine, proline, and histidine amino acids (Tilley and Upton, 1997), which have different associated points of zero charge (PZC). Considine and Drummond (2001) have observed that glycoproteins on the surfaces of oocysts have a tendency to behave like a "polyelectrolytic brush" with charged surfaces extending from the oocysts surface to the solution. Consequently, the more complex surface chemistry of oocysts may make their attachment more pH sensitive in the presence of the quartz-rich Drummer soil than the compositionally simpler microspheres. As was observed in an earlier study involving colloid transport in karst limestone (Harvey et al., 2008), the suitability of microspheres as surrogates for oocysts in soils may depend, at least in part, upon the chemical conditions under which the tests are run.

4.2. Relative transport velocities

For the flow-through column studies, faster transport of the peak concentrations of microspheres and oocysts relative to those for nitrate for the Poamoho, Lalamilo, and Drummer soils (Figs. 3 and 4, and 5, respectively) suggest that transport of the colloids that have not come into contact with grain surfaces may be more rapid than would be predicted by a conservative tracer. The "velocity enhancement" effect for

1.8-μm colloids was particularly noticeable for the Poamoho and Drummer soils (RF_p = 0.72 and 0.67, respectively; Table 4) and is likely due to a combination of preferred flow-path structure and size-exclusion effects. There was statistically significant difference between the arrival times and center of mass between the two replicate experiments.

The Poamoho soil, which is characterized by a high (>50%) clay content and water-stable aggregates, holds substantial amounts of immobile water (Uehara and Gillman, 1981) and exhibits visible macropore structure when re-wetted. The occurrence of preferential flow paths in granular media has been related to clay content in at least one other study (Kjaergaard et al., 2004) and is commonly observed in aggregated packed soils (Biggar and Nielsen, 1962). Macropores important for colloid transport are generally characterized by higher velocities and less tortuosity, the average velocities of the colloids appearing in the eluent would be greater than that of the conservative tracer (Grolimund et al., 1998). Presumably because of the size exclusion effect, the peak concentrations of the larger (4.9-μm) microspheres and oocysts (3.6-μm) eluted from the Poamoho soil earlier than the smaller (1.8-μm) microspheres. In a natural-gradient test conducted in a sandy aquifer in Cape Cod, MA, it was found that 1.7-μm sized microspheres broke through earlier than 0.7-μm microspheres (Harvey et al., 1989). Similarly, it has been reported that the 3-μm colloids arrived earlier than 50 nm microspheres and 25 nm MS2 virus (Keller et al., 2004). Consequently, for Poamoho and Drummer soils, the time required for pathogen breakthrough down gradient would depend, in part, upon the size of the organism. The "velocity enhancement" for the 1.8-μm microspheres relative to that of the conservative tracer in the eluent of the Lalamilo soil column was clearly related to presence or absence of the SOM. Removal of the organic matter by chemical oxidation had the effect of reducing the (RF_p) from 0.85 to 0.54. Although the specific reason(s) for this are not clear, we hypothesize that the SOM, which constitutes up to 46% of the solid phase, occupies much of what would otherwise be preferred flow-path structure. Consequently, its removal by chemical oxidation re-creates more macroporosity capable of transporting colloids.

4.3.   Reversibility of colloidal attachment

The model could capture the tail of the breakthrough curve for the Poamoho and Drummer soils but not for the Lalamilo soil without organic matter. Colloidal attachment appeared to be much weaker in the organic-rich Lalamilo soil than in the other two soils. For the Poamoho soil (Fig. 3), chemically oxidized Lalamilo soil (Fig. 4b), and Drummer soil (Fig. 5), only a modest degree of tailing was evident from the breakthrough curves of the 1.8-μm microspheres relative to those of the conservative tracer. Tailing is generally caused by the release of microspheres previously attached to grain surfaces. A subsequent experiment designed to assess reversibility of colloid detachability in Poamoho and Drummer soils indicated that few attached microspheres detached during the passage of 10 pore volumes of deionized water at pH 7, suggesting that deposition in these soils is essentially irreversible. In contrast, for the 1.8-μm microspheres advecting through Lalamilo soil, the model could only capture a few of the data points because deposition within this soil was found to be highly reversible. This was evidenced by the substantive and protracted tail that was about one half the height of the peak. We hypothesize that the reversibility of colloidal attachment in this soil type is due to the abundance of SOM. This is because attachment was less reversible after the extraction of SOM and the breakthrough curve changed to a steadily decreasing tail (Fig. 5). This suggests that the organic-rich Lalamilo soil may act as a reservoir for the accumulation of pathogens and detachment of colloids into pore water would be likely, following precipitation events. In spite of the high reversibility of microspheres attachment, it is not clear that this soil would be less than effective in permanently removing oocysts. This is largely because of the 3.6 μm (average diameter) oocysts have difficulty passing through these very fine-grained sediments. However, there is a good possibility that smaller pathogens, particularly those <2-microns in size that would be subject to a lesser degree of straining, would slowly advect through this soil largely because of highly reversible attachment. These highly reversible interactions coupled with the fact that viable oocysts can persist in some environments for up to a year (Current, 1988) and ingesting even a few oocysts can cause cryptosporidiosis (Rose, 1997; Casemore et al., 1997), raises the possibility that considerably more caution may need to be exercised when grazing cattle on Lalamilo soil. Although the Poamoho soil in Oahu appears to be an efficient filter in spite of the macropore structure that develops upon rewetting, the efficacy of the organic-rich Lalamilo from the island of Hawaii to remove pathogens is a more complex issue because short-term removal may not guarantee long-term immobilization.

4.4.   Comparison of transport behavior of oocysts and microspheres

All our experiments were conducted to check the efficacy of microspheres in simulating the transport of oocysts. A polydispersed mixture of 1.8-μm, 2.9-μm, and 4.9-μm were used to bracket the size range of oocysts used in our study. It was found that although the 4.9-μm microspheres are slightly larger than the oocysts (3.6-μm), the 4.9-μm microspheres seem to closely match the oocysts behavior in terms of "velocity enhancement" and filtration efficiency. On average, peak arrival times for the oocysts and 4.9-μm microspheres varied by only ± 0.1 PV in both Poamoho and Drummer soils. Moreover, 4.9-μm colloid and oocysts exhibited similar attenuation within the soil. Although microsphere applications have been shown to be safe, the structures of oocysts are much more complex. Also, it has also been reported that microspheres transport is quite different from oocysts and over-predict or under-predict oocysts transport in laboratory and field scale studies (Harvey et al., 2008b). Consequently, findings resulting from the use of microspheres as transport surrogates should be treated with caution.

5.   Conclusions

All three soils examined in our study appear to be good filters for removing *C. parvum* oocysts over the short-term (first few

pore volumes). In particular, the fast attachment rates combined with very slow detachment and high filtration capacities suggest that the red iron-, aluminum-, and clay-rich (Poamoho) soil from Oahu and the reference agricultural (Drummer) soil from Illinois should be highly effective at filtering out *C. parvum* oocysts. However, we hypothesize that the highly reversible attachment behavior caused by the abundant SOM (43%–46% w/w) in the volcanic ash-derived (Lalamilo) soil from the island of Hawaii could serve as a reservoir of oocysts for their reintroduction into surface water over a protracted period. This was in spite of the high iron oxide content of these soils, which should provide favorable sites for the irreversible attachment of microspheres. Clearly, the relative abundance of SOM substantively affects the efficacy of tropical volcanic soils for removing pathogens and needs to be taken into account when optimizing agricultural practices to lessen the potential for pathogen contamination of underlying groundwater resources. Future studies will include the roles of cow manure, surfactants, and clays on oocysts transport behavior in tropical volcanic soils.

Major findings from this study include:

■ Attachment was pH dependent in the Poamoho and Drummer soil but pH independent in the organic-rich Lalamilo soil.

■ "Velocity enhancement" of oocysts relative to a conservative tracer was observed for Poamoho and Drummer soils, indicating that oocysts and oocyst-sized microspheres are transported primarily through preferential flow paths in these complex soils. In spite of the presence of macropores, these soils were efficient filters for removing oocysts and microspheres.

■ 'Tailing' caused by highly reversible attachment dominated the colloidal transport behavior in the volcanic ash (Lalamilo) soil indicating that the fate and transport of colloids within these soils is clearly different from those of Poamoho and Drummer soils. Although oocysts would have difficulty in passing through these fine grained soils, water borne pathogens of smaller diameter (<2-μm) may be subject to steady, but retarded transport because of the highly reversible attachments.

■ Finally, it was found that 4.9-μm carboxylated microspheres appeared to be better surrogates for assessing the abiotic aspects of oocyst transport behavior than the 1.8-μm microspheres, although the suitability of microspheres as surrogates was clearly pH-dependent.

Acknowledgements

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