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Microbial-sized, carboxylate-modified microspheres as surrogate tracers in a variety of subsurface environments: an overview

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Abstract

Since 1986, fluorescent carboxylate-modified polystyrene/latex microspheres (FCM) have been co-injected into aquifers along with conservative tracers and viruses, bacteria, and (or) protozoa. Use of FCM has resulted in new information about subsurface transport behaviors of microorganisms in fractured crystalline rock, karst limestone, soils, and granular aquifers. FCM have been used as surrogates for oocysts of the pathogenic protist *Cryptosporidium parvum* in karst limestone and granular drinking-water aquifers. The advantages of FCM in subsurface transport studies are that they are safe in tracer applications, negatively charged, easy to detect, chemically inert, and available in wide range of sizes. The limitations of FCM are that the quantities needed for some field transport studies can be prohibitively expensive and that their surface characteristics may not match the microorganisms of interest. These limitations may be ameliorated, in part by using chemically modified FCM so that their surface characteristics are a better match to that of the organisms. Also, more sensitive methods of detection may allow using smaller quantities of FCM. To assess how the transport behaviors of FCM and pathogens might compare at the field scale, it is helpful to conduct side-by-side comparisons of their transport behaviors using the geologic media and site-specific conditions that characterize the field site.

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1. Introduction

Since their first use in an injection-and-recovery groundwater transport study in 1986[1], microbial-sized (0.05-5.0 µm, diameter), fluorescent carboxylate-modified polystyrene/latex microspheres (FCM) have been increasingly used as surrogates for microorganisms in field studies to gather information about abiotic aspects of microbial transport

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behavior for a variety of subsurface settings[2]. FCM have been injected in aquifers coincidently with conservative solute tracers, typically halide salts or non-reacting dyes. Comparison of the microsphere and conservative tracer breakthrough curves can yield important information about the "particulate" and "reactive" natures of FCM in determining their transport behavior in various geohydrologic settings. This paper discusses the uses and limitations involving field applications of FCM in order to gain a better understanding of microbial transport behavior in a variety of subsurface environments.

2. FCM and microbial comparisons in subsurface transport studies

Table 1 lists injection-and-recovery transport studies where FCM were injected into aquifers along with viruses, bacteria, or protozoa and comparisons made between their respective transport behaviors. The field studies involved a wide range of aquifer types from well sorted, sandy glacial outwash deposits to fractured granite and karst-limestone. Microorganisms that have been co-injected with FCM and solute tracers have included bacteria-specific viruses (phages), bacteria, and protozoa. Viruses have included the coliphages MS2[3] (26 nm), PRD1[4,5] (62 nm diameter, alternate host *Salmonella typhimurium*), and M1[5] (25 x 110 nm); the marine phages H40[6] (85 nm long) and H4[6], and the cyanophage AS-1[3] (110 nm long). Results from injection tests involving both microspheres and viruses suggest that it would be difficult to make any generalizations concerning the suitability of FMC as surrogates for viruses in subsurface transport studies. FCM may over-predict the rate of virus transport by a factor of up to ~10[3], judging from results of tracer tests involving sandy aquifers at sites in Cape Cod, MA USA[4] and Borden, Ontario Canada[5]. Also, FCM traveled considerably faster than the viruses at the Borden site, but considerably slower than the viruses at the Cape Cod site. For the transport study involving karst limestone in Northwest Switzerland[6], 1 µm (diameter) FCM traveled ~7 times faster than the H40 bacteriophage based upon time of first arrival. However, these results could be affected, at least in part, by the >10-fold difference in the sizes of the two colloids.

Table 1. Use of carboxylate-modified microspheres with microorganisms in subsurface injection-and-recovery tracer tests.

Field test site	Description	Micro-sphere (diameter, µm)	Microbe	Solute tracer	Travel dist.(m)
Crystalline rock					
White Mtns, NH, USA[7]	Fractured-crystalline rock	1.0	*Pseudomonas stutzeri*	^2H_2O	36
Chalk R., Ontario, Canada[8]	Fractured-crystalline rock	1.2, 2.1	*Escherichia coli* K12	Bromide	13
Carbonate rock					
Hochgrat site, Germany[9]	Clastic carbonate conglomerate	1.0	Fecal bacteria	Several dyes	1000
Jura Mtns, NW Switzerland[6]	Karst limestone	1.0	H4 & H40 (phages)	Uranine	1250
Gännsbrunnen, Switzerland[10]	Epikarst (limestone)	1.0	*Ralstonia eutropha* H16	Iodide	10
Soils					
Kintore, Ontario, Canada[11]	Silt/calcareous loam till	1.1, 3.9, 4.8	*Escherichia coli* RS2g	Brillant blue (dye)	0.22
Unconsolidated sediments					
Borden, Ontario, Canada[5]	Well-sorted sand	0.01	PRD1 & M1 (phages)	Chloride	2.0
Falmouth, MA, USA[4]	Well-sorted quartz sand	0.7	PRD1 (phage)	Bromide	12
Falmouth, MA, USA[1]	Well-sorted quartz sand	0.2, 0.7, 1.2	Aquifer community	Bromide	1.7
Falmouth, MA, USA[12]	Well-sorted quartz sand	2.0	*Spumella guttula*(protist)	Bromide	1.0
Ashumet Pond, MA[3]	Pond bottom sediments	1.7	*Synechococcus* sp. IU625, MS2 (coliphage), AS-1 (cyanophage)	Bromide	0.25

Bacteria introduced to subsurface environments have included *Pseudomonas stutzeri*[7] (rod-shaped, motile 1.5-2.2 µm), *Escherichia coli* K12[8], *Ralstonia eutropha* H16 (rod-shaped, motile 0.5 x 1.8-2.6 µm), *Escherichia coli* RS2g[11] (1.2 µm, diameter), *Synechococcus* sp. IU625[3] (2.6 µm), and entire communities of groundwater bacteria (0.2-1.6 µm)[1]. In the last case, the morphologically diverse community of aquifer bacteria were concentrated from groundwater by using continuous-flow centrifugation or tangential-flow filtration, stained with the DNA-specific stain 4',6-diamidino-2-phenylindole (DAPI) and diluted with filtered groundwater before re-introducing them to the aquifer. For the studies listed in Table 1, retardation factors based upon peak concentrations of FCM relative to a conservative tracer were reasonably close (within half a log unit) to those of the bacteria. However, immobilization