Chapter 6 Fluorescent Microspheres as Suprogates in Evaluating the Efficacy of Riverbank Filtration for Removing *Cryptosporidium parvum* Oocysts and Other Pathogens

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Abstract A major benefit of riverbank filtration (RBF) is that it provides a relatively effective means for pathogen removal. There is a need to conduct more injection-and-recovery transport studies at operating RBF sites in order to properly assess the combined effects of the site heterogeneities and ambient physicochemical conditions, which are difficult to replicate in the lab. For field transport studies involving pathogens, there is considerable interest in using fluorescent carboxylated microspheres (FCM) as surrogates, because they are chemically inert, negatively charged, easy to detect, available in a wide variety of sizes, and have been found to be nonhazardous in tracer applications. Although there have been a number of in-situ studies comparing the subsurface transport behaviors of FCM to those of bacteria and viruses, much less is known about their suitability for investigations of protozoa. Oocysts of the intestinal protozoan pathogen *Cryptosporidium* spp are of particular concern for many RBF operations because of their ubiquity and persistence in rivers and high resistance to chlorine disinfection. Although microspheres often have proven to be less-than-ideal analogs for capturing the abiotic transport behavior of viruses and bacteria, there is encouraging recent evidence regarding use of FCM as surrogates for *C. parvum* oocysts. This chapter discusses the potential of fluorescent microspheres as safe and easy-to-detect surrogates for evaluating the efficacy of RBF operations for removing pathogens, particularly *Cryptosporidium*, from source waters at different points along the flow path.

Keywords: Riverbank filtration, bank filtration, fluorescent microspheres, pathogens, *Cryptosporidium*, oocysts,

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1. Introduction

Satisfying the increasing demand for safe drinking water is one of the major environmental/health challenges that humans face. Surface waters, particularly rivers in close proximity to population centers, are typically contaminated with pathogens from treated and un-treated domestic wastewater discharges, as well as runoff that might originate from animal feeding operations and agricultural fields where manure has been applied as fertilizers. Increasingly, river-bank filtration (RBF), an old technology first utilized two centuries ago by the Glasgow Waterworks Company (UK), is being utilized as a cost-effective means of providing pre-treatment and, in some cases, complete treatment of surface water (Ray et al. 2002). One of the major benefits of RBF is it provides a relatively effective means for removal of pathogens as contaminated river water is drawn into wells completed in aquifer materials adjacent to the river (Partinoudi and Collins 2007). In some cases, the microbial quality of water extracted from municipal wells at bank filtration sites exceeds that of water filtration plants (Dash et al. 2008, Gollnitz et al. 2003) and, in at least one case, even that of the local aquifer (Shamrukh and Abdel-Wahab 2008). Also, the passage of river water through the subsurface during RBF has been shown to compensate for peak and shock loads (Malzer et al. 2002). However, the interplay of site-specific parameters, e.g., retention time, pore water velocity, river and groundwater chemistries, and characteristics of the aquifer sediments make pathogen removal difficult to predict based upon colloid filtration theory (Tufenkji et al. 2002). Consequently, the efficacy of each RBF site for removing pathogens must be evaluated separately (Kuehn and Mueller 2000) and, in the United States, regulators are reluctant to assign RBF "treatment credits" without clear evidence showing removal of indicators and pathogens.

Although much has been learned about the transport of viruses, bacteria, and protists through geologic media from column studies (Harvey et al. 2007), it has been shown that lab-scale studies can underestimate considerably, sometimes by many orders of magnitude, transport potential of pathogens in aquifers at scales relevant to RBF (Harvey et al. 2008). Also, it is difficult to accurately replicate in the lab, the various physicochemical conditions and physical heterogeneities that come into play when surface water is drawn through an aquifer to water-supply wells. Consequently, there is a need to conduct more injection-and-recovery transport studies at operating RBF sites in order to properly assess the combined effects of the site heterogeneities and ambient physicochemical conditions. For field transport studies involving pathogens, there is considerable interest in using fluorescent carboxylated microspheres (FCM) as surrogates, because they are chemically inert, negatively charged, easy to detect, available in a wide variety of sizes, and have been found to be nonhazardous in tracer applications (Behrens at al. 2001).

Of particular concern for many RBF operations are oocysts of the intestinal protozoan pathogen *Cryptosporidium* spp. because of their ubiquity in many surface

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water environments (LeChevallier at al. 1991), persistence in aquatic environments for long periods of time (Pokorny et al. 2002), low infective dose rate (Okhuysen at al. 1999), and high resistance to chlorine disinfection (Carpenter et al. 1999). Failures of granular media filtration to remove Cryptosporidium from source water during water treatment have resulted in serious outbreaks of cryptosporidiosis; the most publicized being the massive 1993 outbreak in Milwaukee that resulted in over 400,000 infections (Mac Kenzie et al. 1994). In contrast, definitive data are lacking that link Cryptosporidiosis to RBF operations. However, the potential for oocyst transport from rivers to RBF water-supply wells remains a concern, in part because outbreaks of cryptosporidiosis from consumption of improperly treated, contaminated groundwater are known to occur (e.g., Willocks et al. 1998). Because of the episodic nature of infectious doses and low infective does rate characterizing *Cryptosporidium* contamination events, early detection can be challenging. The use of microbial surrogates is thought to be important in evaluating the efficacy of RBF sites for removing Cryptosporidium (Tufenkji et al. 2002). However, the lack of correlation between the microbial surrogates and pathogenic protists in RBF-produced water (e.g., Gollnitz et al. 2003) suggest that reliable microbial surrogates have yet to be identified.

Fluorescent microspheres may be particularly useful surrogates for use in in-situ transport studies designed to assess the degree of removal of oocysts and other pathogens at RBF sites because they are available in sizes and buoyant densities that are similar to those of oocysts and because their aspect ratios compare favorably to the near-spherical oocysts (Harvey et al. 2008). Microspheres have been used as safe surrogates for oocysts in a variety of bench-scale water-treatment studies, involving water-treatment filters (Brown and Emelko 2009, Dai and Hozalski 2003, Emelko and Huck 2004), carbon-block filtration (Lau et al. 2005), biofilm removal (Stott and Tanner 2005), ozonation (Tang et al. 2005), and sandy media (Tufenkji et al. 2004). However, much less is known about their suitability as surrogates in field-scale studies designed to assess the vulnerability of water-supply wells at RBF sites. This chapter discusses the potential of fluorescent microspheres as safe and easy-to-detect surrogates for evaluating the efficacy of RBF operations for removing pathogens, particularly *Cryptosporidium*, from source waters at different points along the flow path.

2. Comparisons of Microsphere and Microbial Transport Behaviors in the Subsurface

FCM have been used as surrogates in groundwater injection-and-recovery studies since the mid 1980s in order to gain information about the abiotic aspects of subsurface microbial transport behavior in a variety of aquifers (Harvey and Harms 2002). In most of these studies, the microspheres are added to the aquifer concomitantly with a conservative solute tracer, typically a halide salt or non-reacting

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dye. By comparing the breakthrough curves of the conservative tracer and the microspheres, information is gained about the role of the "particulate" and "reactive" natures of the microbial-sized microspheres in determining its transport behavior in various geohydrologic settings. However, in order to relate how transport of microspheres and pathogens might compare at the field scale, it is important to conduct side-by-side transport comparisons of the microorganism and the surrogate microsphere using the site-specific conditions and geologic media that characterize the field site. For pathogens, such comparisons must be done in the lab for safety and permitting reasons. However, the most meaningful transport comparisons are accomplished in the field, because it is often exceedingly difficult to replicate the field geochemical/physical heterogeneities at the bench scale.

Table 6.1 lists groundwater tracer studies where FCM were injected into aquifers along with viruses, bacteria, or protozoa and comparisons made between their respective transport behaviors. The first such test conducted in 1986 involved both forced- and natural-gradient conditions (Harvey et al. 1989). In that test, transport behaviors of a variety of sizes of microspheres were compared to those of the unattached indigenous bacterial communities that had been recovered from a sandy aquifer (Cape Cod, Massachusetts, USA), concentrated, stained with the DNA-specific fluorochrome DAPI (4',6-diamidino-2-phenylindole) and returned to the aquifer with the microspheres and conservative tracers (bromide and chloride). Also in the 1980s, the transport of FCM and the bacterium *Escherichia coli* were assessed in a fractured granite aquifer within the Canadian Shield (Ontario, Canada) (Champ and Schroeter 1988). A more recent subsurface transport study involving the concomitant addition of bacteria and microspheres to a fractured-granite near Mirror Lake, New Hampshire, USA used several morphologically and physicochemically different bacterial strains (*Pseudomonas stuzeri*, *Microbacterium sp.*, and Staphylococcus sp.) (Becker et al. 2003). Also, microspheres and the bacterium *Ralstonia eutropha* were added to unsaturated epikarst/karst limestone at Gännsbrunnen, Switzerland (Sinreich et al. 2009). For the five studies listed in Table 6.1, retardation of the peak concentrations of FCM (relative to a conservative tracer) was reasonably close (within half a log unit) to those of the bacteria. However, immobilization of the microspheres was generally much greater, in some cases by as much as 2 log units (Champ and Schroeter 1988), than that experienced by bacteria traveling through the same flow paths. Consequently, FCM may generally be expected to under-predict bacterial transport potential in a variety of hydrologic settings, including granular systems that are similar in basic structure to some of the alluvial aquifers where RBF operations are sited. However, a greater attenuation of the microspheres may not always be the case, because of the species-to-species differences in surface properties. For example, a greater attenuation of the bacterium than the FCM was observed in the Mirror Lake, NH study, but only for the *Microbacterium* strain (a Gram positive rod) (Becker et al. 2003).

t6.1.1 TABLE 6.1. Field injection-and-recovery studies comparing subsurface transport behaviors of carboxylated microspheres with those of microorganisms.

t6.1.2 Field test site	Media	Microsphere diameter, µm	Microbe (size, µm)	Distance (m)	Relative velocity*	Relative attenuation**	Reference
t6.1.3 Viruses							
t6.1.4 Cape Cod, MA (USA)	well sorted sand	0.7	PRD1, phage (0.06 diameter)	12	-	---	(Bales et al. 1995)
t6.1.5 Borden, Ont. (Canada)	well sorted sand	0.01	PRD1, phage (0.06 diameter)	2	+	---	(Bales et al. 1995)
			M1, phage (0.025 × 0.11)	2	+/-	---	(Bales et al. 1995)
t6.1.6 Jura Mtns. (Switz.)	karst limestone	1.0	H40, marine phage (85nm)	1250	-	+	(Auckenthaler et al. 2002)
t6.1.7 Bacteria							(Harvey et al. 1989)
t6.1.8 Cape Cod, MA (USA)	well sorted sand	0.2, 0.7, 1.2	aquifer community (0.2–1.4)	1.7	+/-	++	(Harvey et al. 1993)
t6.1.9 Cape Cod, MA (USA)	well sorted sand	0.7	aquifer community (0.2–1.6)	6	+/-	++	(Sinreich et al. 2009)
t6.1.10 Gännsbrunnen (Switz.)	limestone (epikarst)	1.0	Ralstonia eutropha (0.5 × 1.8)	10	+/-	+	(Becker et al. 2003)
t6.1.11 White Mtns, NH (USA)	fractured granite	1.0	Pseudomonas stuzeri (1.5–2.2)	36	+/-	+/-	(Becker et al. 2003)
			Microbacterium sp (1.4–1.9)	36	+/-	+	(Becker et al. 2003)
			Staphylococcus sp (0.5–0.8)	36	+/-	-	(Champ and Schroeter
t6.1.12 Chalk R Lab (Canada)	fractured granite	2.0	Escherichia coli (unspecified)	13	+/-	+	1988)
t6.1.13 Protists							
t6.1.14 Cape Cod, MA (USA)	well sorted sand	2.0	Spumella guttula Kent (2–3)	1	+/-	+/-	(Harvey et al. 1995)

t6.1.15 * "Relative retardation" was estimated based upon the arrival times of the peak concentrations relative at the downgradient (sampling) well. "+" means that
t6.1.16 the microspheres traveled >1.5 times faster than the test microorganism. "+/-" means that the microspheres and viruses traveled at velocities that differed
t6.1.17 less than a factor of 1.5. "-" means that the microspheres traveled at a velocity that was at least 1.5 times slower than that of the test microorganism. For the
AQ2 t6.1.18 H4/H40 phage test (Jura Mtns, Switzerland), relative velocity is based upon first detection. Size of H40 phage from {Flynn et al. 2004, #200}.

t6.1.19 ** "Relative attenuation" was estimated from the differences between the microspheres and microorganisms in their respective fractional recoveries. "++"
t6.1.20 means that the fractional loss (immobilization) of microspheres was 1.5–2.4 log units higher than that of the test microbe. "+" means that the fractional loss
t6.1.21 of microspheres was 0.5–1.4 log units higher than that of the test microbe, "+/-" means that there was less than 0.5 log units of difference between fractional
t6.1.22 loss of microspheres and test microbe, "-" means that the fractional loss of microspheres was 0.5–1.4 log units lower than that of the test microbe, "--"
t6.1.23 means that the fractional loss (immobilization) of microspheres was 1.5–2.4 log units lower than that of the test microbe, "---" means that the fractional loss
t6.1.24 (immobilization) of microspheres was at least 2.5 log units lower than that of the test microbe. Relative attenuation for the Borden site study was estimated
t6.1.25 from the changes in peak concentrations as the viruses and microspheres were advected from well ML4-4 to ML5-4 .

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Injection tests involving both microspheres and viruses (Table 6.1) indicate that it would be difficult to make any generalizations about the suitability of microspheres as surrogates for viruses in field injection-and-recovery experiments. However, judging from results of tracer tests involving sandy aquifers at sites in Cape Cod, MA USA (Bales et al. 1995) and at Borden, Ontario Canada (Bales et al. 1997), it is likely that FCM would over-predict the rate of virus transport by a factor of up to ~10^3. Microspheres traveled significantly faster than the phage at the latter site, but considerably slower than the phage at the former site. For a 1250 m-long colloid transport study involving karst limestone (Switzerland), 1 μm microspheres traveled ~7 times faster than the H40 bacteriophage based upon time of first arrival (Auckenthaler et al. 2002). However, the more than ten-fold difference in size of the two colloids would be expected to have at least some affect on time of first detection, judging from the results of other tests performed in karst limestone (e.g., Harvey et al. 2008).

Very little information is available on how the subsurface transport behaviors of FCM compare to those of protozoa. Although there were substantial differences in the transport behaviors of 1 μm FCM and similar sized groundwater bacteria in a well-sorted, sandy glacial outwash aquifer in Cape Cod, Massachusetts (Harvey and Garabedian 1991), larger (2 and 3 μm) FCM reasonably captured the transport behavior of the 2–3 μm groundwater protist (protozoa), *Spumella guttula* Kent in a subsequent study at the same site (Harvey et al. 1995). In particular, the breakthrough of microspheres and protozoa were reasonably similar in terms of both immobilization and retardation (Table 6.1). Also, the FCM did a reasonable job of replicating the multi-peaked pattern of the protozoan breakthrough curves at the sampling wells downgradient from point of injection. Consequently, it was suggested in the latter study that FCM may be useful as abiotic analogs for *C. parvum* oocysts in subsequent field transport tests.

3. Microspheres for Assessing Vulnerability of RBF Wells to *Cryptosporidium* Contamination

3.1. Comparison of Oocyst and Microsphere Properties

A comparison of physicochemical characteristics (buoyant densities, aspect ratios, average diameters, zeta potentials) of *C. parvum* oocysts and oocyst-sized FCM that would affect subsurface transport behavior is detailed in Harvey et al. (2008). In that study, aspect ratios of oocysts were generally slightly greater than those of the microspheres, although both were nearly spherical. The oocysts exhibited a

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range of buoyant densities (1.03–1.07 g/cm^3) that bracketed that of the microspheres (~1.05 g/cm^3). However, under ionic strength (10^{-2}) and near-neutral to slightly alkaline conditions, FCM exhibited substantially more negative zeta potentials than those measured for the C. parvum oocysts. In a preliminary study involving static minicolumns, artificial groundwater, and fragments of crushed limestone core, both oocysts and 4.9 μm microspheres exhibited a tendency for increasing sorption on limestone surfaces in response to increasing dissolved calcium (Osborn et al. 2000).

Unfortunately, a dearth of information exists about surface properties for *C. parvum* oocysts found in natural waters, because of the difficulties in concentrating a large enough number to perform surface measurements. However, the range of surface properties reported for *C. parvum* oocysts suggests that it is probably incorrect to assume that oocysts in aquifers will always be highly charged. A more cautious approach is suggested that assumes some oocysts in natural waters may be characterized by low charge, at least until there is more definitive evidence to the contrary. Although FCM proved useful surrogates in the 2004 Northwest Well Field vulnerability test (Miami, FL), it is recommended that a mixture of different types and sizes of microspheres representing a wider variety of surface charge having car-boxylated, neutral, and carbonyl surfaces be employed in future well field vulnerability assessments regarding this pathogen (Harvey et al. 1989).

3.2. Bench-Scale Comparisons of Microsphere and Oocyst Transport

Several recent laboratory studies have compared the attachment and (or) transport behaviors of oocyst-sized FCM and C. parvum oocysts in the presence of granular porous media (Dai and Hozalski 2003, Emelko et al. 2003, Tufenkji et al. 2004). Emelko et al. (2003) reported similar rates of removal for formalin-inactivated oocysts and 4.7 μm FCM in a pilot-scale dual-composition media (anthracite and sand) filter. Similarly, Tufenkji et al. (2004) noted similar transport behaviors (dimensionless concentration histories in the eluent and fractional removals) for heat-inactivated oocysts and 4.1 μm FCM in clean quartz sand (1 mM ionic strength and pH 5.6–5.8). In contrast, other microsphere-oocyst comparisons suggest that their attachment and transport behaviors in porous media can also differ substantially. Bradford and Bettahar (2005) reported differences in breakthrough and tailing behaviors for transport of oocysts (3–6 μm) and microspheres (3.2 μm) being advected through a sand column. In another study (Brush et al., 1998), 3–4 fold greater attachment was observed for 4.5 μm carboxylated polystyrene microspheres onto polystyrene surfaces at 50 mM ionic strength as compared with DIS-purified oocysts.

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The effectiveness of FCM as surrogates for C. parvum oocysts in in-situ subsurface transport studies depends upon how well the microspheres represent their surface properties for a particular set of chemical and physical conditions. In several studies, zeta potentials (ζ) of the FCM were more negative than those of oocysts under neutral to slightly alkaline conditions (Bradford and Bettahar 2005, Dai and Hozalski 2003, Harvey et al. 2008). Although some reports suggest a very weak surface charge at circumneutral pH (e.g., Brush et al. 1998, Butkus et al. 2003), others (Considine et al. 2000, Hsu and Huang 2002, Ongerth and Pecoraro 1996) suggest that oocysts may carry a more substantive negative charge. Reported differences in oocyst ζ where similar physicochemical conditions were used are probably due to a variety of factors, including source (Butkus et al. 2003), age, exposure to antibiotics, and method of purification (Brush et al. 1998).

Given the variability of surface characteristics of oocysts and differing geologic media from site to site, surrogate microspheres can potentially over-predict and under-predict transport of *C. parvum* oocysts. For a transport study involving intact core samples of lime-stone characterized largely by matrix porosity, 4–6 μm oocysts were transported, respectively, ~4 and ~6 times more readily than 3 and 5 μm FCM (10 mM ionic strength, pH 8) (Harvey et al. 2008). In contrast, recovery of 3 μm FCM in static columns packed with sediments from a RBF site (Russian River, CA) was substantively greater than oocysts of similar size (Metge et al. 2010). Unfortunately, there is little information about surface properties for oocysts found in natural waters, because of the difficulties involved in recovering and concentrating a sufficient number on which to perform surface measurements. However, available evidence suggests that FCM are better surrogates for some *C. parvum* oocysts than others. It is also likely that the suitability of FCM as transport surrogates for *Cryptosporidium* oocysts will vary from species to species.

It is also evident that within the typical *C. parvum* oocyst size range, colloidal diameter can substantively affect their rate of attenuation within geologic media. Figure 6.1 depicts the breakthrough curves for pulse injections for 2.5–6 μm oocysts and a monodisperse suspension of microspheres being advected through 10 cm of repacked aquifer sediments recovered from the Greater Miami River (Ohio, USA) RBF site (Sheets et al. 1989). Average size of the oocysts decreased ~2 fold with time and distance in the column. This suggests that the larger sized oocysts are subject to a higher rate of immobilization. Similarly, 3 μm FCM being advected through in-tact lime-stone cores were attenuated to a lesser degree than were 5 μm FCM (Harvey et al. 2008). The differences in attenuation in the latter study appeared to be due largely, but not entirely, to predicted differences in settling rates.

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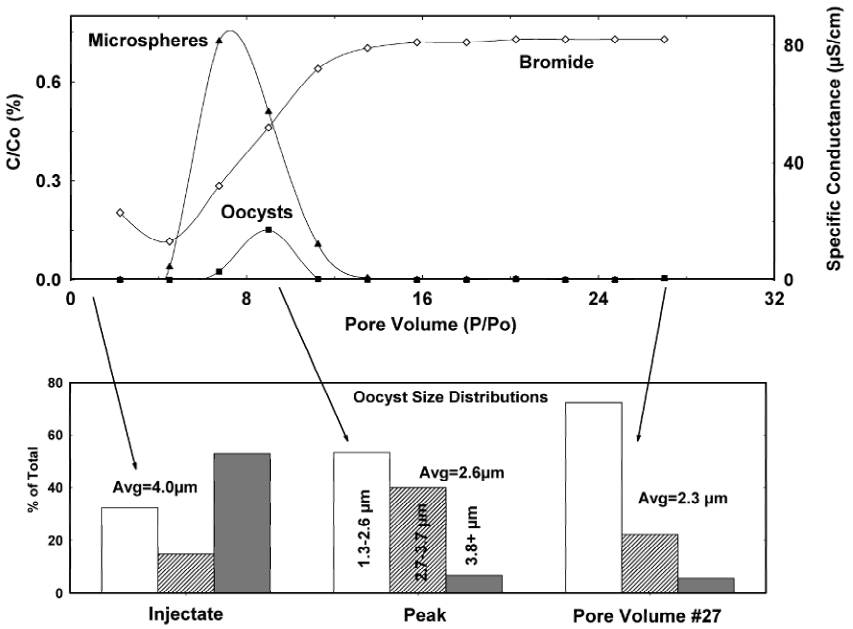

Figure 6.1. Dimensionless concentrations of carboxylated microspheres (1 µm) and 2.5–7 µm C. parvum oocysts in the eluent of a column of aquifer sediment recovered from the Greater Miami River RBF site located in southwestern Ohio, USA. Breakthrough of the constant injection of the conservative tracer (bromide) was measured by specific conductance. The bar graphs indicate the changes in the size classes and average diameter for the oocysts as they were advected through the column.

3.3. Field Transport Studies Using Oocyst-Sized Microshpheres

Several injection-and-recovery tests have been conducted in which the subsurface transport of protozoan-sized microspheres have been assessed. In 1998, the transport potential of 5 and 15 µm microspheres were assessed in a forced-gradient injection and recovery study involving a granular aquifer in Idaho USA (Petrich et al. 1998). More recently, oocyst-sized FCM were used as transport surrogates to assess the vulnerability of two karst-limestone aquifers to Cryptosporidium contamination. In 2005, 5 µm microspheres were added to a cave stream in southern Germany, some of which were recovered 2.5 km away (Goppert and Goldscheider 2008). Although greatly attenuated within the aquifer, the appearance of microspheres at a spring 2.5 km downgradient from point of injection demonstrated that oocyst-sized colloids were capable of traveling quickly over considerable distances in karst limestone settings and could be detected in very low numbers.

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In a forced-gradient, convergent study conducted in 2004, the transport of 2, 3, and 5 μm FCM were assessed relative to that of a conservative solute (SF6) from a borehole placed into the karst limestone aquifer to a water-supply well 97 m away (Harvey et al. 2008). The Miami, FL (USA) study established the utility of a polydispersed suspension of fluorescence microspheres in tracer application in the vicinity of water-supply wells. The early appearance of the center of mass of the three size classes of microspheres relative to the conservative tracer underscored the need to use colloidal tracers in studies assessing the vulnerability of wells to oocyst contamination. Although not a RBF site, the water-supply well drew water from an aquifer that was hydraulically connected to surface water and in the vicinity of borrow pit lakes with similar depths as the well's production zone. Oocyst-sized microspheres could be quantified with reasonable accuracy at a distance of 97 m downgradient using either epifluorescence microscopy or by the less labor-intensive flow cytometry. For the latter method, microspheres in well water samples had to be concentrated by filtration. Lastly, 2, 3, and 5 μm FCM were employed as safe surrogates in order to gather in-formation about the transport potential of oocysts in the shallow bottom sediments 25 m above a lateral collector (Russian River RBF site, Sonoma County, CA, USA) (Metge et al. 2007). The latter study pointed out the effect of site-to-site variability in transport parameters; the smallest microspheres (2 μm diameter) were transported preferentially at a location characterized by relatively lower vertical hydraulic conductivity (and slower vertical flow rate) site, but that the intermediate size microspheres (3 μm diameter) were transported preferentially at a nearby site characterized by higher vertical hydraulic conductivity and faster vertical flow rates.

The experimental design of injection-and-recovery studies involving the use of protozoan-sized microspheres in a given water-supply aquifer depends, in large part, upon their transport potential in a given system. Table 6.2 illustrates the variations in removal efficiency of 2 μm FCM during field studies involving three different types of aquifers. Removal (expressed per meter of travel through the aquifer) were 5–6 log units greater in the poorly sorted, Fe-rich sediments beneath the Russian River (CA) relative to sediments in a glacial outwash aquifer (Cape Cod, MA), in spite of the coarser grains at the former site. These differences reflect both differences in structure (sorting) and differences in extractable (grain-surface) iron. As expected, transport of the same size microspheres through the karst limestone characterized by preferential flow was much greater (by ~77 log units) than in the sandy, outwash aquifer.

t6.2.1 TABLE 6.2. Role of media characteristics in the removal efficiency of 2 μm carboxylated microspheres in field injection and recovery studies.

t6.2.2 Field test site	Depth (m)	Flow (m/d)	Media characteristics			Log_{10} removal	Reference
			Description	Grain size	Extractable Fe		
t6.2.3 Russian R. bank filtration site (Sonoma County, CA, USA)	0–0.5	0.15–0.38	poorly sorted granular	2.74 mm (D_{50}) 0.50 mm (D_{10})	20–34 mg/g*	6.6/m	Metge et al. 2007
t6.2.4 USGS Cape Cod test site (Falmouth, MA, USA)	9.3	0.5	well-sorted sand	0.59 mm (Avg)	0.2–0.3 mg/g**	1.0/m	Harvey et al. 1995
t6.2.5 Northwest Well Field (Miami, FL, USA)	11–13	variable	karst limestone	n/a	ND	0.013/m	Harvey et al. 2008

t6.2.6 *Metge 2010

t6.2.7 **Ryan et al. 1999 and Scholl et al. 1992

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4. Limitations of Microspheres as Surrogates

Although commercially available fluorescent microspheres are easy to detect, available in a variety of sizes (tens of nm to tens of μm), and chemical stable over long periods of time, there are two primary drawbacks that could limit their use as pathogen surrogates in certain field applications. The first is that their surface characteristics and morphology may not match up well with the microorganisms of interest, which can lead to differences in retardation, immobilization, and apparent dispersion. For example, it was observed in forced- and natural-gradient tracer tests performed in a sandy aquifer, that the transport behavior and collision efficiencies of native groundwater bacteria were not well represented by FCM of similar size (Harvey and Garabedian 1991, Harvey et al. 1989). However, it was also observed that a polydispersed suspension (2–5 μm) of FCM could be very useful as a surrogate of C. parvum oocysts, providing that differences in surface characteristics are taken into account (Harvey et al. 2008).

One potential solution for improving the utility of microspheres as surrogates in field applications would be to use microspheres whose surface chemistry more closely match the microorganism of interest. In addition to FCM discussed in this report, there are other types of commercially available microspheres that may be more appropriately suited for a particular application. It was observed that different types of microspheres having carboxylated, carbonyl, and neutral surfaces and injected concomitantly into a sandy aquifer were subject to different degrees of retardation and immobilization (Harvey et al. 1989). In a tracer test that involved addition of both amidine (positively charged) and polystyrene (negatively charged) microspheres to a fractured granite aquifer, the microspheres with carboxylated surfaces were transported much more readily that than the microspheres with positively charged surfaces (Becker et al. 2003). The surfaces of at least some microspheres can be chemically modified relatively easily. For example Pang et al. (2009), chemically modified the surfaces of 20 nm FCM with proteins (bovine milk α_s casein) such that the surface electrical properties were fairly similar to those of the MS2 bacteriophage (virus).

A second drawback for the use of microspheres for RBF field application involves cost. Using a large enough quantity of microspheres that ensures accurate and quantifiable breakthrough at wells downgradient can be very expensive. This is particularly true for the larger (protozoa-sized) microspheres, because the cost (per microsphere) is related to their diameters. The microspheres are manufactured to exact tolerances for such use as instrument calibrations and biomedical applications, both of which require much smaller quantities than a field-scale injection-and-recovery test. However, finding and using microbial surrogates in RBF operations can be problematic often because of permitting and safety issues or because good microbial surrogates do not always exist. Also, some surrogates require analysis within 24-h, which, in many cases, could limit the scope of field transport studies (Goppert and Goldscheider 2008).

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5. Conclusions

The suitability of fluorescent, microbial sized microspheres as field surrogates for pathogens at RBF sites continues to be explored. Although microspheres often have proven to be less-than-ideal analogs for capturing the abiotic transport behavior of viruses and bacteria, encouraging evidence exists regarding use of FCM as surrogates for *C. parvum* oocysts. Suspensions of variably-sized FCM that bracket the size range of oocysts typically found in natural waters can be useful as surrogates in field-scale injection-and-recovery tests at an operating RBF sites (Metge et al. 2007) or other situations where water-supply wells are drawing water, at least in part, from nearby sources of surface water (Harvey et al. 2008). There are commercially available microspheres that have roughly the same aspect ratios and buoyant densities as oocysts. However, the use of microspheres is limited because of the high costs and discrepancies between their surface characteristics. Following a recent test involving the transport of oocyst-sized microspheres through a drinking-water karst limestone aquifer, it was recommended that a mixture of different types of microspheres having a wider variety of surface characteristics be used in future field-scale studies designed to investigate the transport potential of pathogens. Modification of microsphere surfaces with proteins (Pang et al. 2009) may facilitate our ability in the future to construct mixtures of microspheres that would bracket not only the size of pathogen of interest, but also its surface charge. Field-scale studies by Passmore et al. (2010) found that microspheres with similar surface properties and size as microorganisms of interest can be useful surrogates to trace transport pathways of in the subsurface.

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