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Use of Carboxylated Microspheres to Assess Transport Potential of *Cryptosporidium parvum* Oocysts at the Russian River Water Supply Facility, Sonoma County, California

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Carboxylated microspheres were employed as surrogates to assess the transport potential of *Cryptosporidium parvum* oocysts during forced- and natural-gradient tests conducted in July and October 2004. The tests involved poorly-sorted, near-surface sediments where groundwater is pumped from an alluvial aquifer underlying the Russian River, Sonoma County, CA. In an off channel infiltration basin and within the river, a mixture (2-, 3-, and 5-μm diameters) of fluorescently-labeled carboxylated microspheres and bromide tracers were used in two injection and recovery tests to assess sediment removal efficiency for the microspheres. Bottom sediments varied considerably in their filtration efficiency for *Cryptosporidium*.

Keywords bank filtration, microbiology, *Cryptosporidium parvum*, water quality

INTRODUCTION

Riverbank filtration involves drawing surface water through alluvial and aquifer sediments to nearby wells. It is used worldwide as a cost-effective pre-treatment option for substantively reducing the quantity of many microbial and chemical contaminants (Tufenkji et al. 2002). The efficacy of bank or natural filtration systems to remove oocysts of the protistan pathogen, *Cryptosporidium parvum,* is particularly important because of their ubiquity in surface waters (Rose 1997; LeChevallier et al. 1999) and resistance to chemical disinfection by chlorination (Okun 1993; Macler and Merkle 2000). However, the subsurface transport behavior of this pathogen during bank filtration operations is not well understood. The Enhanced Surface Water Treatment Rule (LT2ESWTR) of the USEPA specifies criteria for pathogen removal and grants treatment credits to utilities employing bank filtration as part of their overall drinking water treatment process (EPA 2006). Models have been applied to *C. parvum* oocyst transport (Harter et al. 2000; Darnault et al. 2004), but there is a lack of supporting information from the field, including bank filtration sites. Because of site heterogeneities, accurate predictions of oocyst removal in alluvial sediments during bank filtration are difficult to make. The presence and persistence of pathogens and the degree of their subsurface transport are dependent on a variety of physical, biological and geochemical factors (Harvey and Harms 2001; Schijven et al. 2002). For bank filtration, these factors can include, but are not limited to flow rate, temperature, pH, dissolved organic carbon (DOC), sediment grain size distribution, metal oxide content, microbe size, and the surface characteristics of both microbes and grains (Tufenkji et al. 2002).

Because the occurrence of *C. parvum* oocysts in surface waters are typically episodic and otherwise quite low and because of the physical and geochemical heterogeneities that characterize bank filtration sites, model predictions derived from monitoring

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data or from small-scale laboratory columns may not be adequate to provide much of the more-critical information about oocyst fate and transport in the subsurface. Therefore, carefully controlled field-scale injection and recovery tests at bank filtration or natural filtration sites are needed to help understand the efficacy of these systems in removing oocysts. The first few meters of travel occurs through biologically active, often reducing, bed sediments (Hiscock and Grischek 2002; Tufenkji et al. 2002).

It is important to understand the role of these sediments in controlling oocyst removal during induced infiltration and whether deeper sediments are equally important in the removal processes. Designing and implementing quantitative, field-scale injection and recovery tests involving bottom sediment in rivers or infiltration basins is challenging because the introduction of tracers and subsequent recovery from the bottom sediments need to be carefully controlled. In addition, public health considerations necessitate the employment of surrogates that reasonably approximate the size and surface characteristics of *C. parvum* oocysts. Carboxylated, fluorescent microspheres have been used as surrogates in a variety of field-scale transport studies involving bacteria (Becker et al. 1999; McKay et al. 2000), viruses (Bales et al. 1997), and protozoa (Harvey et al. 1995).

Most recently, microspheres were used as surrogates for *C. parvum* oocysts in a field-scale (97 m) transport test involving a drinking-water aquifer (Harvey et al. in review). Ideally, the sizes, buoyant densities, and surface charges of microsphere surrogates should be similar to those of *C. parvum* oocysts.

This article details results of in situ transport experiments performed using two different methods of introducing polydispersed suspensions of oocyst-sized microspheres into Russian River and infiltration basin bottom sediments at a water supply facility operated by the Sonoma County Water Agency (SCWA). To examine the efficiency of shallow infiltration basin and river sediments for removing microspheres, both a Lee-type half barrel seepage meter (Rosenberry 2005) and an in situ column fitted with interior sampling ports along its length were used to introduce a suspension of microspheres through the sediment-water interface into the near-surface bank-filtration sediments. The 2 devices were employed in riverbed sediments of the Russian River above an operating lateral of a radial collector well and within engineered infiltration basins that were near 3 large-capacity radial collector wells. In all experiments, carboxylated microspheres and potassium bromide (KBr) were added to the bottom sediments as pulses and tracked as they advected downward through the sediments.

The goals of the study described herein included evaluations of: (1) the utility of carboxylated microspheres for assessing oocyst-removal potential in river and infiltration basin bottom sediments, (2) the utilities of the in situ core barrel and the modified seepage meters in colloidal-injection and recovery studies at the bottoms of shallow rivers and infiltration basins at an operating bank-filtration site, (3) the influence of seepage rates upon oocyst-sized colloidal transport in near-surface sediments, and (4) the role of grain-size distributions upon transport of oocyst-sized particles.

Site Characterization. The Russian River, which has a modest (3850 km^2) watershed, flows approximately 160 km from its headwaters in the Coast Range in Mendocino County, California, to the Pacific Ocean near Jenner, California (Rantz and Thompson 1967) (Figure 1). River flow is highly variable seasonally, responding to a winter rainy season and a summer dry season. Two upstream reservoirs provide storage and flood control that buffers the seasonal variability in flow at the downstream study site east of Guerneville, California. Mean daily flows at the USGS gauging station near Guerneville range from 4.5 to 265 $m^3\ s^{-1}$ ⟨http://nwis.waterdata.usgs.gov/ca/nwis/ site no. 11467000⟩. Previous research at the site includes water quality parameters (Anders et al. 2006) and use of heat as a groundwater tracing method (Constantz et al. 2006).

Seepage flux and microsphere surrogate transport were measured near an inflatable dam on the Russian River east of Forestville, California. The Sonoma County Water Agency (SCWA) extracts water from the alluvial aquifer adjacent to and beneath the Russian River via large-volume radial collector wells. The enhanced river stage caused by inflation of a temporary dam, increases the hydraulic gradient between the river and the surficial aquifer while increasing the available surface area for intensive groundwater recharge. This, in turn, allows the collector wells to extract more ground water than would otherwise be possible. Ponded water extends to ∼1 km upstream of the dam and averages ∼85 m in width with a maximum depth of ∼3 m. When the dam is inflated, SCWA also diverts river water to 4 off-channel infiltration basins where diverted surface water infiltrates through the basin sediments and enhances recharge to the alluvial aquifer. Seepage rate and microsphere transport assessments were also conducted in Recharge Basins 2 and 3 (40,500 m^2), which are downstream of the inflatable dam (Figure 1).

Slug tests were conducted in shallow wells adjacent to seepage meters in order to evaluate the hydraulic conductivity of the shallow sediments. Hydraulic conductivity of the shallow sediments beneath the recharge basin was 2×10^{-3} cm s^{-1}, indicative of fine sand or silty medium sand, whereas hydraulic conductivity near the east bank of the riverbed site was 8×10^{-3} cm s^{-1}, indicative of coarser sand. Within this region of the riverbed site, we found different infiltration velocities, which we designate in this paper as "fast" and "slow" zones.

Seepage fluxes were determined with a standard Lee-type half-barrel seepage meter (Lee 1977). The device was modified with large-diameter bag-connection hardware to minimize head loss resulting from high-velocity seepage fluxes (Rosenberry and Morin 2004). This design of seepage meter combined with the use of a thin-wall plastic bag is very efficient, requiring a multiplier of only 1.05 to correct for head loss associated with meter inefficiencies (Rosenberry 2005). Bag shelters were employed at seepage-measurement locations in the Russian River

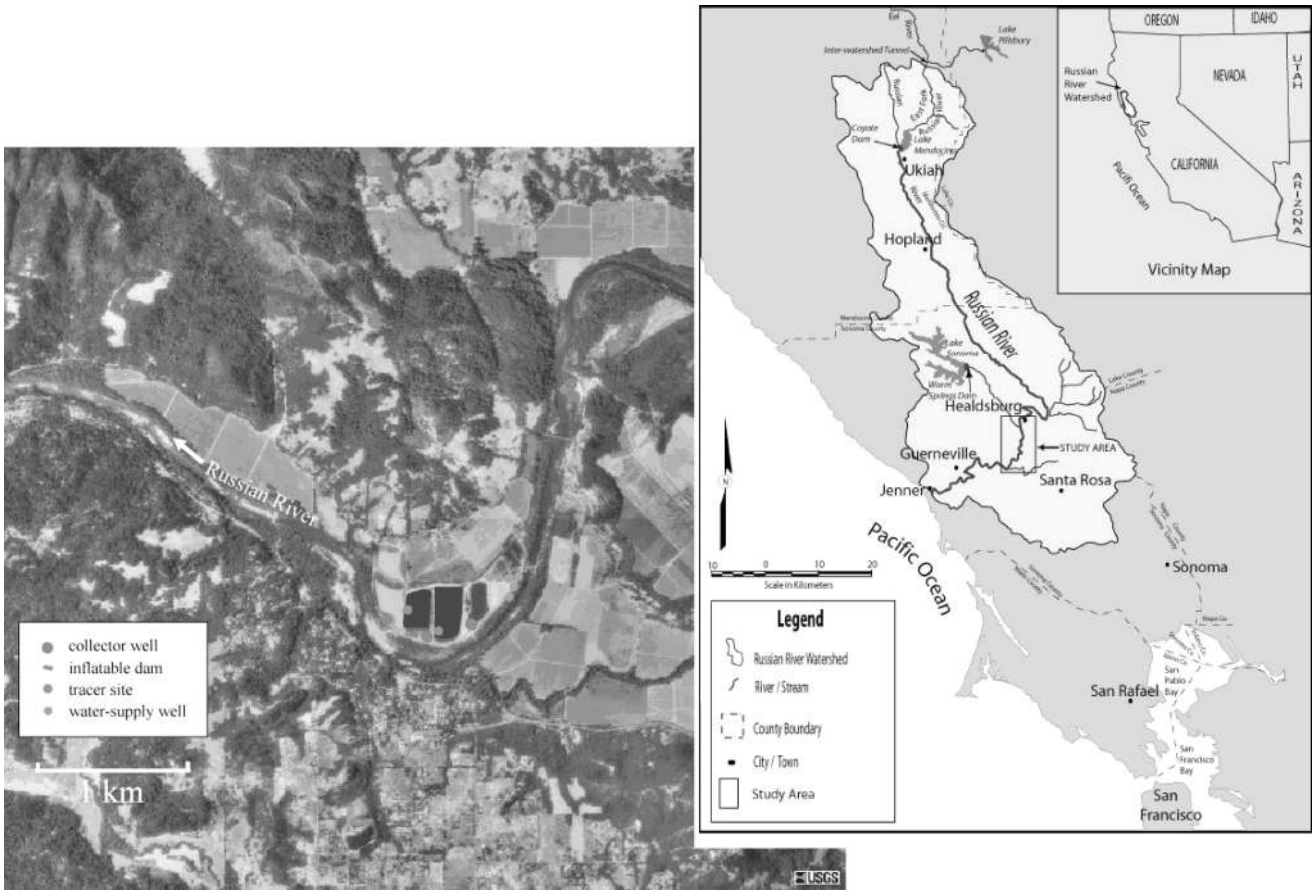


FIG. 1. Location of Russian River in Sonoma County, CA and location of infiltration basin and riverbank injection and recovery tests. Map modified from SCWA, 2004.

to minimize any velocity-head effects from moving water in the river (Sebestyen and Schneider 2001; Shinn et al. 2002). Bag shelters were not necessary in the infiltration basin where the water was exceptionally calm, even during afternoon windy periods.

Background Chemistry, Physical and Hydrological Parameters

A summary of the different water chemistries within the study area is presented in Table 1. Waters within the system are slightly alkaline and have electrical conductivities ranging from ~200 to 250 μS cm^{-1}. A detailed description of the water-quality in the Lower Russian River Basin is presented by Anders et al. (2006). Porosity ranged from 23% (Russian River sediments) to 30% (infiltration basins). Average grain sizes (longest axis) were 2.7 mm in river bottom sediments and 1.6 mm in infiltration basin sediments. Downward seepage rates ranged from 32–68 cm day^{-1} (basins) to 3–150 cm day^{-1} (river sediments). Measurements were made in the late summer to early fall and did not reflect yearly variations in water chemistry, seepage rates or sediment porosity.

MATERIALS AND METHODS

Oocysts and Microspheres

Carboxylated, polystyrene microspheres that are inert, nontoxic (Jung et al. 2000), and were found to transport similarly to groundwater protozoa at another granular field site (Harvey et al. 1995), were employed as surrogates for our transport tests in the vicinity of municipal supply wells. We employed a polydispersed mixture consisting of 3 size classes of fluorescent, carboxylated, polystyrene-type microspheres (Polysciences, Warrington, PA and Invitrogen—Molecular Probes, Carlsbad, CA and Corvallis, OR), i.e., 2-, (actually 1.6 $\pm$ 0.1), 3- (actually 2.9 $\pm$ 0.1), and 5- (actually 4.9 $\pm$ 0.2) μm in diameter. The three size classes were used to create the polydispersed microsphere suspension that collectively bracketed the size range (2.9–4.1 μm) of oocysts that we routinely use in our laboratory.

Use of a different color, i.e., brilliant blue fluorescence (Type BB) for the intermediate-size (3-μm) microspheres facilitated differentiation from the 2- and 5-μm yellow-green fluorescing (type YG) microspheres using epifluorescence microscopy (NIKON Optiphot II). The excitation and emission wavelengths

TABLE 1
Background chemical and physical data from Russian River bank filtration study site

	Basin	River
pH	7.5–8.5	7.3–8.5
Temperature (C)	12.8–22.1	19.7–25.2
Specific Conductance (μS cm^{-1})	223–522	214–598
Dissolved Oxygen (mg L^{-1})	ND*	7.7–11.1
Dissolved organic carbon (mg L^{-1})	5.8	5.4
Ionic Strength (mM) +	ND	5.14
Ionic Strength (mM) ++	3.25	3.23
Porosity	$\sim$ 30%	23%
	Basin Sediments	River Bottom Sediments
Grain size (mm)		
D_{50}	1.56	2.74
D_{10}	0.11	0.50
Seepage rates (cm day^{-1})	32–68	$\sim$ 3−150
Iron (mg g^{-1})	ND	$\sim$ 3–5

*Not determined.
+Estimated from water chemistry data.
++Estimated from specific conductance equation: Ionic Strength = 0.000025*0.59*specific conductance (Hem 1992).

are 360 and 440 nm, respectively, for the Type BB microspheres and 441 nm and 486 nm, respectively, for the Type YG microspheres. The 3 different size classes were also differentiated based on fluorescence intensity and diameter by flow cytometry (see Figure 2). Although fluorescent algae did sometimes appear in our samples, we were able to distinguish autofluorescing algae from microspheres-based upon size and fluorescent signal differences.

We obtained formalin-inactivated *C. parvum* oocysts from Sterling Parasitology Laboratory (SPL), University of Arizona, Tucson. The oocysts were harvested from a calf infected with the "Iowa" isolate of *C. parvum* (Dr. Harvey Moon, National Animal Disease Center, Ames, Iowa) and purified at the SPL. Oocysts employed in the laboratory study, were pelleted and stained with the DNA-specific fluorescent dye 4,6-diamidino-2-phenylindole (DAPI, 0.1 mg L^{-1} final concentration, for 30 minutes) as described by Abudalo et al. (2005). The oocysts were then resuspended at final concentrations of 10^2 to 10^7 oocyst mL^{-1} in water collected from the Russian River or from wells located in the vicinity of the Russian River. The peak excitation

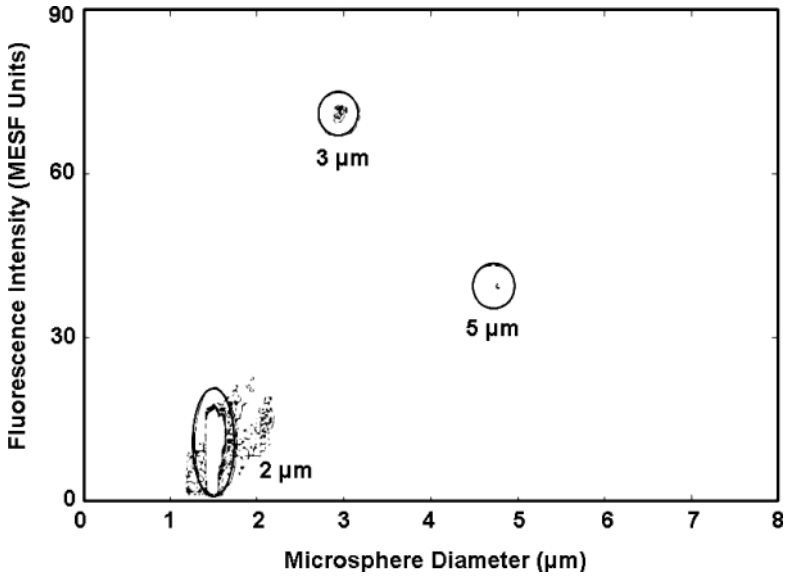


FIG. 2. Plot of fluorescence intensity and fluorescently labeled microspheres of varied size (2, 3 and 5 μm). This plot was obtained using a BIORAD HS-Bryte flow cytometer with a 5 channel (2 light and 3 fluorescent) photomultiplier system, an HBO 103 watt Hg lamp, and 5 μl min^{-1} flow rate.

and emission wavelengths for DAPI-stained oocysts are 359 and 461 nm, respectively.

Biocolloid Buoyant Density

Buoyant-densities of oocysts and fluorescent microspheres were determined by density gradient centrifugation using a method reported by Wolff (1975) and subsequently modified for protozoa (Harvey et al. 1997). This method involves layering of 10^8 microspheres mL^{-1} and 10^7 oocysts mL^{-1} on the pre-formed gradients of colloidal silica solutions and subsequent centrifugation. As centrifugation progresses, the microspheres and oocysts sink to their respective buoyant densities. The buoyant densities of the oocysts or microspheres were then determined from their migration distance relative to brightly colored density-marker beads (Sigma Chemical Company, catalog number DMB10-1KT).

Zeta Potential Measurements

Laser Doppler microelectrophoresis was performed on microspheres and oocysts (Nicomp Particle Sizing Systems, 380 ZLS with Nicomp 388 software, Nicomp Corporation, Santa Barbara, CA) at the University of Colorado, Boulder in order to determine their zeta potentials. All colloidal material suspended in liquid acquires an electrical surface charge. Zeta potential, an important indicator of this charge, can be used to predict and control the stability of colloidal suspensions, and to help predict the degree of interactions with sediment surfaces. A pair of pH-adjusted (7.6–7.8 final pH) solutions was used in determining electrophoretic mobilities.

One solution group was filtered (0.1 μm pore size) Russian River water that had an ionic strength of 5.1×10^{-3} M. The second solution was a filtered NaCl (10^{-3} M) solution. For zeta potential determinations over a range of pH (~3–8), pH adjustments were made with 0.1M HCl or 0.1 M NaOH. Final solution pH levels were measured with a calibrated Beckman model phi-12 pH meter and Beckman pH probe (Beckman Corporation, catalog number 511053, Fullerton, CA) immediately before the electrophoretic mobility measurements. Next, electrophoretic mobility measurements of oocysts and microspheres were made in triplicate using filtered Russian River water as the suspending medium. The electrokinetic data were converted to zeta (ζ) potentials using the Smoluchowski approximation (Abudalo et al. 2005). Isoelectric points (pH at which corresponding zeta potentials are zero) for the microsphere and oocyst suspensions were estimated by trend line extrapolations from plots containing ζ potentials vs. pH.

Smoluchowski approximation:

$$\upsilon_{\mathrm{E}} = 4\pi\varepsilon_0\varepsilon_{\mathrm{r}}(1+\kappa r)\left(\frac{\zeta}{6\pi\mu}\right)$$

In this equation, zeta potential is represented by υ_{E}, ε_0 and ε_{r} are the relative dielectric constant and electrical permittivity of a vacuum, respectively, r is the particle radius, μ is the solution viscosity and $\kappa = 2n_0z^2e^2/\varepsilon_{\mathrm{r}}\varepsilon_0k_BT)^{1/2}$ is the Debye–Hückel parameter. For the latter parameter, n_0 is the bulk ionic concentration, z is the valence of the ion, e is the charge of an electron, k_B is the Boltzmann constant, and T is the absolute temperature.

Sediment Size Fraction Analyses

Sediments were collected from infiltration basin and riverbed areas near the injection and recovery tests. Prior to sieving, the sediments were washed and rinsed three times with Milli-Q water (18 M Ohm) and dried in an oven at 70°C. Approximately 1–2 kg of riverbed and infiltration basin sediments were size-fractionated using mechanical sieving techniques. Grain-size frequency distributions were calculated for the infiltration basin and river sediments (Figures 5a–5b). The respective D_{10} (fraction of sample where 10% of the grains are smaller, by weight, and 90% are larger) and D_{50} (fraction of sample where 50% of the grains are smaller, by weight, and 50% are larger) sizes were also calculated (Table 1).

Retention Under Static Conditions

The effectiveness of riverbed and infiltration basin sediments for retaining oocysts and microspheres was tested by loading different concentrations of each biocolloid onto sediments contained within static minicolumns (Scholl and Harvey 1992; Metge et al. 1995). This method uses 15 g of rinsed sediments added to acid-washed and baked 20 mL glass syringe barrels (20 mL, Perfektum Micromate interchangeable, Popper and Sons). The syringes were fitted with 2-way plastic valves. One to two pore volumes (4–8 mL) of filtered water collected from the Russian River was added to each syringe before sediment addition. Varying abundances of oocysts and microspheres were added to the minicolumn sediments. After a 3-hour contact time at 15°C, each column was rinsed with approximately 10 pore volumes of filtered river water. Rinse solutions were collected in acid-washed and baked glass vials and the solutions were immediately analyzed for microspheres and oocysts by flow cytometry. Two control sets were run with each experiment. One control had microspheres and oocysts in the absence of sediments; the other control had filtered river water alone.

FIELD EXPERIMENTS

Seepage Meter Injection and Recovery Experiments

Seepage meters (Lee 1977) were carefully placed in infiltration basin or riverbed sediments to prevent or minimize disturbance of sediments. Seepage flux measurements were made several times with river or basin water to obtain an average seepage rate. During the injection and recovery experiment, a 1-liter bag containing the polydispersed mixture of microspheres and KBr conservative tracer was attached to the seepage meter (Figure 3). The mixture was allowed, under natural seepage conditions, to enter the seepage meter. Samples were collected initially (T_0)

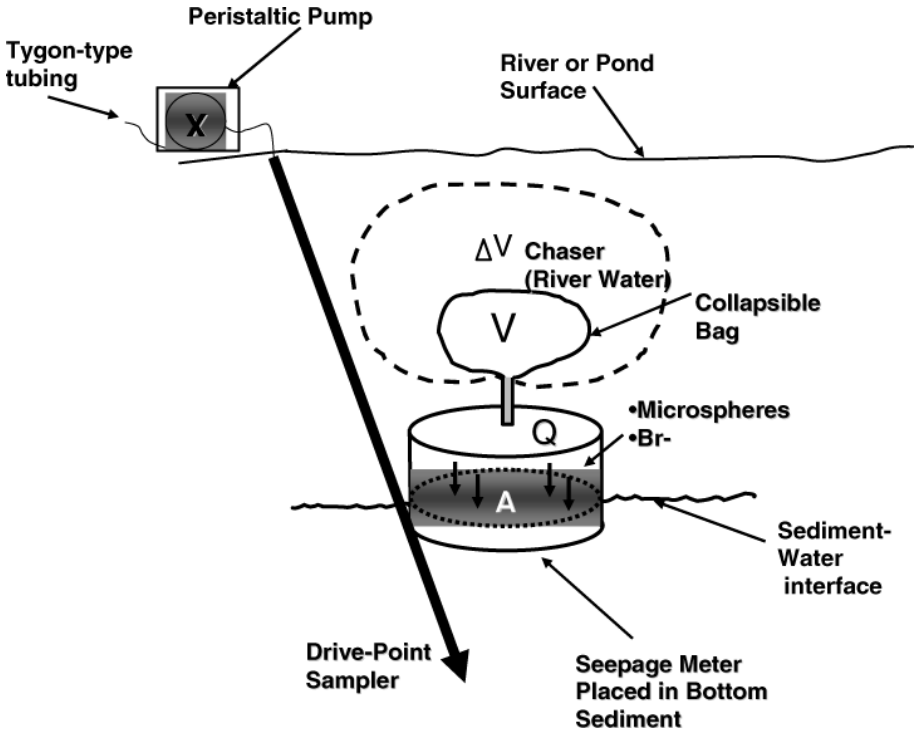


FIG. 3. Schematic of seepage meter illustrating components used in injection and recovery experiments with fluorescently labeled microspheres. Dimensions of seepage meter (~70 cm wide × 24 cm high), approximate volume of meter when placed (66 L), drive point sampling wells (15 cm long) were placed at 25 and 50 cm below river or basin sediment surface. Collapsible bag was filled with 1 L of injection mixture and attached to seepage meter; an instantaneous seepage rate could be obtained by noting changes in volume over time.

and at subsequent time intervals from the seepage meter using a peristaltic pump.

Changes in the concentration of conservative tracer within the seepage meter provided information about mixing and disappearance of the injectate solution within the seepage meter. Drive point samplers (3–4 cm long screened interval by 1 cm diameter, Geoprobes) were gently tapped at an angle into the sediments until the sampling ports were ~25 and ~50 cm below the sediment-water interface within the seepage meter. Water samples were pulled from the drive-point samplers with a peristaltic pump (Geotech model Geopump II Denver, CO) and collected in 60-mL acid-washed high-density polyethylene bottles. Sampling frequency was every 5 minutes in the infiltration basin test and 15–20 minutes for the riverbed test. In the riverbed sediment test, two different sites were chosen that represented a range of infiltration rates. Flow from the river to the sediment within the slower zone ranged from 3–30 cm day^{-1} with an average of about 15 cm day^{-1} whereas downward velocity rates at the faster zone ranged from 49 to over 100 cm day^{-1} with an average of ~68 cm day^{-1}.

Approximate initial concentrations of the 2-, 3- and 5-μm microspheres were 4.1×10^6 mL^{-1}, 4.1×10^4 mL^{-1}, and 5.0×10^3 mL^{-1}, respectively. The initial concentration of the bromide (Br^-) tracer was 90.9 mg L^{-1}. Bromide samples were analyzed by ion chromatography at the USGS laboratory in San Diego, CA. Samples containing microspheres were assayed at the USGS laboratory in Boulder, CO using flow cytometry. An HS-Bryte (Biorad Corp., Hercules, CA) 3-color flow cytometer with Apogee 1.3 software (Apogee, London, UK) were used to enumerate microspheres (Harvey and others in review). A mercury lamp (OSRAM, HBO 103 W/2) within the flow cytometer created excitation spectra of the fluorescent dyes within the microspheres. BG1 and GR1 (Biorad, Hercules, CA) filter cubes separate the specific emission spectra to respective photomultiplier tubes. Three separate measurements of microspheres concentration were made for each sample; the mean of each measurement were used in microsphere recovery calculations.

In Situ Column Injection and Recovery Experiments

A stainless steel column consisting of outer and inner sleeves and with dimensions shown in Figure 4 was designed and constructed specifically for injection and recovery tests at riverbank filtration sites. This test also employed microspheres as oocyst surrogates and bromide as a conservative tracer. The outer core barrel is 152 cm long and inner hollow barrel is 142 cm long. There are five ports within the inner sleeve that allow for discrete sampling from sediments within the inner sleeve. A port at the lower end of instrument allows introduction of compressed liquid CO_2 gas and freezing of the sediments at the distal end of the column.

Once the instrument was advanced into the infiltration basin (Pond 2) sediments using heavy equipment, compressed liquid

TABLE 2
Comparison of fluorescent microspheres, oocyst diameter, aspect ratio, zero point of charge (ZPC), zeta potential, buoyant density

Colloid	Diameter (μm)	Aspect Ratio*	pH_{zpc}	Zeta Potential at Russian River pH (mv)**	Buoyant Density (g cm^{-1})+
Fluorescent microspheres					
2 μm	1.62 ± 0.03	1.02	1.2	−14.4	1.055
3 μm	2.95 ± 0.06	1.16	2.1	−21.1	1.055
5 μm	5.06 ± 0.03	1.12	3.2	−19.2	1.056
Cryptosporidium parvum oocysts	3.61 ± 0.05	1.22	3.2–3.4	−10.9	1.064

*Determined by epifluorescent microscopy with computer coupled image analysis ratio of longest axis to shortest axis.
+Percoll gradient Method (Harvey et al. 1997).
**Significant Student's *t*-test differences between *Cryptosporidium parvum* and FMS measured zeta potentials ranged from p values of 0.002–0.02.

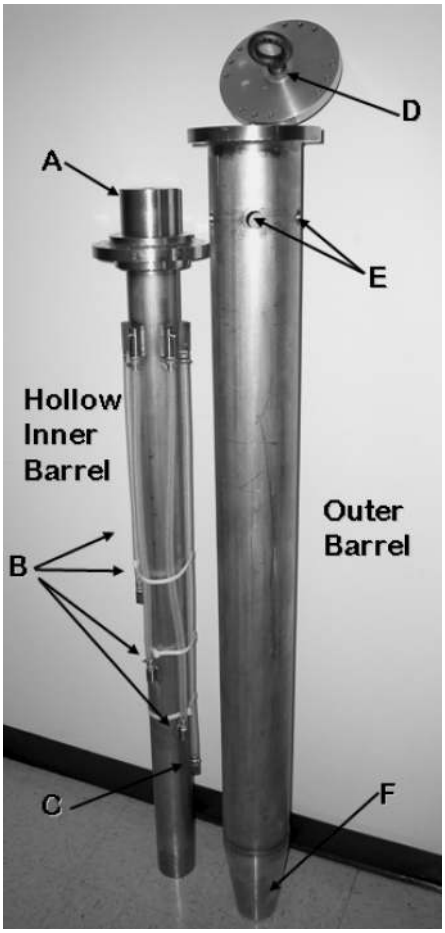


FIG. 4. USGS in situ column with dimensions and location of components: Fitting for hydraulic hammer (A), sampling ports (B-spaced 27 cm apart), port for injecting liquid CO_2 (C), retrieval plate (D-15.2 cm diameter), ports for sample tube adapters (E-3 cm diameter), and nose cone (F-25.2 cm length). Outer core barrel is 152 cm long and inner hollow barrel is 142 cm long.

CO_2 (General Air, Boulder, CO) was injected into the lowermost port of the column in order to freeze the bottom part of the core after insertion into basin sediments. This allowed a sediment plug at the base of the column to freeze and precluded water from the aquifer from entering the column when the ports were pumped. Also, this facilitated control on the apparent infiltration rate during the in situ column test. The approximate injectate concentrations of the 2-, 3- and 5- μm microspheres were 13.7×10^7, 7.8×10^4, and 2.3×10^4 mL^{-1}, respectively. The initial concentration of the bromide tracer was 250 mg L^{-1}.

The bromide and microsphere solutions were placed in the overlying fluid of the column and introduced, as a pulse, to the infiltration basin sediments within the in situ column. This pulse injection, at a constant flow velocity of about 2.5 m day^{-1}, was maintained within the column sediments by pumping water from a lower port at a constant rate. As the pulse injection was drawn into infiltration basin sediments, overlying fluids were replaced by infiltration basin water moving into the top of the column. Samples were taken at 20-minute intervals until appearance of elevated conductivity (background $\sim$ 220–230 μS cm^{-1}) as measured by a handheld specific conductance meter (Checkmate II, Catalog number EW-58912-00, Cole Parmer, Vernon Hills, IL). At that point, samples were collected every 2 to 3 minutes. Bromide was later analyzed by ion chromatography at the USGS laboratory at San Diego, CA and microspheres by flow cytometry at the USGS laboratory in Boulder, CO.

RESULTS

Comparison of *C. parvum* Oocysts and Microsphere Surrogates

The average diameters, aspect ratios, pH_{zpc}, zeta potentials at river pH, and buoyant densities for *C. parvum* oocysts and the 3 size classes of microspheres are summarized in Table 2. The oocysts, which had an average diameter of 3.6 ± 0.1 μm, were

closest in size to the 3-μm class of fluorescent microspheres, which had a measured diameter of 3.0 ± 0.1 and a manufacture's specified diameter of 2.9 μm. Relative to the microspheres, the oocysts were slightly less spherical (aspect ratio of 1.2), somewhat ($\sim$1%) denser, and substantially less charged at the slightly alkaline conditions of the Russian River. The zeta potential for the oocysts (-10.9 mV) at a measured pH of 7.6 and an estimated ionic strength of about 5 mM was only about half that exhibited by the 3-μm size class of microspheres (-21.1 mV), based upon electrokinetic data. Although the pH_{zpc} for the oocysts (3.2–3.4) was higher than those measured for the smaller size classes of microspheres by 1–2 pH units, it was reasonably similar to that estimated for the larger (5 μm) size class of microspheres.

Riverbed and Basin Sediment Retention of Fluorescent Microspheres (FMS) and Oocysts

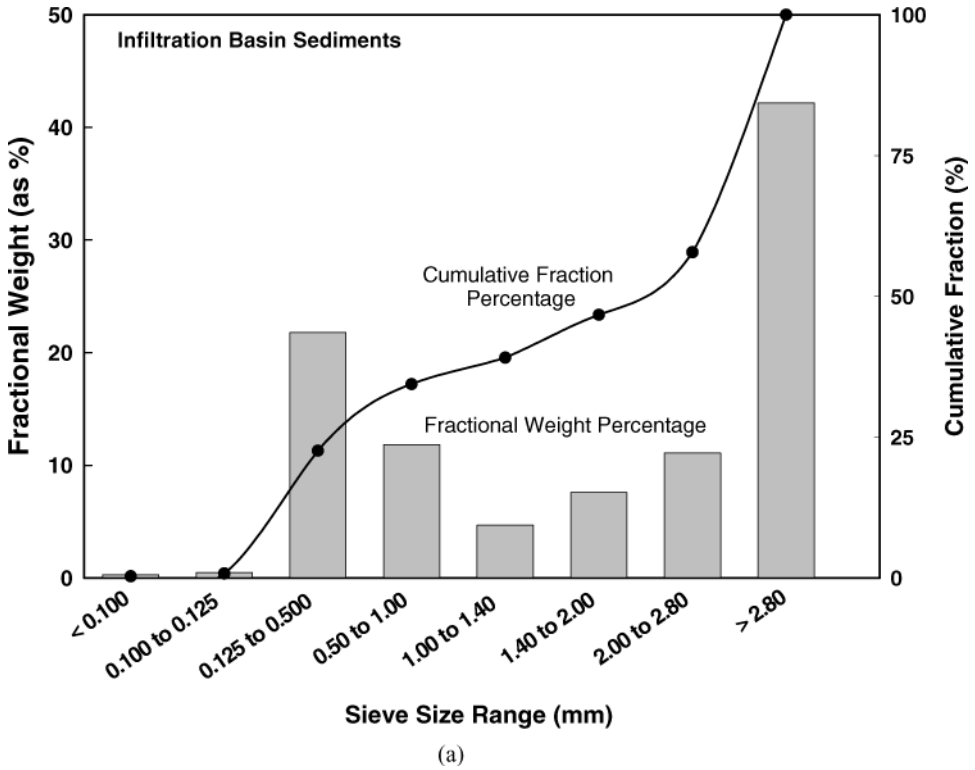
Grain-size frequency distributions for riverbed and infiltration basin sediments collected from the vicinity of the transport test locations are depicted, respectively, in Figures 5a and 5b. The size frequency distributions show bimodal distributions of grain sizes at both sites, with substantive fractions of both fine (<0.5 mm) and coarse (>2.8 mm) materials. However, the Russian River sediments were generally coarser than those sampled from the infiltration basin. The average grain sizes (D_{50}) for riverbed and infiltration basin sediments were 2.74 mm and 1.56 mm, respectively (Table 1).

Column Tests

The abilities of the aforementioned sediments to retain oocysts and 3-, and 5-μm microspheres within repacked columns are depicted in Figures 6a and 6b for the infiltration basin and riverbed sediments, respectively. In general, 99 to 99.9% of the oocysts and microspheres were retained within the sediments. Fractional recoveries for the microspheres were similar to those observed for *C. parvum* oocysts. For both microspheres and *C. parvum* oocysts, there was a significant linear relationship between colloid load and colloid recovery over a range of several log units.

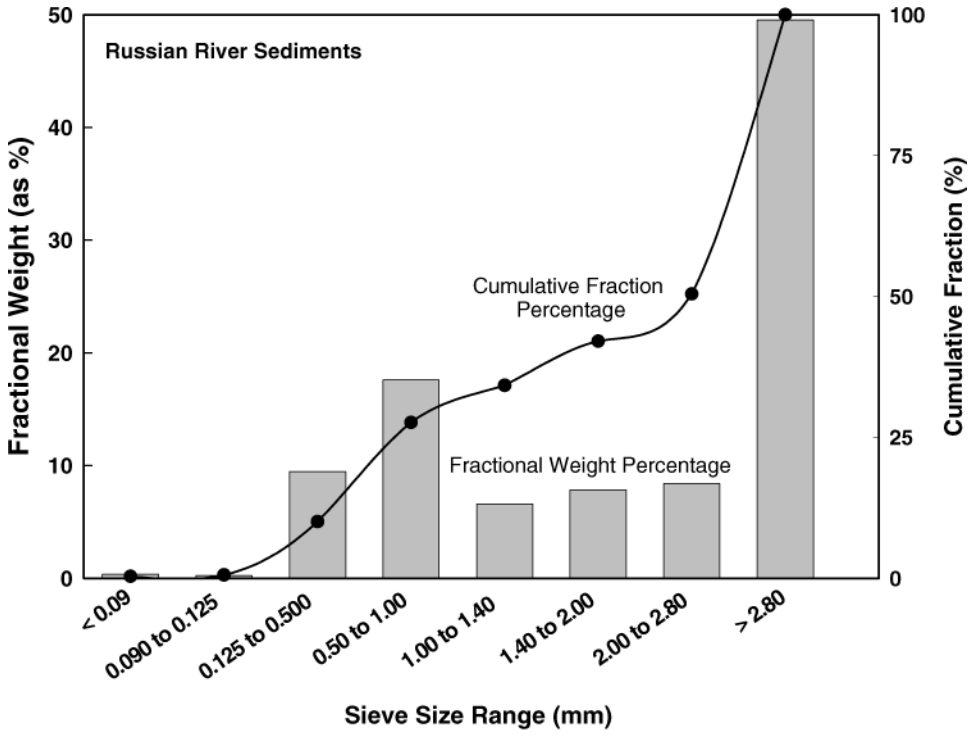
In Situ Tests

Breakthrough of the conservative solute (bromide) and the 2-, 3-, and 5-μm microspheres at 25 cm below the sediment-water interface in the infiltration basin transport study are depicted in Figure 7a and 7b, respectively, for the in situ column and seepage meter devices. For both test devices, there was an unexpected inverse relation between the size of the microspheres and the removal rate within the sediments. Transport potential for 5-μm sized particles for the 2 infiltration test types seemed reasonably similar, despite differences in devices and a 5-fold difference in pore velocity (Table 3). Relative recoveries of the 5-μm diameter microspheres after in situ transport through 25 cm of near-surface sediments was 33.5% using the seepage meter and



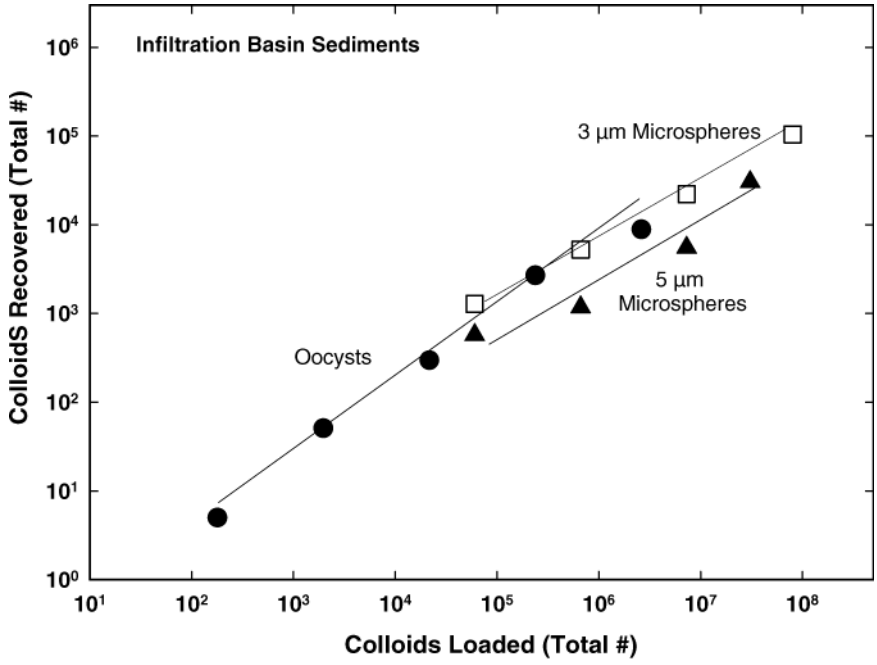
(a)

FIG. 5a. Sediment size fractions (as %) and cumulative fraction within Russian River basin sediments. Approximately 2 kg of sediment were fractionated using USA Standard Testing [ASTME] sieve plates (21.5 cm inner diameter) and sieving for approximately 3 hours.



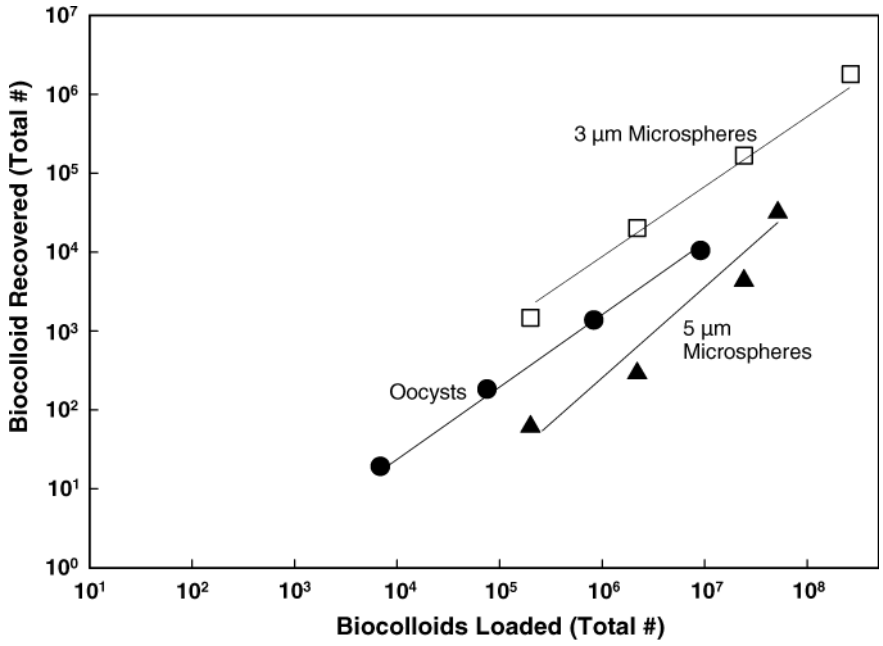
(b)

FIG. 5b. Sediment Size Fractions (as %) and cumulative fraction within Russian River Sediments. Approximately 2 kg of sediment were fractionated using USA standard testing [ASTME] sieve plates (21.5 cm inner diameter) and sieving for approximately 3 hours.



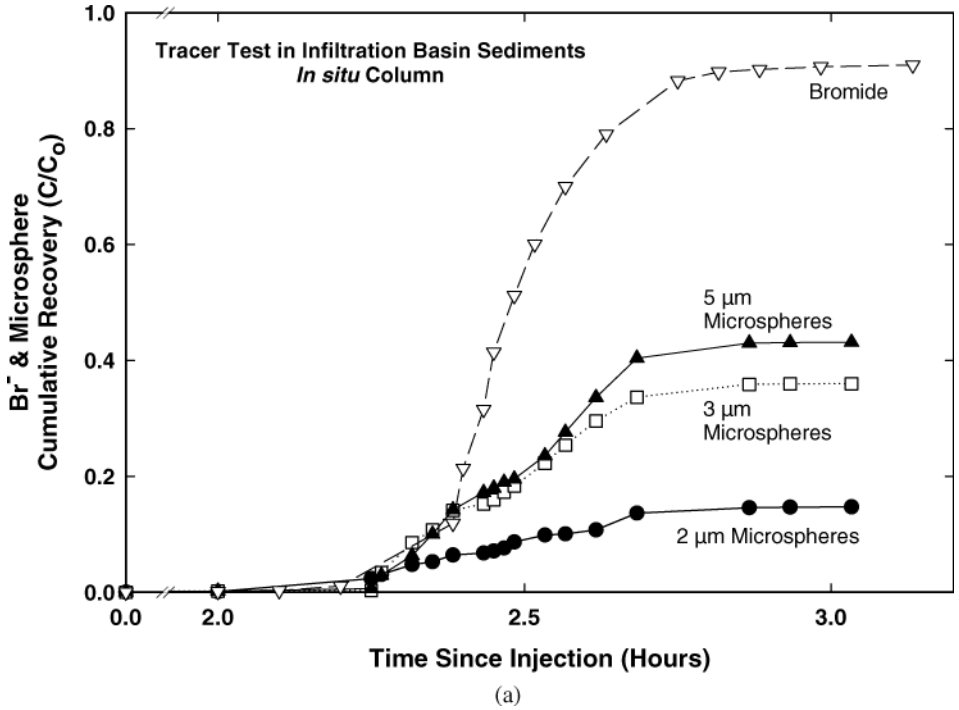
(a)

FIG. 6a. Comparison of *Cryptosporidium parvum* oocysts, 3-μm and 5-μm fluorescent microspheres loaded and desorbed from Russian River infiltration basin sediments. Experiment performed in static minicolumns with 15 grams of sediment at 15°C over a 3-hour incubation time. Concentrations determined using flow cytometry (Biorad HS-Bryte with 103W HBO Hg lamp, and UV FITC excitation filter cube with GR1 and OR1 emission wavelength discrimination cubes and standard forward and side scatter photomultipliers).



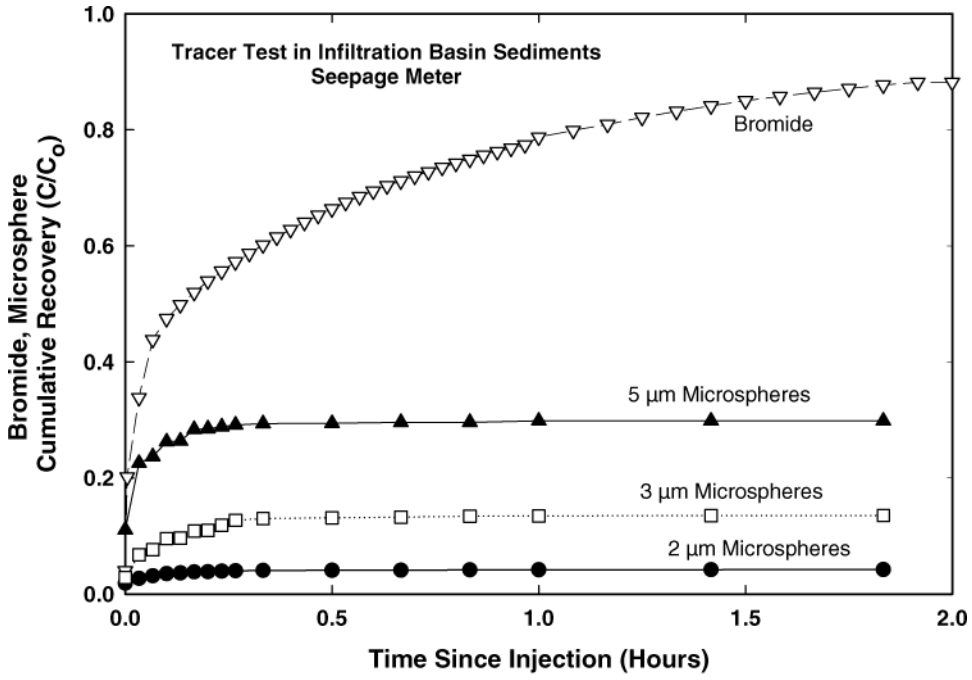
(b)

FIG. 6b. Comparison of *Cryptosporidium parvum* oocysts, 3-μm and 5-μm fluorescent microspheres loaded and desorbed from Russian River infiltration basin sediments. Experiment performed in static minicolumns with 15 grams of sediment at 15°C over a 3-hour incubation time. Concentrations determined using flow cytometry (Biorad HS-Bryte with 103W HBO Hg lamp, and UV FITC excitation filter cube with GR1 and OR1 emission wavelength discrimination cubes and standard forward and side-scatter photomultipliers).



(a)

FIG. 7a. Cumulative recoveries for fluorescent microspheres and bromide within infiltration basin sediments using in situ column test technique.



(b)

FIG. 7b. Cumulative recoveries for fluorescent microspheres and bromide within infiltration basin sediments using the seepage meter test technique.

47.4% using the in situ column. However, retardation factors for the 3 microsphere types relative to the conservative tracer were 0.9–1.0 using the in situ column, whereas retardation rates for the microspheres in tests performed using the seepage meter were considerably less than unity.

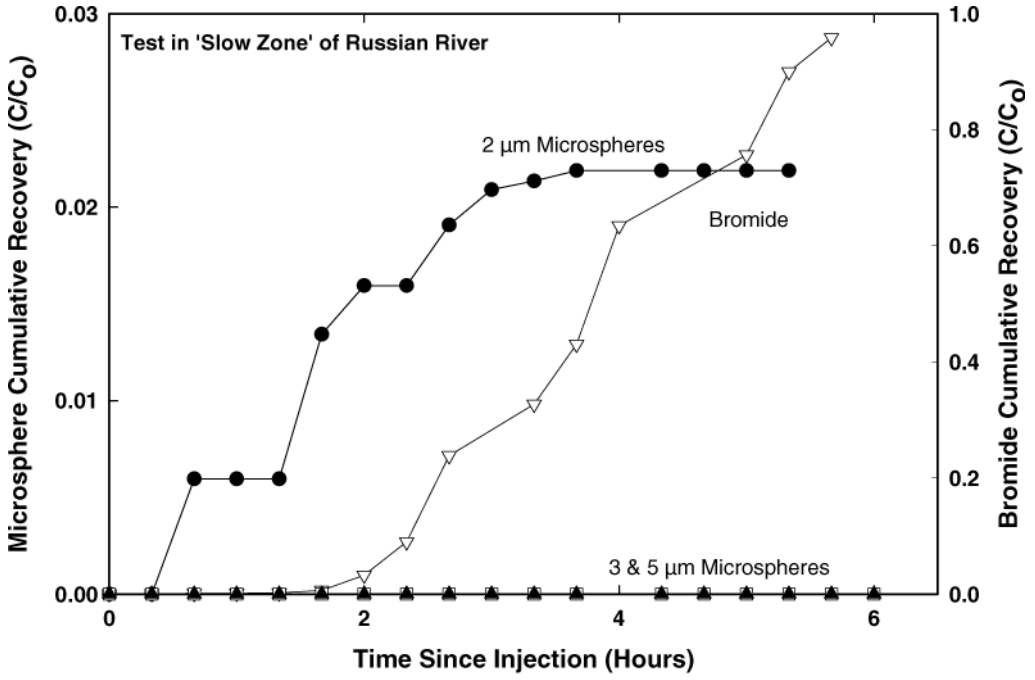
For the 2 riverbed sediment field experiments performed with the seepage meters, there was a 25-fold difference in cumulative microsphere recovery between the size classes of microspheres (Figure 8a and 8b). At the "slower" site characterized by lower seepage rates (0.15–0.38 m d^{-1}), only the smallest (2 μm) microspheres traveled to the first drive point well (25 cm below the surface). About 2% of the 2-μm microspheres added to the seepage meters were recovered at depth, whereas none of the larger microspheres were recovered. By comparison, at the "faster" site characterized by higher measured seepage velocities (0.38–0.60 m day^{-1}), all three microsphere types were detected at ~25 cm below the sediment-water interface.

The highest relative recovery (more than 55%) occurred for the 3-μm size class; the 5-μm diameter microspheres were found to be the least effectively transported. Centers of mass for the microspheres appeared well before that of the bromide tracer. In contrast to the infiltration basin tests, there was no clear relation between total recovery of microspheres and size. For example, the 3-μm diameter microspheres were transported more effectively in the faster zone, whereas the 2-μm microspheres were transported more effectively in the slower zone.

TABLE 3
Comparison of sites and test type—relative recovery, log removal rates for infiltration basin and river sediment injection and recovery tests

Location	Type of test	Downward flow rate (m day^{-1})	Diameter (μm)	Relative breakthrough (%)	Removal (log $meter^{-1}$)
Basin	In situ Column	2.5	4.9	47.4	1.3
	Seepage Meter	0.5	4.9	33.5	1.9
River	Seepage Meter	0.38–0.60	2.9	55.3	1.0
	Seepage Meter	0.15-0.38	1.6	2.2	6.6

Removal rates calculated using a first-order equation.



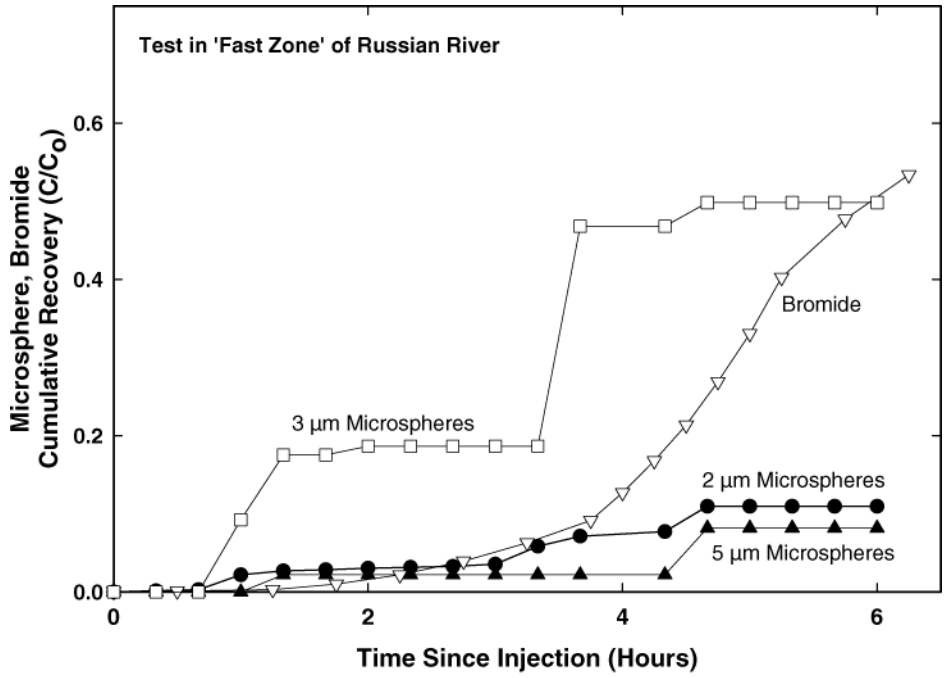
(a)

FIG. 8a. Cumulative recoveries of fluorescent microspheres and bromide in seepage meter test within Russian River sediments ("slow zone").

DISCUSSION

Microspheres as Oocyst Surrogates

The utility of carboxylated polystyrene microspheres, as abiotic surrogates for microorganisms in granular drinking-water aquifers, has been evaluated in earlier, in situ injection and recovery tests (Harvey et al. 1989, 1995). Although small microspheres (from 0.2–1.0 μm-diameter sizes) were found to be poor transport analogs of bacterial transport properties (Harvey et al. 1989); larger microspheres (2–3 μm-diameter size classes)



(b)

FIG. 8b. Cumulative recoveries of fluorescent microspheres and bromide in seepage meter test within Russian River sediments ("fast zone").

exhibited transport behavior reasonable similar to those exhibited by a groundwater nanoflagellate (protozoa) (Harvey et al. 1995). It was suggested in the latter paper that microspheres may be useful in assessing the transport potential of *C. parvum* oocysts. In a recent glass-bead column study, it was reported that 4.1 μm carboxylated microspheres exhibited transport behavior similar to that of *C. parvum* oocysts (Tufenkji et al. 2004). For field applications, the utility of carboxylated microspheres as surrogates for *C. parvum* oocysts would depend primarily on how well the microspheres represent oocyst surface properties under ambient chemical and physical conditions.

This study used commercially available, fluorescent, carboxylated, polystyrene microspheres as surrogates for *C. parvum* oocysts for small-scale, in situ column and seepage meter injection-and-recovery experiments at the SCWA's Russian River site. We employed microspheres in our field tests because they are relatively non-toxic, easy to detect, and available in a wide variety of size classes. Carboxylated microspheres and *C. parvum* oocysts share several important physicochemical properties that affect transport behavior, i.e., near-spherical morphology, similar buoyant densities, and a net-negative charge over pH values typical of many rivers employed in bank filtration.

However, the differences in surface properties between the microspheres and oocysts, particularly the difference in the magnitude of the negative charge under neutral to slightly alkaline conditions, need to be taken into account. In our study, the carboxylated polystyrene microspheres suspended in water collected from the Russian River exhibited a more negative zeta (ζ) potential than similarly sized *C. parvum* oocysts (Table 2). Similarly, in studies described by Dai and Hozalski (2003) and Bradford et al. (2002), where ζ potentials of oocysts and carboxylated microspheres were compared under similar chemical conditions and using similar methods, carboxylated microspheres were substantially more electronegative than the oocysts. Consequently, microspheres should be more reactive in the presence of aquifer grain surfaces characterized by both positive and negative charges.

In a separate transport study involving the use of microspheres as surrogates for *C. parvum* oocysts in the Biscayne Aquifer, it was found that 3-μm and 5-μm polystyrene microspheres had a higher propensity for attachment to limestone surfaces and were less likely to be transported than similarly-sized *C. parvum* oocysts (Harvey et al. in review). Thus, it is likely that microsphere surrogates may underestimate the degree of oocyst transport, at least for some granular aquifers. Also, it is not known how the surface characteristics of oocysts obtained from university and commercial laboratories compare to those of oocysts found in natural waters. Consequently, caution should be exercised in interpreting microsphere transport results from bank-filtration field studies.

Researchers have recognized that oocyst source and preparative methods, use of antibiotics as preservatives, exposure to high ionic strength solutions, and extensive washing can substantively alter oocyst surface charges (Brush et al. 1998; Butkus et al. 2003). Microspheres used in our study have well-defined, rather simple carboxylated polystyrene surfaces, whereas the surfaces of oocysts are a great deal more complex and are characterized by the presence of surface proteins (Kuznar and Elimelech 2005) and a glycocalyx-like immunogenic surface (Harris and Petry 1999; Butkus et al. 2003). Also, it has been recognized that the oocyst sorption to metal oxides can be affected by changes in cyst wall integrity and consequential redistribution of nucleic acids from the interior of the oocyst to the exterior (Walker and Montemagno 1999). Nevertheless, this study suggests that the use of a polydispersed mixture of microspheres has utility in assessing the efficacy of alluvial sediments for removing *C. parvum* oocysts, providing that differences in surface characteristics are taken into account.

Oocyst Transport Potential in Russian River Sediments

Laboratory experiments, conducted with 3- to 5-μm diameter microspheres and fluorescently labeled oocysts in contact with riverbed or infiltration basin sediments saturated with water collected from the Russian River, suggested that at least 99% of the introduced oocysts and microspheres would be retained within the shallow sediments. Removal rates were linearly related to loading over several orders of magnitude (Figures 6a and 6b), indicating that sorptive-filtration may play a more important role than straining in oocyst immobilization in Russian River sediments. Because of grain angularity, Bradford et al. (2002), suggested that straining of oocysts may start to occur at a ratio of colloid to average grain diameter (D_p/D_g) of only 0.0017, which is considerably lower than previously believed, i.e., 0.05 (McDowell-Boyer et al. 1986).

Tufenkji et al. (2004) suggested a higher ratio of at least 0.018 for sediments composed of angular grains in order for straining of oocysts to occur. However, a microscopic examination of sediment grains from the 2 sites suggested that rounded grains were more prevalent in Russian River sediments. Given the rounded and coarse (1.6–2.7 mm average grain size) nature of the sediments, it is unlikely that straining is a major removal mechanism of oocysts, at least for the near-surface basin and river sediments that we examined.

Microsphere Transport in Russian River Infiltration Basin Sediments

Laboratory experiments, conducted with 3- to 5-μm diameter microspheres in contact with riverbed or infiltration basin sediments, suggested that 1–7 log units of removal of the introduced microspheres are likely to occur within the first m of travel through the near-surface sediments. Given the differences in the ζ potential between oocysts and microspheres when suspended in water collected from the Russian River (this study) and previous observations of higher fractional removal of microspheres in flow-through column tests (Harvey et al. in preparation), oocysts may be transported through the bottom sediments to a greater

extent than microspheres. However, results of column experiments suggest removal rates for *C. parvum* oocysts on the order of at least ~1 log unit per meter of travel through near-surface Russian River sediments is likely.

Results from the July and October 2004 field tests indicated that all 3 size classes of our microsphere surrogates are readily removed within river and infiltration basin sediments from the Russian River, CA (Figures 7a and 7b, 8a and 8b). For the infiltration basin study, the degree of removal was inversely related to microsphere diameter. The latter observations are not consistent with filtration theory (Rajagopalan and Tien 1976; Yao and others 1971). This predicts that for the 3- to 5-μm colloidal size range, where contact with grain surfaces is governed largely by settling and physical interception, the removal of the microspheres by the media should be directly related to size.

There were large differences in the time of initial appearance of the tracers (slightly more than 2 hrs for the in situ column versus less than a minute for the seepage meter). The calculated retardation factors (R_f) for the populations of microspheres appearing at sampled ports within the in situ column test were all nearly 1.0, suggesting that the microspheres and conservative solute were accessing the same pore volumes. In contrast, for the transport experiments involving seepage meters, the appearance of the center of mass for the population of microspheres that were recovered at 0.25 m below the sediment-water interface preceded that of the conservative tracer. The R_f for microspheres employed in latter tests were substantively less than 1, ranging from 0.15 to 0.25, suggesting a velocity enhancement possibly due to preferred flow-path structure. An apparent velocity enhancement of *C. parvum* oocysts relative to a conservative solute tracer was reported for sand-packed columns (Harter et al. 2000).

In the latter study, the velocity enhancement was substantially larger for the columns packed with coarse sand relative to those packed with either fine or medium sand. For a natural-gradient, in situ tracer test performed in another sandy aquifer (Harvey and others 1989), it was observed that the largest size class of microspheres was transported more rapidly than the two smaller size classes. This suggests that pore-size exclusion can be more significant for larger versus smaller sized colloids. An apparent enhancement of velocity relative to a conservative tracer was also observed for other studies involving microbes in granular media (Sinton et al. 2000; Ginn et al. 2002) and has been attributed to hydrodynamic retardation of the solute relative to the microorganism.

It appears that the near-surface Russian River sediments have a substantial capacity for retaining oocysts. Under static conditions in the laboratory, >99 % of the oocysts were retained in basin sediments over the tested range of loading (4 log units for the infiltration basin sediments and 5 log units for the river sediments). Because of the limited ranges of loading, the retentive capacity of the sediments for oocysts could not be determined. However, it appears that the removal capacity of the sediments may be at least 1.7×10^5 oocysts g^{-1} for the infiltration basin sediments and 6.1×10^5 oocysts g^{-1} for the river basin sediments (Figures 6a and 6b).

CONCLUSIONS AND FUTURE WORK

Despite uncertainties in surface properties of oocysts isolated from natural waters, our study nevertheless demonstrated the utility of polydispersed mixtures of carboxylated microspheres in assessing subsurface transport potential of *C. parvum* oocysts at an operating bank filtration site. The differences in surface characteristics and complexity between oocysts and microspheres may limit the utility of microspheres as oocyst surrogates for some applications.

Consequently, more research is needed to ascertain the limitations of microspheres as surrogates in a variety of geohydrologic settings and groundwater chemistries and to develop surrogates with surface characteristics that more nearly match *C. parvum* oocysts. The present study focused on oocyst-sized colloid removal within shallow sediments less than 1 m from the sediment-water interface. The transport behavior of oocysts and microspheres in deeper sediments is unknown, but worthy of further study. Also, more studies are needed to ascertain the roles of the poorly sorted nature of the sediments, depth-specific metal oxide content, and seasonal changes in the near-surface river sediments at the Russian River site upon oocyst removal potential.

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